OPTIMIZATION OF SPRAY DRYING CONDITIONS FOR PREPARATION OF PROBIOTIC DIRECT VAT SET STARTERS

THESIS SUBMITTED TO THE

ICAR-NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL

(DEEMED UNIVERSITY)

IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE AWARD OF THE DEGREE OF

MASTER OF TECHNOLOGY

IN

DAIRY MICROBIOLOGY

BY

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This is to certify that the thesis entitled: **"OPTIMIZATION OF SPRAY DRYING CONDITIONS FOR PREPARATION OF PROBIOTIC DIRECT VAT SET STARTERS"** submitted by Miss. **WALDE NEHA RAVI** towards the partial fulfilment of the requirement for award of the degree of **MASTER OF TECHNOLOGY** in **DAIRY MICROBIOLOGY** of the **ICAR-NATIONAL DAIRY RESEARCH INSTITUTE (DEEMED UNIVERSITY), KARNAL-132001** (HARYANA) is a bonafide research work carried out by her under my supervision and guidance. The work embodied in this thesis is original and no part has been submitted in part or full for the award of any diploma or degree of this or any other university.

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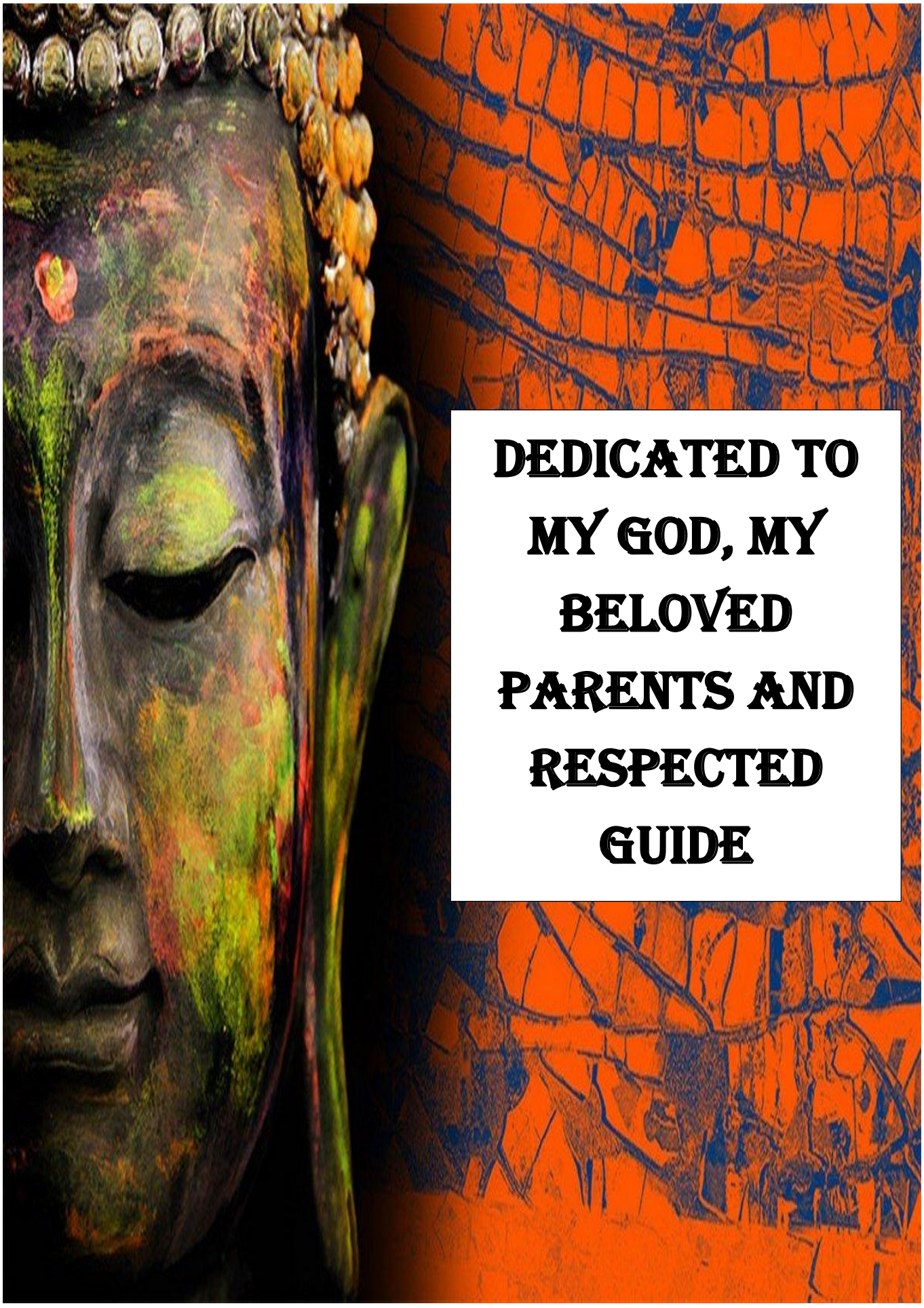

DEDICATED TO MY GOD, MY BELOVED PARENTS AND RESPECTED GUIDE

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DATE : /02/2023 **Miss. Walde Neha Ravi**

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LIST OF SYMBOLS AND ABBREVIATIONS

Symbols/ Abbreviations	Full Forms	Symbols/ Abbreviations	Full Forms
%	Percent	i.e.	That is
:	Ratio	LAB	Lactic Acid Bacteria
~	Approximately	MRS	De Man, Rogosa and Sharpe agar
≤	Less than or Equal to	mm	milimeter
≥	Greater than or Equal to	Mg	Milligram
cm	Centimeter	mL	Millilitre
CFU	Colony Forming Unit	min.	Minute(s)
DVS	Direct Vat Set	NaOH	Sodium Hydroxide
DVI	Direct Vat Inoculation	N	Normality
DMSO	Dimethyl Sulfoxide	nm	Nanometer
g/L	Gram per Liter	NaCl	Sodium Chloride
FDA	Food and Drug Administration	FD	Freeze Drying
g	Gram (s)	NDRI	National Dairy Research Institute
GRAS	Generally Recognised as Safe	℃	Degree Celsius
H+	Hydrogen Ions	rpm	Revolutions per Minute
h	Hour(s)	RSM	Reconstituted Skim Milk
ICAR	Indian Council of Agriculture Research	SD	Standard Deviation
SD	Spray drying	CAGR	Compound Annual Growth Rate

ABSTRACT

Direct vat set (DVS) starters are concentrated form (10^{11}-10^{14} CFU/g), available in both freeze-dried and frozen state. Freeze drying is most commonly used for the production of probiotic DVS on a commercial scale which is a costly and time-consuming process. On the other hand, spray drying is a low-cost, high-yield technology being explored in the food sector to prepare large amounts of dried probiotics. The present study was aimed to select protective agents and optimize spray drying conditions to prepare DVS starters of probiotic *Lactiplantibacillus plantarum* strains. Two probiotic strains i.e. *Lactiplantibacillus plantarum* CRD7 and *Lp. plantarum* HD48 were selected based on their health-promoting features and evaluated for techno-functional attributes in milk for preparation of *dahi.* Significance difference ($p<0.05$) was observed w.r.t. techno-functional (i.e. curd setting time (14.33±0.02 h), pH (4.26±0.01), titratable acidity (0.7±0.01 % LA) and total probiotic counts (8.71±0.01 Log CFU/mL), overall acceptability sensory score (9.0±0.0) and texture profile (firmness 2.30±0.0 N), of probiotic *dahi* prepared with *Lp. plantarum* CRD7 compared to *Lp. plantarum* HD48 respectively. Based on better techno functional performance, two *Lp. plantarum* strains were subjected for heat challenge experiment using to optimize spray drying parameters i.e. protective agents and inlet temperature to determine their heat tolerance for the better survivability during spray drying of probiotic for probiotic DVS powder preparation. Three protective agents *i.e.* lactose, maltodextrin and sorbitol were assessed at varied concentrations and temperature combinations for their heat tolerance to enhance the survivability of selected probiotic *Lp. plantarum* strains. Evaluation of probiotic DVS prepared with maltodextrin @ 2.5% concentration showed better survivability rate exposed to 1 min (95.27±0.03) and 5 min (95.15±0.03) at 55°C compared to sorbitol and lactose. The cell survivability of selected probiotic *Lactobacillus* strains was found to be affected by the type and concentration of the protective agents. Inoculum levels @ 0.002% and 0.004% (w/v) of three different spray dried probiotic DVS was optimized for preparation of *dahi*. Observations on storage stability of probiotic DVS packed in three packaging (aluminium laminate, LDPE and EVOH) materials and stored at -20°C and 4°C exhibited no significant difference in techno-functional performance up to two months of storage. It is concluded that optimized spray drying conditions for preparation of probiotic DVS starters of *Lp. plantarum* CRD7 could be utilized for preparation of health-promoting fermented dairy foods such as *dahi*, *lassi* etc.

सारांश

डायरेक्ट वैट सेट (DVS) स्टार्टर्स केंद्रित रूप (1011-1014CFU/g) होते हैं, जो फ्रीज-ड्रइंग और फ्रोजन दोनों अवस्था में उपलब्ध होते हैं। फ्रीज ड्रइंग का उपयोग आमतौर पर व्यावसायिक पैमाने पर प्रोबायोटिक डीवीएस के उत्पादन के लिए किया जाता है जो एक महंगी और समय लेने वाली प्रक्रिया है। दूसरी ओर, स्प्रे ड्रइंग एक कम लागत वाली, उच्च उपज वाली तकनीक है जिसे खाद्य क्षेत्र में बड़ी मात्रा में सूखे प्रोबायोटिक्स तैयार करने के लिए खोजा जा रहा है। वर्तमान अध्ययन का उद्देश्य प्रोबायोटिक लैक्टिप्लांटिबैसिलस प्लांटारम स्ट्रेन के डीवीएस स्टार्टर्स तैयार करने के लिए सुरक्षात्मक एजेंटों का चयन करना और स्प्रे ड्रइंग की स्थिति को अनुकूलित करना था। दो प्रोबायोटिक स्ट्रेन यानी लैक्टिप्लांटिबैसिलस प्लांटारम CRD7 और Lp. प्लांटारम एचडी48 को उनके स्वास्थ्य को बढ़ावा देने वाली विशेषताओं के आधार पर चुना गया था और दही की तैयारी के लिए दूध में तकनीकी-कार्यात्मक विशेषताओं के लिए मूल्यांकन किया गया था। महत्वपूर्ण अंतर (पी<0.05) संदभ से तकनीकी-कार्यात्मक (यानी दही सेटिंग समय (14.33 ± 0.02 हॉर्स), पीएच (4.26 ± 0.01), अनुमापनीय अम्लता (0.72 ± 0.01% लैक्टिक एसिड) और कुल प्रोबायोटिक गणना (8.71 ± 0.01 लॉग सीएफयू/एमएल), समग्र स्वीकार्यता संवेदी स्कोर (9.0 ± 0.0) और बनावट प्रोफाइल (दृढ़ता 2.30 ± 0.0 एन), एलपी प्लांटारम सीआरडी 7 के साथ तैयार प्रोबायोटिक दही की क्रमशः एलपी प्लांटारम एचडी 48 की तुलना में देखा गया था। बेहतर तकनीकी कार्यात्मक प्रदर्शन के आधार पर, दो एलपी. प्लांटारम उपभेदों का उपयोग करके गर्मी चुनौती प्रयोग के अधीन प्रोबायोटिक डीवीएस पाउडर तैयार करने के लिए प्रोबायोटिक के स्प्रे ड्रइंग के दौरान बेहतर उत्तरजीविता के लिए, उनकी गर्मी सहनशीलता निर्धारित करने के लिए स्प्रे ड्रइंग के मापदंडों यानी सुरक्षात्मक एजेंटों और इनलेट तापमान को अनुकूलित किया गया था । तीन सुरक्षात्मक एजेंटों यानी लैक्टोज, माल्टोडेक्सट्रिन और सोर्बिटोल का मूल्यांकन उनकी गर्मी सहनशीलता के लिए विभिन्न सांद्रता और तापमान संयोजनों पर चयनित प्रोबायोटिक एलपी. प्लांटरम उपभेदों की उत्तरजीविता बढ़ाने के लिए किया गया था। 2.5% एकाग्रता माल्टोडेक्सट्रिन के साथ तैयार प्रोबायोटिक डीवीएस के मूल्यांकन ने बेहतर उत्तरजीवी दिखाया सोर्बिटोल और लैक्टोज की तुलना में 55 डिग्री सेल्सियस पर, 1 मिनट (95.27 ± 0.03) और 5 मिनट (95.15 ± 0.03) के संपर्क में आने की व्यवहार्यता दर। चयनित प्रोबायोटिक लैक्टोबैसिलस उपभेदों की कोशिका उत्तरजीविता सुरक्षात्मक एजेंटों के प्रकार और एकाग्रता से प्रभावित पाई गई। दही तैयार करने के लिए तीन अलग-अलग स्प्रे ड्राइड प्रोबायोटिक डीवीएस के इनोकुलम स्तर 0.002% और 0.004% (w/v) को अनुकूलित किया गया था। तीन पैकेजिंग (एल्यूमीनियम लैमिनेट, एलडीपीई और ईवीओएच) सामग्री में पैक प्रोबायोटिक डीवीएस की भंडारण स्थिरता पर टिप्पणियों और -20 डिग्री सेल्सियस और 4 डिग्री सेल्सियस पर संग्रहीत दो महीने के भंडारण तक तकनीकी-कार्यात्मक प्रदर्शन में कोई महत्वपूर्ण अंतर नहीं दिखाया। यह निष्कर्ष निकाला गया है कि एलपी के प्रोबायोटिक डीवीएस स्टार्टर्स की तैयारी के लिए अनुकूलित स्प्रे ड्रइंग की स्थिति लैक्टिप्लांटिबैसिलस प्लांटारम CRD7 है, जिसका उपयोग स्वास्थ्य को बढ़ावा देने वाले किण्वित डेयरी खाद्य पदार्थ जैसे दही, लस्सी आदि की तैयारी के लिए किया जा सकता है।

CHAPTER –1

Introduction

INTR0DUCTION

The rapid growth in the dairy processing industry coupled with upsurged demand for fermented dairy products is the key factor in boosting the growth of the dairy starters market globally. The global dairy starters culture market was worth US$ 1,124.3 million in 2018 and it is predicted to increase at compound annual growth rate (CAGR) of 4.5 percent from 2019 to 2027, which is expected to reach US$ 1,670.2 million by 2027. Lactic dairy starters are the 'heart' of the dairy industry and a key component in the production of high quality fermented dairy foods. Dairy starters used for production of fermented products should consist of harmless food-grade microorganisms. Lactic acid bacteria (LAB) employed in preparation of fermented dairy foods comprise single or multiple strains (Ebing , 2006). These are carefully selected groups of harmless food-grade strains of one or more species, deliberately added to milk, whey or other formulated media to initiate desired fermentation under controlled conditions (Evivie *et al*., 2017). Various fermented milk products *viz., dahi, Lassi, shrikhand,* yogurt, *kefir,* cheese, butter and many dairy-based beverages are manufactured by application of lactic dairy starters to improve their flavour, texture and taste, shelf life and safety. Lactic acid bacteria are used for centuries in food fermentation due to generally recognized as safe (GRAS) status for improving quality, shelf life and safety. *Lactococcus, Lactobacillus, Leuconstocs, Pediococcus* and *Streptococcus,* the major genera used in dairy food fermentation produce lactic acid as the major end product of sugar fermentation. There has been an increasing interest in screening of LAB with improved or novel properties. Broadly based on optimum growth temperature, lactic starters are classified into thermophilic (37-42°C) and mesophilic (25-30°C).

Genus Lactobacillus constitute Gram's positive, microaerophilic, non-motile, rod shaped, catalase negative, non spore forming bacteria and best grow at pH 6.0. These are major part of lactic acid bacteria group because of their ability to convert milk sugar to lactic acid. *Lactobacillus* forms biofilms in vagina and gastrointestinal tract that allow them to persist during harsh environmental condition and maintain their ample population to exert health promoting effect to the host. The genus was subdivided by Orla-Jensen (1919) into three groups i.e. *Thermobacterium, Streptobacterium* and *Betabacterium* on the basis of fermentation of glucose. *Lactobacillus* exhibits a mutualistic relationship with the human body, as it protects the host against potential invasion by pathogen and in turn,

the host provide a source of nutrients. *Lactobacillus* is the most common probiotic widely used in dairy food fermentation such as yoghurt and it is diverse in its application to maintain human well-being, as it can treat diarrhea, vaginal infection and skin disorders such as eczema. Many lactobacilli are homo-fermentative i.e., produce only lactic acid and some species are hetero-fermentative i.e., produce either alcohol or lactic acid from sugar.

'Probiotics' are defined as living organisms that upon ingestion in certain numbers, exert health benefits to host (FAO/WHO, 2002). Probiotic products must have a live count of ≥6 log CFU/g at the end of shelf-life to provide their claimed benefits (Garcia-Ruiz *et al.,* 2011). The most commonly used probiotics belonging to the genera of *Lactobacillus* and *Bifidobacterium* generally, do not exhibit any harmful effects in contrast to other gut microorganism (Mombelli & Gismondo, 2000 and Kimoto-Nira *et al.,* 2007). Species of probiotic lactobacilli include *L. acidophilus, L. rhamnosus , L. casei, L. delbrueckii ssp. bulgaricus, L. johnsonii , L. reuteri, L. brevis, L. cellobiosus, L. curvatus, L. fermentum, L. gasseri and L. plantarum* (Meurman & Stamatova, 2007). Probiotics LAB also include other genera *viz., Streptococcus, Lactococcus, Enterococcus, Leuconostoc, Propionibacterium* and *Pediococcus* (Power *et al.,* 2008).

To provide beneficial health effects to the host, probiotic bacteria must survive through the gastrointestinal tract passage, tolerating acid, bile and gastric enzymes with adherence and colonization ability on the intestinal epithelium (Huang & Adams, 2004). The ingestion of foods supplemented with probiotics bacteria has been associated with a variety of benefits to human health as like anticarcinogenic improve lactose intolerance, antibiotic associated diarrhoea, hypercholesteraemic effect etc.

The conventional method of starter culture propagation is the simple microbiological technique wherein starters are propagated in the laboratory from the stock cultures. These cannot be added directly in the milk to initiate fermentation because of being in an in-active or dormant state due to prolonged storage. They need to be brought into active form and also scale up to prepare bulk quantities. This method has certain drawbacks *viz.,* require skilled manpower, cumbersome, costly process and prone to contamination by bacteriophage and other contaminants that results in poor quality fermented dairy foods that cause economic loss to the dairy industry. However, this problem can be overcome by application of direct vat set starters (DVS). Now-a-days, the DVS cultures have been widely used to resolve problems associated with liquid starters, especially in small plants and to substitute a bulk starter in fermented milk and cheese

production. The DVS starters are used in structured dairies to manufacture high-quality fermented dairy products. These are concentrated and active either in freeze-dried and frozen formats consist that at least 10^{11}-10^{13} CFU/g. They eliminate the in-plant sub culturing, thereby reduce costs associated with bulk culture preparation, and lower the risk of bacteriophage infection.

Different methods like liquid starter, spray drying, fluidized bed drying, microencapsulation and lyophilization are applied to manufacture DVS cultures with good viability and better shelf life, which is essential for industrial point of view. Nowadays, liquid starter cultures a large extent have been replaced by commercial concentrated freeze dried starter cultures. However, major disadvantage associated with freeze-drying is expensive and complex process, and can take days to complete for large product loads due to slow energy and water transfer that needed to dry the material. Therefore, many attempts have been made to develop alternate drying processes i.e., spray drying with lower production costs with a reasonable viability.

Spray drying is commonly used technique for industrial food drying, which can also be used for drying of dairy starters. The cost of spray drying is approximately 10 times less than that of freeze drying. The versatility of the process and the considerable progress made through technical innovation have led to greater flexibility in meeting biotechnological requirements, especially low-heat treatments that help avoid loss of viability (Schuck *et al*., 2013). Incorporation of LAB into dairy and non-dairy foods, as well as development of dried formulations for a variety of industrial applications, has become common practices now a day. However, a number of factors i.e. spray drying medium, strain type, physiological state of the cells and rehydration conditions influence the viability of spray-dried DVS. Thus, manufacturers and researchers have placed a high priority on developing technologies that enhance fermentation productivity, cell viability and probiotic functionality during spray drying and storage.

Therefore, keeping in view of merits of spray drying process for preparation of probiotic DVS present investigation was aimed to evaluate effects of types and level of protectant for better survivability of *Latiplantibacillus plantarum* strains with the following objectives:

I. Selection of suitable protectants / carrier agents for better survivability of DVS

II. Elucidation of spray drying parameters and packaging for improved techno – functional performance of probiotic direct vat set preparation.

CHAPTER –2

Review of Literature

2.1 LACTIC ACID BACTERIA

Lactic acid bacteria (LAB) are used for production of fermented dairy products worldwide in the dairy and food industry. The most important application of LAB in the dairy industry for the production of an enormous variety of fermented dairy products next to fermented meat and vegetable products industry. Besides food production, LAB are used in the production of lactic acid, high-value metabolites involved in flavor and texture development, probiotic products and antimicrobial peptides. The LAB widely known for their use as starter cultures in the manufacture of fermented dairy products such as acidophilus milk, Dahi, Lassi, Shrikhand, Yogurt, buttermilk, cottage cheese, hard cheeses (Cheddar, Provolone, Romano, and Edam) and soft cheeses.

The term lactic acid bacteria were gently accepted at the beginning of the 20th century (Carol *et. al*., 2010). Other terms as "milk souring" and "lactic acid producing" bacteria had also been in use earlier used for the same bacteria. Classification of LAB genera were based upon morphology, mode of glucose fermentation, growth at certain temperatures, configuration of the lactic acid produced, ability to grow at high salt concentrations and acid or alkaline tolerance (Khalid, 2011) and sugar utilization. The LAB compose a group of bacteria which have morphological, metabolic and physiological similarities and they are also closely related phylogenetically. The general description of the LAB within the group is Gram's-positive, non-sporulating, non-respiring cocci or rods through fermentation of carbohydrates produce lactic acid as their major end product (Bintsis, 2018). This core group consisting of four genera; *Lactobacillus, Leuconostoc, Lactococcus, Pediococcus* and *Streptococcus*. Taxonomic revisions have proposed several new genera and other group comprises: *Aerococcus, , Carnobacterium, Dolosigranulum, Enterococcus, Lactococcus, Oenococcus, Tetragenococcus, Vagococcus* and *Weissella. Lactobacilli, Carnobacteria* and *Weissella* are rods and remaining genera are cocci (Axelsson *et al*., 2004).

The physiology of lactic acid bacteria has been of interest as these are LAB involved in acidification of food and food products. The LAB physiology, such as metabolism and nutrient utilization has been constant to achieve more controlled processes (Khalid, 2011). The LAB are generally collaborated with habitats rich in nutrients, such as various food products *viz.*, milk, meat and vegetables, but some are also flora of mouth,

intestine and vagina of mammals (Khalid, 2011). *Lactobacillus* exhibits a mutualistic relationship with the human body, as it protects the host against potential invasion by pathogen and in turn, the host provide a source of nutrients. *Lactobacillus* is the most common probiotic widely used in dairy food fermentation and has diverse application to maintain human well-being.

2.2 LACTIC ACID BACTERIA AS PROBIOTICS

A number of microorganisms are presently used as probiotics. However, the most commonly used LAB are belonging to the genera *Lactobacillus*, the first and largest group of microorganisms to be regarded as probiotics (Mombelli & Gismondo, 2000) and *Bifidobacterium* (Wolfson, 1999). In contrast to other gut bacteria, they are known to have no negative effects on human health (Kimoto-Nira *et al.*, 2007). The most commonly lactobacilli species investigated as probiotics are *Lactobacillus acidophilus, L. rhamnosus, L. casei, L. delbrueckii ssp. bulgaricus, L. johnsonii , L. reuteri, L. brevis, L. cellobiosus, L. curvatus, L. fermentum, L. gasseri and L. plantarum* (Krasaekoopt *et al.*,2003; Meurman & Stamatova, 2007). The most commonly used bifidobacteria species are *Bifidobacterium breve*, *Bifidobacterium anima*lis subsp *lactis*, formerly *Bifidobacterium lactis* (Masco *et al*., 2004), and *Bifidobacterium longum* biotypes *infantis* and *longum* (Masco *et al*., 2005). Other LAB from genera such as *Streptococcus, Lactococcus, Enterococcus, Leuconostoc, Propionibacterium,* and *Pediococcus* are now included in probiotics (Krasaekoopt *et al.*, 2003; O'Sullivan *et al.,* 1992; Power *et al.,* 2008; Vandenplas *et al.,* 2007; Vinderola & Reinheimer, 2003). Bacteria such as non-pathogenic *E. coli* Nissle-1917 and *Clostridium butyricum* (Harish & Varghese, 2006), yeasts (*Saccharomyces boulardii*), filamentous fungi (*Aspergillus oryzae*) and some spore forming bacilli were also reported to be used as probiotics (Fuller *et al*., 2003). The consumption of probiotic-enriched foods has been linked to a number of health benefits as described in **Table 2.1.**

Table 2.1: Health benefits of probiotic microorganisms.

Diseases	Function	References
Colon cancer	Antimutagenic effects	Hirayma and Rafter, 2000
Irritable bowel syndrome(IBS)	Decrease in symptoms	Niedzielin *et al.*, 2001
Antibiotic associated diarrhea	Normalization of an unbalanced gastrointestinal flora.	Cremonini *et al.*, 2002
Helicobacter pylori infection	Supressive effect in an active group of 20 patients	Cats *et al.*, 2003
Hypocholesterolemic effect	Lowered serum cholesterol levels	Ouwehand *et al.*, 2002

2.3 STARTER CONCENTRATES

The traditional method of propagating starter cultures is a simple microbiological technique in which starters are propagated in the laboratory from stock cultures. Because they are inactive or dormant and they cannot be added directly to milk to initiate fermentation. They need to be brought into active form and also scale up to prepare bulk quantities. This method has certain drawbacks *viz.,* require skilled manpower, cumbersome, costly process and prone to contamination by bacteriophage and other contaminants that results in poor quality fermented dairy foods that cause economic loss to the dairy industry. However, this problem can be overcome by application of direct vat starters set (DVS) starters. Now-a-days, starter cell concentrates labelled as DVS cultures have been widely used to resolve problems associated with liquid starters, especially in small plants and to substitute a bulk starter in cheese and fermented milk production. Concentrated starters contain high cell density in the range of 10^{11}-10^{13} CFU/g. These are used as starter concentrates in the form of DVS/DVI cultures. The cell concentration of starter in milk reaches around 10^{9} CFU/mL after fermentation under normal growth conditions.

Concentrated starters are those that have been grown under strict aseptic controlled conditions, concentrated into a small volume, and frozen or dried for storage and transportation. These have resulted in improved starter cultures performance that are easier to use in the production of cultured foods than traditional starters. Earlier stock cultures were maintained in processing plants by regular subculturing in the plant laboratory in the traditional or conventional way. It was necessary to gradually increase the volume of the culture in order to have enough volume to inoculate the product to be prepared. However, now-days using concentrated cultures, many traditional fermented foods products have been successfully manufactured. The use of cheese whey as a base medium for producing starter cell biomass has been appealing, owing to the fact that it is essentially a waste by product that is relatively cheap (Stanley, 1995).

2.4 METHODS FOR PRODUCTION OF CONCENTRATED STARTER CULTURES

2.4.1 FROZEN LACTIC CULTURES

To achieve a high cell population, cells are first concentrated at approximately 10^{12}- 10^{13} CFU/mL, after which they are immediately frozen in liquid nitrogen. Freezing

can damage the cell preservation that can be prevented by appropriate level of protective agents. This helps to preserve the culture for a longer period of time. Direct-vat-set (DVS) cultures are often used for prepartion of fermented foods.

Thunell and Sandine (1984), prepared unconcentrated individual frozen cultures of *Streptococcus cremoris* strains in an internal-pH-control medium. Frozen cultures were stored for 3 months, with and without a cryoprotectant, assayed monthly for cell viability and activity. Individual strains showed variations in their ability to survive during storage at -20°C. Addition of glycerol as cryoprotectant helped to preserve cell viability and activity during storage at -20°C. However, storage at -40 and -80°C preserved activity and viability without need of cryoprotectants. Unfrozen cultures retained original activity and viability after 1 month refrigerated storage. Frozen starters stored at -40 and -80°C had been used successfully in Cheddar and Cottage cheese making for over 1 year (Thunell *et al*., 1984).

Baumann and Reonbold (1964) studied conditions influencing the survival of frozen single-strain, mixed-strain and multiple strain lactic cultures. These researchers reported that freezing and thawing rates affect its activity and viability then, fast freezing followed by fast thawing resulted best survival. Cultures grown at 26°C to 32°C had significantly greater activities after 4 weeks of storage at -20 °C than cultures grown at 15 or 21°C. Yeast extract, glycerol and N-Z Amine A (an enzyme digest of casein processed to a high degree of hydrolysis) was found to be more effective than sucrose, dimethyl sulfoxide, egg white and egg albumen in protecting cells from freezing damage at -20 °C. The survival of cultures stored at -196°C was unaffected by additives. Experiments with Cheddar cheese showed that freezing and storing cultures at -196 °C is beneficial for production.

Frozen starter cultures have the disadvantage of requiring very low transportation or storage temperatures such as -20 to -40 °C (Gilliland, 1985). Beside from the risk of thawing, high transportation costs limited economically market of frozen starter cultures in distant areas or countries.

2.4.2 DRIED LACTIC CULTURES

The conventional drying process for lactic starter by freeze drying consists of two major steps. Cells are typically frozen at -196 °C before being dried by sublimation under high vacuum. It has been discovered that inactivation is mostly associated with the freezing

step and 60-70 percent of cells that survived the freezing step can survive the dehydration step. In contrast to the extremely low temperatures used in freeze drying, alternative drying processes can be divided into two major groups based on drying temperature: (i) processes that operate at low temperatures, such as fluidized bed drying and vacuum drying. (ii) high-temperature processes, such as spray drying. The inactivation mechanisms of these two different groups act differently on the cell. Among these processes, spray drying is the most extensively investigated since the pioneering work of Rogers, (1914). This may be due to its industrial establishment as a predominant processing tool used in the dairy industry, rapidity of drying, and continuous production capability which is very useful for the drying of a large quantities of starter cultures.

2.4.2.1 FREEZE DRIED LACTIC CULTURES

Freeze-dried lactic starters are ready-to-use culture concentrates for direct vat inoculations in milk that have piqued researchers and industrial interest. The viability of cells in freeze-dried starters is influenced by a number of factors such as type strain, freeze-drying suspending media, freeze-drying parameters, rehydration conditions and physiological state of the cells. Freeze drying is a preferred method due to its low operating temperature and pressure which help to retain the native structure, biochemical properties, and activities of bacterial cells (Fatemeh *et al*., 2011). To minimize cell damage during freeze drying and to achieve high cell viability yields, protectants known as cryoprotectants can be added to the biomaterial before drying. A good cryoprotectant is easily vitrifiable and protects the embedded bacterial cells during the entire freeze-drying process and subsequent storage. The key challenge is identifying the appropriate protective agents to improve cellular survival during storage (Savini *et al*., 2010). Disaccharides (saccharose, lactose, trehalose), polyols (mannitol, sorbitol), and polysaccharides (maltodextrin, dextran, inulin) are common probiotic cryoprotectants (Aschenbrenner *et al*., 2015). Freeze dried DVS is unique for its high cell concentration and functional activity, including its prolonged storage life.

The freeze-drying process is costly and complex wherein large product loads can take days to complete due to the slow energy and water transfer required to dry the material. This, along with the growing commercial interest in dairy starters, explains why researchers are working to develop alternative drying techniques (Silva *et al*., 2011).

Papavasiliou *et al*., (2008) produced freeze-dried cultures and studied various cryoprotective agents and cooling rates. Fermented whey proved to be an effective cryoprotective medium, allowing the kefir culture to survive for 86% and demonstrated satisfactory metabolic activity in freeze-dried cultures. The freeze-dried culture was evaluated for carbohydrate fermentations which showed a high operational stability during repeated batch fermentations.

Ledenbach *et al*., (2009) investigated the use of dairy industry by-products in the practical production and long-term storage of industrial microbial cultures. The cryoprotectants provided no significant protection during freeze-drying process and use of rennet casein reduced viability significantly of freeze dried starters. In comparison to the control culture, the efficiency of the preservatives in preserving viability during storage was very successful. Casein-enriched cultures had a high water activity and moisture content, but their viability remained high during storage. Different cryoprotectants were used to differentiate the powder morphologies of the cultures. The particle sizes of the cultures were correlated with their moisture content. Storage increased particle size and distribution. According to thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC), addition of casein protein increased the degradation temperature of the cultures. The use of casein made the production of freeze-dried cultures more difficult. However, it was generally agreed that the addition of dairy by-products as a protective agent could improve the protective properties of skimmed milk used for the preservation of *L. plantarum*. Maitriot *et al*., (1997) immobilized *Bifidobacterium longum* in k-carrageenan/locust bean gum, gel beads and cultured in subsequent MRS broth and whey-permeate. The same beads were incubated for five batch fermentations and then freeze-dried after being mixed with a protective solution. Viable population in the beads increased from 8.3×10^{7} to 4.73×10^{10} CFU/g after three batch fermentations, but there was no further increase in viable cell population in the final two fermentations. The freeze-dried culture contained 3.3×10^{10} CFU/g with a survival rate of 10% approximately. Immobilized cell survival to freeze-drying was comparable to that of classical free-cell cultures. Stability of freeze-dried cultures during storage at -17, 4 and 20°C was not influenced by immobilization.

Many attempts have been made to develop alternative drying processes with lower costs as mentioned in **Table 2.2** and some authors have also reported a reasonable viability after the drying.

Table 2.2: Costs of drying processes referenced to freeze drying.

Drying Processes	Fixed costs (%)	Manufacturing costs (%)
Freeze drying	100.0	100.0
Vacuum drying	52.2	51.6
Spray drying	12.0	20.0
Drum drying	9.3	24.1
Fluidized bed drying	8.8	17.9
Air drying	5.3	17.9

(Santivarangkna *et al*.,2007)

2.4.3 FLUIDIZED BED DRYING

A fluidized bed is a bed of solid particles with a stream of air or gas blowing upward through the particles at a rate high enough to set them in motion. As the air travels through the particle bed, it imparts fluid-like behavior to the bed and provides rapid mixing of solids. Particles are freely suspended in the air stream and dehydrated simultaneously by rapid exchange of heat and mass with air. The costs of fluidized bed drying are comparable or slightly lower than those of spray drying, and the process is also suitable for large-scale continuous production. Fluidized bed drying time varied from 1min to 2 hours and is longer than of spray drying, but heat inactivation can be minimized and easier controlled by using relatively low air temperatures. However, the use of fluidized bed dryers for many types of food is limited due to irregular particle sizes and the sticky nature of the granulated materials, which can result in an inhomogeneous bed, agglomerated particles, and a decreased drying rate. Fluidized bed drying of microorganisms has been studied in yeast, and the process is now used to produce commercial dry yeast (Akbari *et al*., 2012). The disadvantage is that only granulatable materials can be dried, so cells must be entrapped or encapsulated in support materials like skim milk, potato starch, alginate, and casein. As the support materials are combined with the cells, they should be compatible with the fermented foods, such as alginate or skim milk for fermented milk, maltodextrin for fermented sausages, and starch or flour for sourdough bread (Shantivarangkna *et al*., 2007).

2.4.4 VACUUM DRYING

Vacuum drying is established as a heat-sensitive material-friendly process. Since the drying operates under vacuum, moisture can be removed from the materials at a low temperature. For example, while the boiling point of water at 100 kPa (1 atm) is 100 °C, at 1 kPa it is approximately 8 °C (Parikh, 2015). The oxidation reactions during drying can be minimized for oxygen sensitive lactic acid bacteria. The basic vacuum dryer is made up of a chamber with heated shelves. Water is removed in a vacuum pump and condensed at a condenser after trays containing wet materials are placed on shelves. Major drawback of freeze drying is that, it is confined as a batch process and relatively have long drying time compared to spray or fluidized bed drying which range from 20 to over 100 h.

2.4.5 SPRAY DRYING

Spray drying is an ancient and widely used concentration technique for particles smaller ≤40 μm in size. Polysaccharides (maltodextrins, starches, arabic gum, and corn syrups), lipids (monoglycerides, diglycerides and stearic acid), and proteins (casein, gelatin, soy, wheat and milk serum) are the most commonly used encapsulating agents for spray drying. The spray drying process involves atomizing homogenized carrier material (1:4) into a drying gas, resulting in dry powder capsules that are primarily controlled by product feed, gas flow, and temperature. Spray drying is highly suitable for industrial applications due to its rapidity, reasonable low cost and higher reproducibility. The main disadvantage of spray drying that is not compatible with bacterial viability is the use of high temperatures (Bustos *et al*., 2013). Spray drying produces a dry powder by atomizing the liquid at high velocity and directing the spray of droplets into a flow of hot air, e.g. 150-200 °C. When exposed, the atomized droplets have a very large surface area in the form of millions of micrometer-sized droplets (10-200 μm), resulting in a very short drying time to hot air in a drying chamber.

2.5 FACTORS AFFECTING SURVIVAL OF CONCENTRATED DRIED CULTURES DURING SPRAY DRYING PROCESS

2.5.1 Effects of growth phase

The growth phase has been shown to be an important factor in cellular survival in the face of various stresses. However, few studies have looked at the impact of the growth phase on cell resistance to spray drying and powder storage. Teixeira *et al*., (2005) found that *L. bulgaricus* cells harvested in the stationary phase survived spray drying better than

cells harvested in the exponential phase. Corcoran *et al*., (2004) also demonstrated that stationary phase *Lactobacillus rhamosus* cultures were more resistant to spray drying and subsequent storage at 4, 15, and 37°C than exponential phase cultures. Zamora *et al*., (2006) reported complete recovery of 10^{12} CFU/g of LAB strains harvested and spray-dried during the exponential phase. The highest resistance of stationary phase was found linked to nutrient deficiency (Santivarangkna *et al*., 2007) and cross protection conferred by the low pH attained during growth under uncontrolled pH conditions (Silva *et al*., 2011).

Lactobacillus rhamnosus GG was spray-dried in the lag, early log and stationary phases of growth in reconstituted skim milk (RSM) 20 and 10% (w/v), polydextrose (PD) 10%(w/v) mixture at an outlet temperature of 85-90°C. They investigated the effect of growth phase and inclusion of a prebiotic substance in the feed media on probiotic viability during spray-drying. Stationary phase cultures survived best (31–50%) in both feed media and were the most stable during powder storage at 4 and 37ºC over 8 weeks, with 30 to 140-fold reductions in cell viability at 37ºC in RSM and PD/RSM powders, respectively (Corcoran *et al*.,2004)

2.5.2 Effects of growth media

The presence of an amino group, a secondary alcohol group, or both confers the ability of a protective agent to preserve the viability of dried cells during the desiccation process (Font de voldez *et al*., 1985). As a result, accumulation of compatible solutes would be expected to improve survival during those processes. Small organic osmolytes such as sugars, polyols, amino acids, and their derivatives are examples of compatible solutes. Even at molar concentrations, they are compatible with cell metabolism. A variety of organisms synthesize or take up compatible solutes when adapting to extreme environments (Roberts *et al*., 2005). Because compatible solutes are unlikely to be accumulated by microorganisms during the short drying period, these solutes should be accumulated prior to drying i.e., during the growth phase (Silva *et al*., 2005). In addition to osmotic stress, cells are subjected to high temperatures during spray drying. Cells are subjected to high temperatures during spray drying, in addition to osmotic stress. In a variety of organisms, the accumulation of compatible solutes has been linked to increased thermotolerance (Welsh, 2000). Sheehan *et al*., (2006) demonstrated that improving the betaine uptake system in *L. salivarius* UCC118 increased resistance to osmo, cryo, baro, and chill stress, as well as resistance to spray drying.

Several authors have referred to the addition of various compounds to the growth medium prior to drying as a mechanism capable of modifying microorganism survival through drying and subsequent storage (Desmond *et al*., 2002). However, the degree of protection provided by a given protective solute during storage was reported to be species and strain dependent as well as stress dependent (Siaterlis *et al*., 2009). Sucrose has the advantage of being a nonreducing sugar that does not undergo maillard reactions with protein amino groups as an osmolyte accumulated by stressed bacteria ((Ferreira *et al*., 2005). The case-by-case selection of an appropriate growth medium is critical to maximizing organism survival during drying storage. Silva *et al*., (2005) demonstrated that *L. bulgaricus* cultures grown in uncontrolled pH conditions were more resistant to heating and spray drying than cultures grown in controlled pH conditions of 6.5. Thus, selection of an appropriate drying medium is critical to improved bacterial survival rates during the drying process and subsequent storage (Sunny-Roberts and Knorr, 2009). The effect of solids concentration on microorganism survival during spray drying was also reported in several studies (Boza *et al*., 2004).

Barbosa *et al.,* (2016) studied the effect of different conditions of growth and storage on the cell counts of two lactic acid bacteria after spray drying in RSM. Consumers are increasingly demanding innovative food products that provide health benefits. In this context, they investigated whether different sugars added to the culture media used for growth of two LAB contributed to their protection during spray drying in RSM and subsequent storage under different conditions of temperature, light exposure and water activity. Cell viability was also investigated during passage through simulated gastro-intestinal conditions. Cells grown in fructose-containing culture medium had the lowest survival rates during storage. Cells grown in the presence of lactose showed highest survival rate, followed by glucose. The survival of dried LAB was enhanced at 4°C storage with water activity of 0.03 and absence of daylight. Cells grown in standard culture medium, dried and stored at 4°C for 12 months showed cell viability of 10^9 CFU/ml. Viabilities of both LAB reduced by approximately 2 log-units after stimulated gastro-intestinal tract passage. Investigations on optimization of growth and storage conditions proved that it is possible to improve survival rate of probiotic LAB in RSM powder with shelf life of 12 months at 4°C.

Teixeira *et al*., (1995) compared spray drying and freeze drying methods for concentration of *Lactobacillus bulgaricus* starter cultures in terms of viability, lag phase

until pH decrease onset, and total acid production. There were no significant differences between the methods for the experimental conditions used. The effect of spray drying on *Lactobacillus bulgaricus* cell membrane was investigated. Five methods were used to investigate the theory that spray drying causes cell membrane damage: three for leakage of intracellular components from the cell into the surrounding environment (260 and 280 nm absorbing materials, potassium ions, and proteins), and two for increased cell permeability (increased sensitivity to NaCl and increased permeability to o-nitrophenyl-P-D-galactopyranoside (ONPG). There was some cytoplasmic material loss from the damaged cells. The dried cells became NaCl sensitive and ONPG permeable. Heat shock increased exponential cell survival compared to controls but did not result in the normal levels seen in unshocked stationary phase cells. Stationary phase cells were unaffected by heat shock. Slow rehydration increased survival when compared to other rehydration methods and media.

Gardiner *et al.*, (2002) studied spray drying of culture for probiotic Cheddar cheese manufacture. Spray-dried probiotic milk powder was produced at pilot scale from 300 L of 20% (w/v) reconstituted skim milk containing a rifampicin resistant variant of the probiotic *Lactobacillus paracasei* NFBC 338. During powder manufacture, air inlet and outlet temperatures of 175ºC and 68ºC, respectively, were used, which yielded a probiotic survival of 84.5%. The powder, which contained 1×10^9 CFU/g of *Lb. paracasei* NFBC 338 Rifr was used as an adjunct inoculum @ 0.1% (w/v) during probiotic Cheddar cheese manufacture. Probiotic numbers were 2×10^7 CFU/g in the cheese on day 1 and grew to 7.7×10^7 CFU /g after 3 months of ripening, without adversely affecting cheese quality.

2.5.3 Effects of drying medium

The choice of an appropriate drying medium is crucial for enhanced the survival rates of bacteria during drying process and subsequent storage (Sunny-Roberts and Knorr, 2009). Espina and Packard (1979) reported that *L. acidophilus* survival was higher in milk with 25% than 40% solids content. Riveros *et al*., (2009) discovered no significant differences in *L. acidophilus* viability during spray drying at feed concentrations ranging from 10% to 30%. These authors also reported that feed concentration could not be exceed 30% to avoid nozzle spray blocking. The drying time was insufficient to evaporate the water content in the feed solution for values less than 12%. Solutions of nonfat dry milk solids, maltodextrin, soluble starch, gum arabic, whey and gelatin and more commonly skim milk have been used as drying media (Carvalho *et al*., 2004).

Lian *et al*., (2002) investigated the effect of gum arabic, gelatin, and soluble starch on the survival of bifidobacteria after spray drying and discovered that *Bifidobacterium infantis* strains survived better with gum arabic than with gelatin or soluble starch. *Bifidobacterium longum* strains, fared better with gelatin as a carrier than with gum arabic or soluble starch. Further testing with *B. infantis* CCRC 14633 and *B. longum* B6 revealed that when dried in skim milk, the percentage of survival increased to 16.0 percent and 82.6 percent, respectively. In fact, nowadays, several LAB strains and/or starter cultures are successfully commercially available in the dried form, and the positive effect of skim milk as a suspending agent before drying has been well accepted and reported by several authors (Lian *et al*., 2002; Carvalho *et al.*, 2004; Manojlovic *et al*., 2010). Skim milk can prevent cellular injury by stabilizing the cell membrane, forming a structure that is easy to rehydrate after drying, and protecting the cells with proteins that provide a protective coating. Reddy *et al*., (2009) discovered that maltodextrin in combination with a dilute suspension of cells protected the probiotic properties of the organisms better than skim milk, despite the lower viability. Furthermore, supplementing the cells in skim milk with protective agents prior to drying may significantly improve viability during the drying and storage processes. This cellular protection is affected by the ingredients used, the strain tested, and the drying process (Carvalho *et al*., 2004; Silva *et al*., 2005; Ananta *et al*., 2005). However, some authors believe that the protective effect of some compounds during drying processes is masked by the protective effect of milk components (Carvalho *et al*. 2002). It is common to add protective agents to the drying medium, which can improve cellular protection during drying and subsequent storage (Carvalho *et al*., 2004; Ferreira *et al*., 2005).

Corcoran *et al*., (2004) evaluated the survival of several probiotic lactobacilli during spray drying in the presence of prebiotics (polydextrose and inulin) and powder storage. Powders with high number of viable micro-organisms were attained when the drying media contained skim milk but not in the presence of prebiotics alone. Skim milk with polydextrose was found to be more protective than skim milk with inulin. De Vos *et al*., (2010) examined the use of encapsulation for bioactive materials such as probiotic bacteria and targeted release within the gastrointestinal system. Prebiotic carbohydrates such as chitosan in combination with alginates were suggested to resist acid conditions in the stomach, as well as future research on inulin (Except for bifidobacteria in the large intestine, this prebiotic is virtually indigestible) as a cheap and abundant encapsulating material.

Table 2.3 Effects of protectants in drying media and spray drying conditions on survivability and moisture content of DVS starters

Protectants/Drying media	Inlet temp (ºC)	Outlet temp (ºC)	Moisture content (%)	Survival rate (%)	References
Gum arabic (5%)	180	80	2.90	37.6	Eratte *et al*.,(2015)
Maltodextrin (20%)	135	90	4 - 8	10	Perdana *et al*., (2014)
Trehalose (20%) + monosodium glutamate	150	65 - 70	3.8	80.8	Sunny-Robert and Knorr (2009)
Gelatin	100	50	10.0	54.3	Lian *et al*., (2002)
RSM (20%)	170	85-90	3.8 3.7 2.7	30 50 1	Corcoran *et al*., (2004)
Gum arabic, gelatine, soluble starch	100	50	-	0.08–15.99	Lian *et al*., (2002)
Skim milk	100	50	-	10.79 – 82.59	Lian *et al*., (2002)
Milk	100	70	-	3.32	Roelans *et al.*, (1990)
20% reconstituted skim milk, 4% reconstituted whey, 0.5% yeast extract	160 180 200	68 72 80	-	58.9-68.7	Mauriello *et al.*, (1999)

Protectants/Drying media	Inlet temp (ºC)	Outlet temp (ºC)	Moisture content (%)	Survival rate (%)	References
Maltodextrin (15%) & lactose (5%),sodium phosphate (0.2-0.5%)	220	82 & 120	-	15	Johnson *et al.,* (1995)
Reconstituted skim milk (10%) & gum acacia (10%)	170	95	-	1.7	Desmond *et al.,* (2002)
Reconstituted skim milk (10%) & polydextrose or inulin	80	80	-	65	Ananta *et al.,* (2005)
Reconstituted skim milk (11%)	180	70	-	9.09	Golowczyc *et al*., (2010)
Skim milk (10%) & Maltodextrin(15%)	135	80	-	11.38	Boza *et al*., (2004)
RSM (20% w/v) + 0.5% (w/v) yeast extract	170	95	-	95.7	Desmond *et al*., (2001)
Skim milk(11%)	200	70	-	3.78	Silva *et al*., (2005)
Skim milk (20%) + yeast extract(0.5%)	170	75	-	3	Gardiner *et al*., (2002)
Reconstituted skim milk (20%)	170	85	-	13	Simpson *et al*., (2005)

Polysaccharides, proteins, and their combinations have been investigated as carrier agents for spray drying matrices. Prebiotic polysaccharides such as glucose, lactose, and maltodextrin have been used to protect probiotic bacteria during spray drying and storage (Bhagwat *et al*., 2020). Maltodextrin is a polysaccharide that is created through the acidic or enzymatic hydrolysis of starch. It has a nutritional value of only 4 calories per gram. Maltodextrins are widely used in the food industry for a variety of reasons, including texture improvement, sweetness modifying agents, controlling non-enzymatic browning, lowering the freezing point of mixtures, and as carrier materials (Behboudi - Jobbehdar *et al*; 2021). Using a protein-carbohydrate mixture improves probiotic survival during spray drying. Yoha *et al.,* (2020) discovered that spray drying of *Lactobacillus plantarum* microencapsulated with fructo-oligosaccharides (FOS) improved encapsulation efficiency and preserved 96 percent viability

Anal *et al.,* (2007) studied microencapsulation of probiotics for industrial applications and targeted delivery in which they used carrageenan, a natural polysaccharide that is extracted from marine macroalgae and is commonly used as a food additive. Elevated temperatures (60-80 ºC) are needed to dissolve the polymer at concentrations ranging from 2 to 5%. Alginic acid, a natural polymer, is a polyuronic acid that is extracted from seaweeds and is composed of various proportions of 1-4 linked β-D-mannuronic and α-L-guluronic acids. The proportions of these residues vared depending on the source of the alginic acid. Researchers prefer gel entrapment using natural biopolymers such as calcium alginate, carrageenan, gellan gum, and chitosan in almost all cases (Martau *et al*., 2019).

2.5.4 Effects of inlet and outlet temperature of spray drying temperature

The inlet temperature is the temperature of the heated drying gas just before it enters the drying chamber. Higher inlet temperatures allow faster solvent evaporation. The inlet temperature should not be increased solely to improve drying performance because it also affects the wet-bulb temperature of the surrounding air. Lower inlet temperatures result in lower wet-bulb temperatures in the surrounding air, preventing thermal degradation of the final product. The temperature of the air containing the dried particles just before it is piped into the collection devices is referred to as the outlet temperature. The outlet temperature is theoretically the highest temperature to which the dried powder can be heated.

Labuza *et al*., (1970) discovered that survival ratio of yeast decreased linearly with increased outlet air temperature, regardless of air inlet temperature. Peri and De Cesari (1974) concluded that the intensity of the osmotic shock caused by an increase in drying rate and a decrease in residual humidity below the critical value of 10% had a greater impact on microorganism survival than an increase in drying temperature. Silva *et al*., (2011) observed survival rates of 26, 46.9, and 19.5 % for *L. lactis, S. thermophilus,* and *L. bulgaricus*, respectively, at 70-72°C outlet air temperature and 1.8, 12.1, and 4.7 % at 82-84°C outlet air temperature when inlet air temperature was 190°C. Investigation on survival of LAB during spray drying of yoghurt, Kim and Bhowmik (1990) concluded that outlet air temperature was a major parameter that affected the number of survivors as inlet air temperature exhibited a much less effect than outlet air temperature. It was reported that 10°C rise in inlet air temperature was equivalent to that of 1.8 and 2.4°C increase in outlet air temperature for *S. thermophilus* and for *L. bulgaricus*, respectively. Lievense (1991) strongly correlated survival of bacterial cells during spray drying to outlet air temperature. However, it was not directly related to the inlet air temperature in the drier, because of the evaporative cooling effect in the first part of the drying process. Teixeira *et al*., (1995) documented the survival of *L. bulgaricus* during spray drying at outlet temperatures ranging from 62 to 105°C, decreased linearly with increased outlet air temperature. Spray-dried powder produced at a lower outlet air temperature resulted in higher cellular stability during storage, as too low an outlet air temperature, resulted in high residual moisture contents (Desmond *et al*., 2001). Similarly, Kim and Bhowmik (1990) reported decreased survival of several LAB strains in yoghurt powder with increased outlet or inlet air temperatures during spray drying as outlet air temperature affected the cellular viability to a greater extent. Zhu *et al*., (2008) evaluated the viability of *Bacillus thuringiensis* under different spray drying conditions. These authors observed that increased inlet, outlet air temperature and atomizing air pressure decreased the viability of the spray-dried cells. Golowczyc *et al*., (2010) decreased survival of probiotic strains of *L. kefir* and *S. lipolytica* during spray drying and subsequent storage as drying outlet air temperatures increased. The variations in viability at the tested outlet temperatures were found strain dependent. The moisture content of the dried culture should be <5%. The suspending medium must be fortified with maltodextrin, gelatin, soluble starch etc after long- term preservation of spray dried cultures.

Bielecka *et al*., (2000) investigated the effect of yoghurt spray drying temperature on starter culture survival, moisture content, and sensoric properties of yoghurt powder. A synergistic combination of *Lactobacillus delbrueckii subsp. bulgaricus* 151 and *Streptococcus thermophilus* MK-10 was preserved using spray drying. The effect of outlet air temperature in the range of 60-80 °C on the survival of yoghurt cultures, as well as the moisture content and sensory properties of yoghurt powder was studied. The survival of yoghurt cultures was greatest at 60 and 65°C, but excessive moisture (10.2 percent) in yoghurt powder harmed its texture. At 80°C, the moisture content of the powder was low (4.4 percent), but sensoric faults appeared, and bacteria survival was significantly reduced. Temperatures in the 70-75°C range ensured satisfactory yoghurt culture survival (*L. delbrueckii subsp. bulgaricus*, 13.7- 15.8 percent; *S. thermophilus*, 51.6- 54.7 percent), maintained strain proportion (L:S-1:3), satisfactory moisture content (5.1-6.3 percent), and good sensoric properties of yoghurt powder.

Zhang *et al*., (2015) studied the effects of spray drying on *Lactobacillus plantarum* BM-1 viability, resistance to simulated gastrointestinal digestion and storage stability. The purpose of study was to assess the suitability of spray drying for producing high viability powder *of L. plantarum* BM-1. Different protectants were added before drying to improve the survival of *L. plantarum* BM-1 during spray drying. The combination of reconstituted skim milk (RSM) and equal weight of sucrose as a protective agent resulted in the highest survival rate of 75.70 percent and the lowest moisture content of 3.67 percent. The reduction in cell counts of free *L. plantarum* BM-1 after 1 minute of exposure to 60°C was 2.58 log CFU/mL, whereas the reduction in spray-dried cells protected by RSM and sucrose was only 0.08 log CFU/mL. After 120 minutes' incubation under simulated gastric conditions, cell counts of free *L. plantarum* BM-1 decreased by approximately 1.4 log CFU/mL, whereas spray dried cells showed no significant ($p > 0.05$) reduction. Spray-dried cells also outlived free cells when subjected to bile salts stress. Spray drying had no effect on *L. plantarum* BM-1 bacteriocin production. The survival rate of spray-dried powder was achieved to 98 percent after 2 months of storage at 4°C and a nitrogen replacement package outperformed a vacuum package and an air-sealed package at room temperature. These investigations demonstrated that combination of equal weight RSM and sucrose a promising *L. plantarum* BM-1 protective agent during spray drying.

Koc *et al*., (2010) investigated spray drying of yoghurt: optimized process conditions to improve viability and other quality attributes. Plain yoghurt was spray dried

to determine the optimal processing conditions for maximum lactic acid bacteria survival ratio, maximum overall sensory attributes, minimal color change, and acceptable moisture content. The independent factors were inlet (150-180°C) and outlet air temperatures (60-90°C), as well as the feed temperature (4-30°C). A pilot-scale spray dryer was used to carry out a series of drying experiments, with the process conditions chosen using the central composite rotatable design (CCRD). The effects of spray-drying conditions on the resulting yoghurt powder for some physical properties *viz.*, water activity, titratable acidity (%LA), and pH on each condition was also measured. Scanning electron microscopy (SEM) was used to examine the powder's morphological structure. Using the desirability function method, the optimum processing condition was determined to be an air inlet temperature of 171°C, an air outlet temperature of 60.5°C and a feed temperature of 15°C.

2.5.5 Residence time inside drying chamber:

The residence time of the atomized droplets inside the drying chamber is another important factor that has a direct impact on the final product quality. The residence time should be long enough to ensure to achieve main goal of drying stage. Maintenance of product characteristics is critical, longer residence times of dried particles resulted thermal degradation, particularly with heat-sensitive materials e.g, fine particles should not stay inside the drying chamber for more than 10- 15 seconds.

2.5.6 Atomization pressure:

When nozzle atomizers are used, the atomization stage is carried out under pressure. The pressure applied during this process influences droplet size, which decreases with increasing pressure for a given atomizer device and feed solution.

2.5.7 Feed flow rate:

A controlled amount of feedstock solution is pumped into the atomizer. When the atomization pressure is held constant, the droplet size grows as the feed flow rate increases.

2.5.8 Feed viscosity:

When the feed viscosity is increased, a large portion of the atomization energy supplied to the nozzle is used to overcome the solution's strong viscous forces. As a result, only a small amount of energy is available for droplet fission, resulting in larger droplet sizes.

2.5.9 Effects of rehydration conditions

It is possible that an organism that survives various stages of spray drying or storage, may lose viability during rehydration process because previously cells have been subjected to a sublethal injury that make them unable to repair in inappropriate rehydration conditions. Thus, rehydration is a critical step in the recovery of dried microorganisms (Costa *et al*., 2000).

Teixeira *et al*., (1995) reported that soaking of spray dried powder of *L. bulgaricus* for 30 minutes yielded a higher recovery than vigorous shaking for 2 minutes. Similar findings had been reported previously and attributed it to less osmotic shock damage. These authors also reported that the temperature of rehydration affect the viability of spray dried *L. bulgaricus* survival rate which increased linearly with temperature ranging from 4 to 50°C.

Teixeira *et al*., (1995) did not find any significant differences in the recoveries of dried *L. bulgaricus* cells in rehydration media such as Skim milk, MRS broth, deionized water or phosphate buffer. Abadias *et al*., (2001), documented that viability of freeze-dried *Candida sake* was increased when the same solution was used as a protectant and a rehydration medium; best survival was obtained using 10% lactose + 10% SM as a protecting solution and rehydration in skim milk.

Overall, the rehydration solution (in terms of pH and nutrients) and the rehydration conditions (temperature and volume) s significantly affect the rate of micro-organisms' recovery and subsequently the apparent survival rates (Carvalho *et al*., 2004; Zhao *et al*, 2009).

2.6 Storage conditions and packaging

The storage conditions of dried cells are critical for their viability. It is well known that storage temperature is a critical factor influencing the survival of dried cells. Generally, the number of viable bacteria inactivated in a given period is affected by storage temperature type of strain (Simpson *et al*., 2005; Reddy *et al*., 2009; Golowczyc *et al*., 2010). Foster (1962) reported best storage at 18℃ of spray-dried *L. lactis* under nitrogen packaging. Storage at refrigeration temperatures was demonstrated to be a most suitable storage temperature for several dried cultures (Riveros *et al*., 2009).

The relative humidity (RH) of dried culture storage was also identified as a major factor influencing survival of dried starters. Peri and Pompei(1976) reported highest

survival of spray-dried *yoghurt* starters at 5% RH and at 5°C storage. The sorption of *S. cerevisiae* cells showed that at RHs lower than 5% during storage Peri (1976). This indicates humidity level as critical factor for the survival of the microorganisms. Teixeira *et al*., (1995) estimated the survival of dried cells of *L. bulgaricus* during storage at various water activities; the survival rate was not linearly related to a_w. These authors came to the conclusion that environments with 0.11 and 0.23 had the highest survival rates.

Carvalho *et al*., (2004), reported better survival of *S. thermophilus* and *B. longum* in laminated pouches than in deoxidant and desiccant-containing glass or polyester bottles. Espina and Packard (1979) found that *L. acidophilus* survived slightly better when stored in nitrogen than atmospheric air. Wang *et al*., (2004) demonstrated better survival in laminated pouches than in glass or polyester bottles containing deoxidants and desiccant *S. thermophilus* and *B. longum*. Golowczyc *et al*., (2010), discovered that three spray-dried probiotic organisms fared worse in vacuum storage than in air storage. Because the preserved organisms are oxygen sensitive, vacuum or modified atmosphere packaging of the dried cultures was strongly advised. However, the glass vial is the most common type of packaging material for dried cultures, followed by the laminated, aluminium foil sachet.

Licari and Potter (1970) investigated the effect of milk droplet and dried particle size on *Salmonella* survival when feed-atomizing pressure was varied. No significant differences were observed, in variations of particle

CHAPTER –3

Materials & Methods

The present research was conducted at Synbiotic Functional Foods and Bioremediation research laboratory, Dairy Microbiology Division ICAR – National Dairy Research Institute (Deemed University), Karnal – 132001, Haryana, India. A detailed description on optimization of spray drying condition for preparation of spray dried probiotic direct vat set starters and selection of protective agents along with reagents and instruments and apparatus used in present investigation are documented in this chapter.

3.1 CHEMICAL & REAGENTS

All chemicals used in the present project were of analytical grade (AR) and purchased from reputable suppliers as given below. The reagents used were freshly prepared using standard procedures.

MRS broth and agar (Hi-media), Dextrose monohydrate (Hi-media), Magnesium sulphate, Manganese sulphate, dipotassium hydrogen phosphate, Sodium acetate, Ammonium citrate, Tween 80, Yeast extract (Hi-media), Sodium chloride (Hi-media), Lactose monohydrate (Hi-media), Maltodextrin (Hi-media), Hydrochloric acid (HCL) 90244 (AR grade, Thomas Baker Pvt. Ltd., Mumbai, India), Sodium hydroxide (Hi-media), Phenolphthalein (Hi-media), Skim milk powder (Farmer fresh), Litmus milk indicator (Hi-media), Nigrosine (Hi-media), D-sorbitol powder (SRL-Sisco Research Laboratories Pvt. Ltd., Maharashtra, India), Paneer whey obtained from Experimental dairy ICAR-National Dairy Research Institute ,Distilled water and Milli-Q water obtained from Milli-Q water purification system (Millipore) located at central instruments facility Dairy Microbiology Division, ICAR- National Dairy Research Institute, Karnal-132001, Haryana.

3.2 PROBIOTIC CULTURES

Two probiotic cultures i.e. *Lactiplantibacillus plantarum* CRD7 and *Lactiplantibacillus plantarum* HD 48 were selected based on our previous compatibility studies carried out at Synbiotic Functional Foods & Bioremediation Research Laboratory, Dairy Microbiology Division, ICAR- National Dairy Research Institute (Deemed University), Karnal-132001, Haryana, India as they revealed health promoting attributes. These cultures were sub-cultured in newly developed cost effective whey based medium

at 37°C for 16-18 h to determine their suitability for preparation of direct vat set (DVS) culture by spray drying method for preparation of fermented dairy products.

3.3 MAINTENANCE, PRESERVATION AND PROPOGATION OF PROBIOTICS CULTURES

Lactiplantibacillus plantarum CRD7 and *L. plantarum* HD48 were maintained in de Man Rogosa Sharpe (MRS) broth at 4°C after sub-culturing weekly at 37°C for 16-18 h. The probiotic strains were also preserved in 40% glycerol as stock cultures at -20°C for long term preservation. The cultures were also maintained at 4°C in sterilized litmus chalk milk after fortnight propagation at 37°C/ 16-18 h. All cultures were sub-cultured twice in MRS broth at 37°C/18 h prior to each experiment.

3.4 PURITY EVALUATION OF PROBIOTIC *LACTOBACILLUS* CULTURES

The probiotic *L. plantarum* CRD 7 and *L. plantarum* HD 48 strains were sub-cultured twice in whey based medium at 37°C/18-24h and subjected to Gram's and negative staining, catalase test for their purity evaluation.

3.4.1 GRAM'S STAINING

When the bacteria are stained with the primary stain crystal violet and fixed with the mordant, some of the bacteria retain the primary stain while others are decolorized by alcohol. Gram-positive bacteria have a thick layer of protein-sugar complexes called peptidoglycan on their cell walls, and their lipid content is low. Decolorizing the cell causes the thick cell wall to dehydrate and shrink, closing the pores in the cell wall and preventing the stain from exiting the cell. As a result, ethanol cannot remove the Crystal Violet-Iodine complex, which is bound to the thick layer of peptidoglycan in gram's positive bacteria and appears blue or purple in colour.

PROTOCOL

- Thin smear of active *Lactobacillus* culture activated in whey based medium (WBM) were prepared on clean grease free glass slides.
- The smear was air dried and stained with crystal violet for 1 minute and then washed the slide with stream of running water and kept for drying
- Applied Gram's iodine for 1 minute and then washed with a stream of running water, then smear was decolorized with 95% ethanol for not > 30 seconds and washed the slide with a stream of running water.

- Applied counter stain safranin for 1 minute and then washed the slide and dried with bloating paper
- Slide was viewed in 100 X objective magnification using an oil immersion lens and observed Gram positive rods in pairs/chains with varied morphology.

3.4.2 NEGATIVE STAINING

Nigrosin is a highly acidic stain. This means the stain readily releases a hydrogen ion (proton) and the dye's chromophore becomes negatively charged. Because the surface of most bacterial cells is negatively charged, the stain is repellent. The glass of slide will stain, but the bacterial cells will not. Bacteria will appear as clear spots on a dark background.

PROTOCOL

- Clean glass slide was washed with a detergent solution and allowed to dry. Thin smear of active *Lactobacillus* cultures activated in whey based medium (WBM) was prepared on glass slides. The smear was stained with one drop of nigrosin solution and allowed it to air dry. Slide was then viewed in 100 X objective magnification using an oil immersion lens. Bacterial cells appear bright against a dark background.

3.4.3 CATALASE TEST

The enzyme catalase mediates the breakdown of hydrogen peroxide into oxygen and water. The presence of the enzyme in a bacterial isolate is evident when a small inoculum is introduced into hydrogen peroxide, and the rapid elaboration of oxygen bubbles occurs. The lack of catalase is evident by a lack of or weak bubble production. The culture should not be more than 24 hours old. Bacteria thereby protect themselves from the lethal effect of hydrogen peroxide which is accumulated as an end product of aerobic carbohydrate metabolism. The presence of catalase indicates contamination with spoilage and pathogen.

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

PROTOCOL

The protocol of Norris *et al*., (1981) was adopted for catalase test.

- Two to three drops of actively growing culture were transferred onto a clean glass slide.

- Equal volume of 3% hydrogen peroxide was poured on it and mixed to observe for effervescence. Presence of effervescence indicates a positive catalase reaction, whereas no effervescence was taken as negative test.

3.5 DEVELOPMENT OF WHEY BASED MEDIUM FOR GROWTH OF PROBIOTICS

Whey based medium for growth of probiotic *L. plantarum* CRD 7 and *L. plantarum* HD 48 strains was developed by supplementation of various ingredients (dextrose; yeast extract; Tween 80; ammonium citrate; sodium citrate; manganese sulphate; magnesium sulphate; di-potassium hydrogen sulphate) to paneer whey and adjusted the pH 6.5±0.2. Fresh paneer whey was collected from experimental dairy, ICAR-National Dairy Research Institute, Karnal-132001, Haryana and processed as per the procedure given below for development of cost-effective growth medium for cultivation of probiotic lactobacilli.

Paneer whey

↓

Heat treatment (85-90°C)

↓

Filtered by cream separator

↓

Addition of supplement as mentioned

↓

Adjusted pH (6.5±0.2)

↓

Autoclaved at 121°C/15 min/15 PSI

3.6 DETERMINATION OF THERMAL TOLERANCE OF PROBIOTIC *LACTIPLANTIBACILLUS PLANTARUM* STRAINS AND SELECTION OF PROTECTIVE AGENT FOR BETTER SURVIVABILITY DURING SPRAY DRYING

- Heat challenge protocol of Stumbo (1965) was followed to determine thermal tolerance of *Lactiplantibacillus plantarum* CRD7 and *L. plantarum* HD48 in 30% reconstituted skim milk (RSM). The 30% RSM was supplemented with three protectant agents i.e. lactose, sorbitol and maltodextrin individually @ 0, 0.5, 2.5, 5.0, 7.0, 10 %(w/v). This was autoclaved at 121℃/15 min and checked for its sterility at 37℃ for 72 h.

- Each 50-mL of supplemented RSM was dispensed aseptically into two 100-mL bottles at the appropriate test temperatures of 37°C (control), 55°C, 60°C and 65°C. After temperature equilibration, 10^{10} CFU inoculum of selected culture was added to the second bottle.
- One bottle was used to monitor the temperature and another was used for heat tolerance determination.
- One mL aliquots at time intervals of 1 min and 5 min, were taken from the test bottles and analyzed for total probiotic counts by pour plate technique. The aliquots were serially diluted and pour plated in whey based medium. The plates were incubated at 37℃ for 24-48 h for enumeration of survivors.

3.7 PRODUCTION OF SPRAY DRIED PROBIOTIC DVS OF *LACTIPLANTIBACILLUS PLANTARUM* CRD-7 AND PACKAGING FOR IMPROVED TECHNO – FUNCTIONAL PERFORMANCE.

3.7.1 Biomass production and preparation for probiotic DVS preparation by spray drying

Active biomass of selected probiotic *L. plantarum* CRD 7 was obtained in whey based medium by inoculation @ 2% incubation at 37℃/16 h. The probiotic cell biomass was harvested by centrifugation at 10,000 rpm for 5 min at 4℃. It was washed thrice with sterile phosphate buffer saline and harvested and washed cell biomass act as inoculum for feed preparation for production of probiotic DVS starter.

- Cells biomass produced was suspended in 1 L 30% sterilized RSM supplemented with maltodextrin at concentrations of 2.5% as optimized by heat challenge experiment. However, comparison purpose 0, 0.5 & 5 % was taken. These were subjected to spray drying (The pilot scale single stage spray dryer (Technosearch instrument, thane, Maharashtra, India) having stainless steel (SS-304) as material of construction for the drying material contact surface, was used for the preparation of spray dried sample. The plant has maximum evaporation capacity of 5L water vapour per hour. The hot air for drying was produced by the inbuilt electric air heater. Hepa filter are provided to control the quantity of inlet air. The two-fluid nozzle was provided to atomize the sample at compressed air pressure at 0.43 bar. The powder collection chamber was designed to collect powder at ambient temperature. The wet cleaning was followed after every experiment to ensure the

purity of sample by eliminating cross contamination with powder from previous batch.) at inlet temp of 170°C, 180°C & 190°C keeping feed rate constant.

- The DVS obtained by spray drying was packed in three packaging materials i.e. aluminium laminates, LDPE & EVOH. This was stored -20°C at 4°C. This was assessed for its techno-functional performance by *dahi* preparation, moisture content, water activity and total probiotic counts.

3.8 EVALUATION OF QUALITY PARAMETERS OF SPRAY DRIED DIRECT STARTERS

Quality of spray dried probiotic DVS of *L. plantarum* CRD 7 was assessed w.r.t the following parameters.

3.8.1 Determination of total probiotic count in spray dried DVS culture

Dried one g probiotic DVS preparation was dehydrated in 9 mL of sterilized peptone water and further serial dilutions were prepared. One mL of the selected serially dilute DVS samples was transferred aseptically in the sterile petri plates in triplicates. Previously melted and cooled to 45°C about 10-15 mL whey based agar was poured to each petri plate. The contents of the plates were mixed properly by rotating clockwise and anticlockwise. These were allowed to set at room temperature for about 25-30 min. The solidified plates were inverted and incubated at 37°C / 24 - 48 h and recorded the colony counts as colony forming units (CFU)/g.

3.8.2 Determination of moisture content of probiotic direct vat set starters

Moisture content of spray dried probiotic DVS culture was determined by gravimetric method. Two gams of dried probiotic DVS powder were taken in a clean, dry and previously weighed moisture dish. Dish was placed in a hot air oven and dried at 100±2ºC for 4-5 h. Dish was cooled in a desiccator and weighed till constant weighed was obtained. Moisture content was calculated as follows:

$$\textbf{Moisture (\% by weight)} = W1 - W2 / W1 - W \times 100$$

W = weight of empty dish (g); W1= weight of the dish with the sample before drying (g);

W2 = weight of the dish with dried sample (g).

3.9 OPTIMIZATION OF INOCULUM LEVELS OF SPRAY DRIED PROBIOTIC DVS

The inoculum levels of spray dried probiotic DVS with varied conc. 0.25, 0.50,

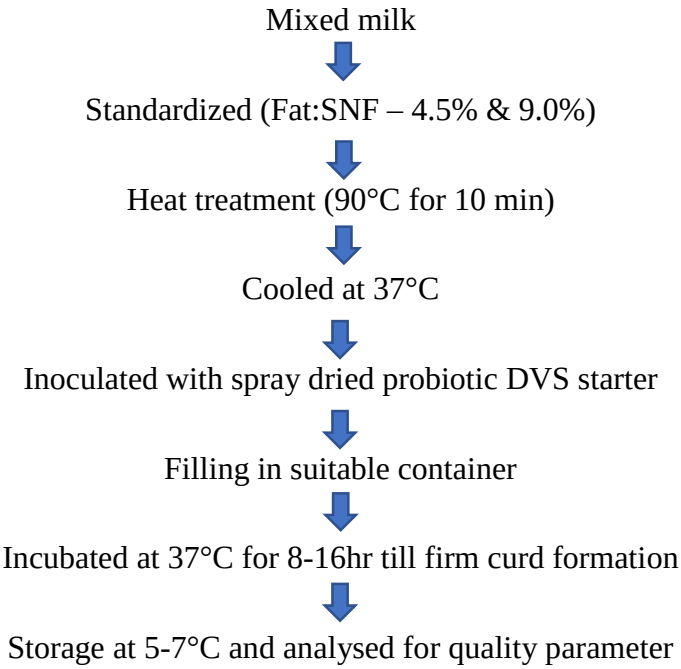
0.75 and 1%(w/v) was optimised by inoculation of standardized and heat treated milk (90℃/10 min). Techno-functional performance of probiotic DVS w.r.t curd setting time (h), pH, titrable acidity, probiotic count, sensory and texture profile was assessed as per protocol mentioned in section 3.0 chapter 3.

3.10 EVALUATION OF TECHNO- FUNCTIONAL PERFORMANCE OF SPRAY DRIED DVS STARTERS

Techno-functional attributes of spray dried probiotic DVS starter was determined by preparation of *Dahi* and assessing its quality w.r.t. physico-chemical, microbiological and textured parameter**.**

3.10.1 Preparation of *Dahi* with spray dried probiotic DVS

Briefly mixed milk was collected from Experimental Dairy, ICAR-NDRI, Karnal-132001. Fat and SNF (solid not fat) was adjusted according to Food safety and standard authority of India (FSSAI) standard for *dahi*. The *dahi* was prepared by the method of De (1980). Milk was heated to 90°C for 10 min and allowed to cool at 37°C. The cooled milk was inoculated by spray dried probiotic DVS starter @ 0.25, 0.50, 0.75 and 1%(w/v) this was mixed thoroughly under aseptic conditions, and incubated at 37°C/ 8-16 hours by filling in 100 m L sterile PVC containers. The activity of spray dried probiotic DVS was assessed for techno functional attributes with respect to curd setting time(h) pH 4.5, titrable acidity sensory and textured profile.



3.10.2 Microbiological quality assessment of probiotic *dahi*

Techno functional performance of spray dried probiotic DVS of *Lactiplantibacillus plantarum* CRD 7

3.10.2.1 Total probiotic counts

- The total probiotic count was enumerated by taking one ml probiotic Dahi sample in 9 ml saline. Subsequent serial dilution upto 10^{-11} was prepared. One ml of respective dilution was transferred aseptically in whey based agar petri plates. The plates were then allowed to solidify at room temp. and incubated at 37℃/ 24-48 h. The plates were then observed for colonies.

3.10.2.2 Coliforms count

Coliform are capable to produce acid and gas from lactose in the presence of bile salts and basic dyes. These are considered hygiene indicator of the process and their presence lead to spoilage of dairy products. Coliforms were enumerated using violet red bile agar (VRBA) after preparation of serial dilution of *dahi*. Plating was done on VRBA taking 1 mL of diluted sample. The plates were incubated at 37°C for 24 hrs and observed for developed colonies.

3.10.2.3 Yeast and molds counts

- Yeast and molds count were enumerated using acidified (pH 3.5) potato dextrose agar. The pH was adjusted with 10% tartaric acid solution. Plating was done aseptically on PDA by taking 1 mL of diluted sample. The plates were incubated at 25°C for 3-5 days' observation were recorded on developed yeast and mold colonies on plates.

3.10.3 Sensory analysis

Dahi samples were evaluated for sensory attributes such as body and texture, flavour, colour and appearance and overall acceptance on 10-point composite score card (flavour – 50, body and texture – 35, colour and appearance – 15).

3.10.3.1 Chemical analysis of probiotic *Dahi*

Probiotic *dahi* prepared by spray dried DVS was assessed for chemical quality w.r.t titrable acid and pH

3.10.3.2 Titrable acidity

- Titrable acidity of probiotic *dahi* was determined by taking 10 mL sample in clean glass beaker. This was diluted with 10 mL distilled water mixed well and added 2-3 drops of phenolphthalein indicator solution. This was titrated against 0.1 N NaOH until faint pink colour formed.
- Noted initial and final reading of 0.1 N NaOH in mL used. Titrable acidity was calculated by following formula

Lactic acid % (LA) = (9 × N × V)/W

Where,

N = Normality of NaOH

V = Volume of NaOH required

W = Volume of sample

3.10.3.3 pH

The pH of probiotic dahi was determined by using calibrated pH meter (Thermo scientific pH meter)

3.10.3.4 Texture profiling

Texture profile analysis method described by the Kumar and Mishra (2003) TPA tests were performed using a TAXT2 Texture Analyzer (Texture Technologies Corp., UK, Model TA.XT2 I, version 05.26 equipped with 5 Kg load cell). Experiments were carried out by compression tests that generated a plot of force (grams) *vs* time(S). A 25 mm diameter perplex cylindrical probe (P25) was used to measure textural profile of set *Dahi* samples prepared in a 100mL beaker at the temperature of 25±1°C, performing three repetitions. Rheological parameters include Firmness/(g), Consistency/(g.sec), Cohesiveness/(g) and Work of Cohesion/(g.sec).

3.11 EVALUATION OF STORAGE STABILITY OF DVS CULTURE

- Storage stability of DVS produced under optimized spray drying conditions will be packed in the above-mentioned packaging materials and stored at 37°C, 4°C and -20°C. It will be analysed for probiotic count and activity performance at 30 days' time interval.

- Performance of probiotic DVS for product quality will be assessed by preparation of dahi with respect to sensory, physicochemical, microbial (probiotic, coliforms, yeast/mold), textural parameters.

3.11.1 PACKAGING MATERIALS AND STORAGE STUDIES

Total of three packaging materials *viz*., aluminium laminates, low-density polyethene and EVOH were used for packaging of probiotic DVS starters. Two grams of freeze-dried probiotic DVS powder was placed in each packaging material. These were stored at 4°C and 37°C to assess their shelf life at two-month intervals. The probiotic DVS was inoculated @1% in sterilized standardized buffalo milk and incubated at 37°C /8h. The *dahi* obtained was subjected to chemical, microbiological, sensory and textural profiling as discussed earlier section

3.11.2 ALUMINUM LAMINATES:

Aluminium laminate is a sheet metal of a very thin gauge. It is produced by the cold reduction process through which pure aluminium is pressed to reduce its thickness to less than 0.152 mm and annealed to give folding properties. Aluminium laminate is used in the form of cups and trays, laminated foil pouches as alternatives to cans or jars, collapsible aluminium tubes for pastes, and aluminium barrels. Size of the alminium laminates is 2×3 cm (W×L), Thickness:100GSM.

Source: Vijay Packaging System. No.3213, Ram Bazar, Mori Gate, Delhi-110006, India.

3.11.3 LOW-DENSITY POLYETHYLENE (LDPE):

LDPE is defined by a density range of 0.910-0.940 g/cm^3.It has a high degree of short and long-chain branching, which means that the chains do not pack into the crystal structure as well. It has therefore less strong intermolecular forces.

Pack if packaging Shop No.1 Bazida Road, Doon Valley Road, Brahmanand Chownk, Karnal-132001, Haryana, India.

The general properties of LDPE:

S No	Property	LDPE
1	Yield Stress	1250 – 2000 psi
2	Yield Elongation	16-20%

3	Ultimate Elongation	200-600%
4	Impact Strength (200-gauge film)	4.5
5	Hardness	41-43
6	Softening Point	85-87^{0}C
7	Tearing Strength (gm / mil)	150
8	WVTR (gm / m^2 / day)	18
9	Oxygen Transmission Rate (cc/cm2/day)	15
10	CO_2 Transmission Rate (cc/cm^2/day)	55
11	Nitrogen Transmission Rate (cc/cm^2/day)	5
12	Turpentine Grease Proof Test	2 hours

3.11.4 ETHYLENE VINYL ALCOHOL (EVOH)

EVOH is a hydrolyzed copolymer of vinyl alcohol and ethylene. EVOH films are strong, have good clarity, are heat-sealable, and have excellent odour, gas, and moisture barrier characteristics. The major disadvantage of EVOH films is that they are hydrophilic and hygroscopic. When they absorb moisture at high relative humidity, the absorbed moisture acts as a plasticizer and the gas barrier properties of the film decrease. This can be overcome by increasing the ethylene content of the film, laminating it between two films that protect it against moisture, or c. Adding a desiccant to the tie layer. EVOH is commonly used in laminated structures where high gas and moisture barrier characteristics are desired, e.g., modified atmosphere packaging applications. packaging pouch of EVOH having this properties Water vapour transmission rate (WVTR)=100 in g m^{-2} /24 h at tropical conditions of 90% RH at 38°C and gas permeability in cm3 m−2 /24 hrs and Oxygen transmission rate=0.5 cc/cm2/day.

Source: Aarpee Packaging C-504, Umiya Tirth Residency, Nr Trishala Appt, R C Technical Road, Chandlodiya, Ahmedabad- 380061, Gujarat, India.

CHAPTER –4

Results and Discussion

RESULT AND DISCUSSION

Conventional technique for dairy starter propagation and maintenance are laborious, prone to contamination, and susceptible to deterioration at different phases of production. Poor-quality fermented dairy products are produced as a result of the contaminated dairy starter, which causes financial losses for the dairy fermentation business. Direct vat set (DVS) probiotic dairy starters can be produced to address the issue of starter contamination and the associated poor performance of the starter. The current study was aimed suitable protective agents and to optimize the spray dried parameters for the production of probiotic DVS with better survivability. The DVS starter observations w.r.t. purity, selection of protective agents and their levels, optimization spray drying conditions, techno-functional evaluation of probiotic DVS prepared, selection of packaging material and storage stability have been presented in this chapter in the form of tables and figures.

OBJECTIVE 1:

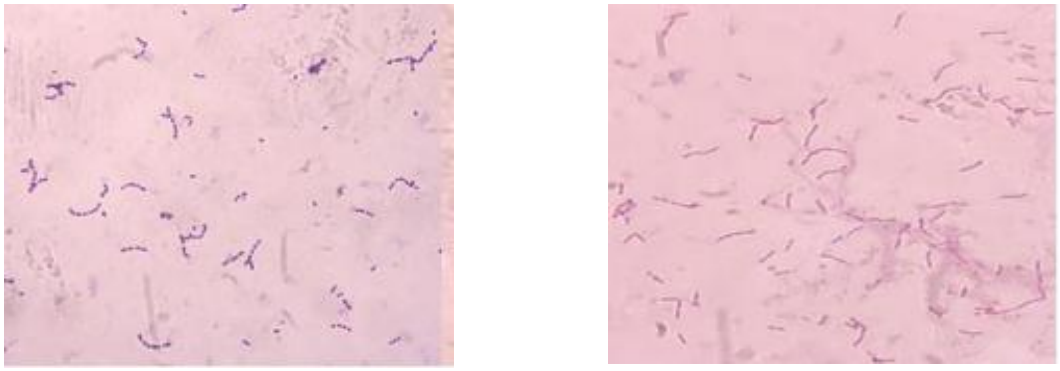
4.1 SELECTION OF SUITABLE PROTECTANTS FOR BETTER SURVIVABILITY DURING SPRAY DRYING

4.1.1 PURITY EVALUATION OF PROBIOTIC *LACTIPLANTIBACILLUS* CULTURES:

Two probiotic *Lactiplantibacillus* cultures listed in **Table 4.1** were selected based on their health benefits features. Evaluated earlier at Synbiotic Functional Food and Bioremediation Research Laboratory, Dairy Microbiology Division, ICAR-National Dairy Research Institute (Deemed University), Karnal-132001, Haryana, India. Selected probiotic strains were sub-cultured in whey-based medium, skim milk for their microscopic and catalase test for purity evaluation. Morphological and biochemical parameters details of selected strains have been presented in **Table 4.1** and **figure 4.1 (a) and (b)**. The selected probiotic *Lactiplantibacillus* strains were found Gram's positive, long or short rods arranged in single chains. Selected probiotic Lactobacilli were found catalase negative. These morphological and biochemical observations reveled their purity for further investigations.

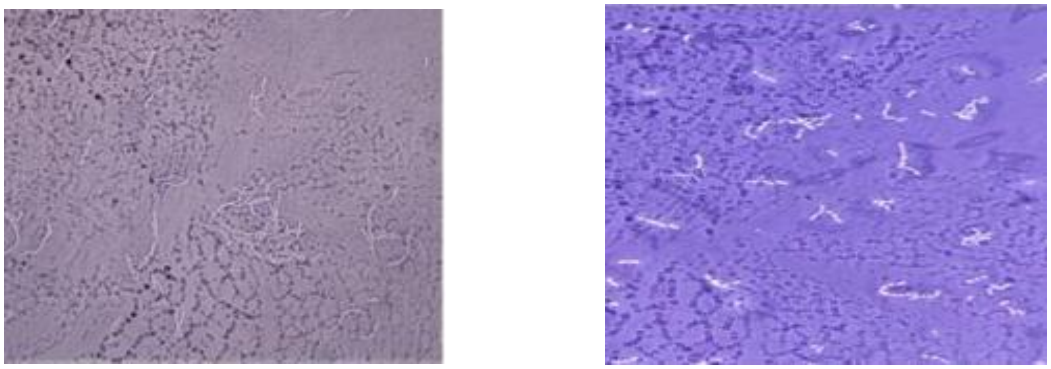
Table 4.1. Morphological and biochemical characteristics of probiotic *Lactiplantibacillus plantarum* cultures.

Probiotic *Lactobacillus* strains	Negative staining	Gram's staining	Catalase test
Lp. plantarum CRD7	Short rods in chains	+ve	-ve
Lp. plantarum HD48	Short rods in chains	+ve	-ve



(a). *Lp. plantarum* CRD7 (**b**). *Lp. plantarum* HD48

Fig 4.1 (a): Microscopic and catalase test observations of probiotic *Lactiplantibacillus* plantarum cultures.



(a). *Lp. plantarum* CRD7 (**b**). *Lp. plantarum* HD48

Figure 4.1 (b): Microscopic observations (Negative staining) of selected probiotic *Lactobacillus* cultures

4.1.2: TECHNO-FUNCTIONAL ATTRIBUTES OF PROBIOTIC *DAHI* PREPARED BY SINGLE *LACTOBACILLUS* CULTURE

Data on techno-functional attributes of *dahi* prepared with single strains have been presented in **Table 4.1.1 (a)** and **Figure 4.1.1 (a).** Probiotic *dahi* was prepared with

standardized milk, heated to 90°C/10min. Milk was cooled to 37°C and inoculated with 1% inoculum. This was incubated at 37°C and observed for curd setting, pH, titratable acidity and probiotic counts. Probiotic *dahi* was also analysed for sensory and textural attributes. The curd setting time taken by single probiotic *Lactiplantibacillus plantarum* strains for the preparation of *dahi* ranged 14.33±0.02 -15.03± h, pH and titratable acidity ranged between 4.26±0.01-4.12±0.01 and 0.72±0.03-0.74±0.02 (% LA) and total probiotic counts ranged from 8.71±0.01 to 8.66±0.01(Log CFU/mL). These observations revealed their survivability to be used as probiotic dairy starters. On basis of minimum curd setting time, faster reduction in pH and acidity development, and highest probiotic counts, selected probiotic strains were explored to optimize spray drying conditions and selection of suitable protective agents for the preparation of DVS starters.

Table 4.1.1 (a): Techno-functional attributes of *dahi* prepared by probiotic *Lactiplantibacillus plantarum* cultures.

Probiotic *Lactiplantibacillus plantarum* cultures	**Curd setting time (h)**	**Ph**	**Titratable acidity (% LA)**	**Total probiotic counts (Log CFU/mL)**
Lp. plantarum CRD7	14.33±0.02^{a}	4.26±0.01^{a}	0.72±0.03^{a}	8.71±0.02^{a}
Lp. plantarum HD48	15.03±0.03^{b}	4.12±0.01^{b}	0.74±0.02^{b}	8.66±0.01^{b}

Data are represented as Mean ± SE; (p<0.05); n=3.

a-b (along the column) values with different superscripts are significantly different from each others.

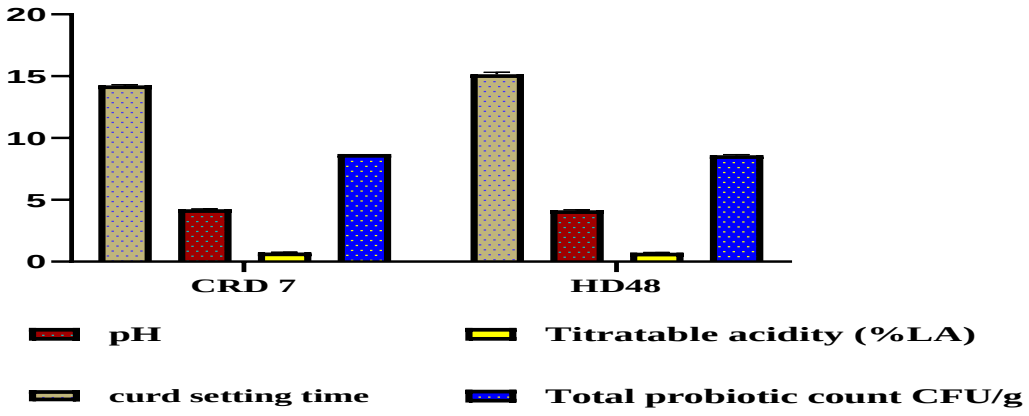


Figure 4.1.1 (a): Techno-functional attributes of *dahi* prepared by probiotic *Lactiplantibacillus plantarum* cultures.

4.1.3 SENSORY SCORES

Observations related to sensory parameters such as colour and appearance (10), flavour (10), body and texture (10), overall acceptability scores of probiotic *dahi* prepared using individual probiotic *Lactiplantibacillus plantarum* cultures are mentioned in **Table 4.1.1 (b)** and **Figure 4.1.1 (b)**.

The highest score for colour and appearance *i.e.,* 9.3±0.73 and 8.6±0.28 was obtained for probiotic *dahi* prepared with *Lp. plantarum* CRD7 and *Lp. plantarum* HD48, respectively. Significant difference in sensory scores was obtained for probiotic *dahi* prepared with different cultures.

The highest score 8.6±0.36 for flavour was obtained for *dahi* prepared with *Lp. plantarum* CRD7. Significant difference in the flavour score of probiotic *dahi* prepared using *Lp. plantarum* HD48 was noticed. Weak and moderate diacetyl favour in *dahi* is more acceptable whereas, high concentration of diacetyl production is not acceptable by consumers as it may leads to off flavours. The formation of methyl aldehydes in milk by the metabolic activity of *L. lactis ssp. lactis* has been recognized as the cause of off-flavours in cheddar cheese (Urbach *et al*.,1997).

The maximum score for body and texture *i.e.,* 9.3±1.02 and 9.0±0.65 in probiotic *dahi* was recorded for *Lp. plantarum* CRD7 and *Lp. plantarum* HD48. There was significant difference in the scores obtained for probiotic dahi prepared with single probiotic *Lactobacillus* cultures.

The highest score for overall acceptability *i.e.,* 9.3±2.03 and 9.0±1.02 was obtained for probiotic *dahi* prepared from *Lp. plantarum* CRD7 and *Lp. plantarum* HD48, respectively. Significant difference in the scores was obtained for probiotic *dahi* prepared with different *Lp. Plantarum cultures.*

Table 4.1.1 (b): Sensory scores of *dahi* prepared by probiotic *Lactiplantibacillus plantarum* cultures.

Probiotic *Lactiplantibacillus plantarum* cultures	**Colour and appearance**	**Flavour**	**Body and texture**	**Overall acceptability**
Lp. plantarum CRD7	9.3±0.70 [a]	8.6±0.36[a]	9.3±1.02[a]	9.3±2.03[a]
Lp. plantarum HD48	8.6±0.28[b]	7.3±0.75[b]	9.0±0.65[b]	9.0±1.02[b]

Data are represented as Mean ± SE; (p<0.05); n=3.
a-b (along the column) values with different superscripts are significantly different from each others.

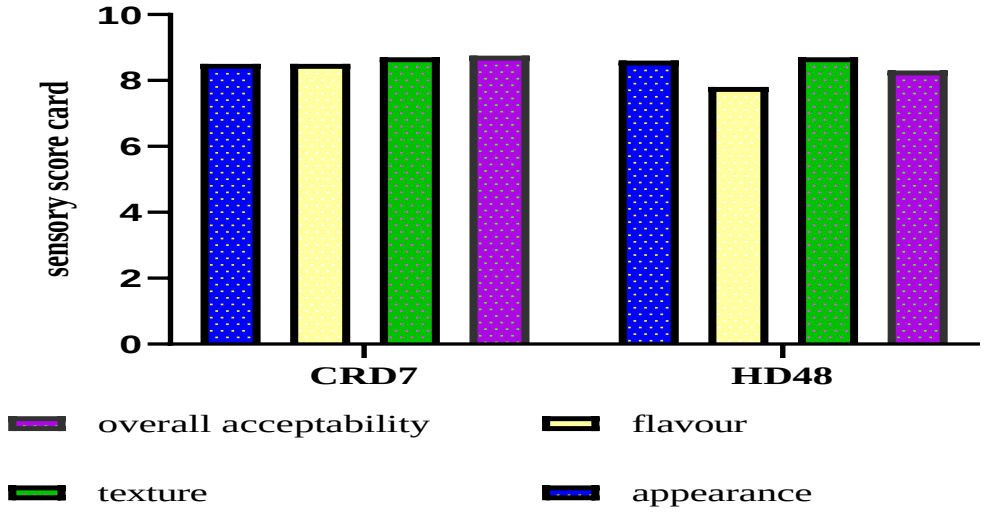


Figure 4.1.1 (b): Sensory scores of *dahi* prepared by probiotic *Lactiplantibacillus plantarum* cultures.

4.1.4 TEXTURE PROFILES

Hardness or firmness, is the most important characteristics in determining texture of dairy products *vis-à-vis* fermented dairy foods. It is regarded as the force required to attain a certain deformation and is considered a measure of hardness of fermented milk products such as yoghurt. Results obtained on texture profile have been presented in **Table 4.1.1 (c)** and **Figure 4.1.1 (c)**. Texture parameters such as firmness (N), consistency (N.s), cohesiveness (N) and work of cohesion (N.s) of probiotic *dahi* prepared by application of individual probiotic *Lactiplantibacillus plantarum* cultures ranged from 2.20±0.02 to 2.28±0.03. The highest score for firmness was obtained for *dahi* prepared with *Lp. plantarum* CRD7 (2.28±0.03) and *Lp. plantarum* HD48 (2.20±0.02). Significant differences in the firmness of probiotic *dahi* were prepared with notices single culture.

Table 4.1.1 (c): Texture profile of *dahi* prepared by probiotic *Lactiplantibacillus plantarum* cultures.

Probiotic *Lactiplantibacillus plantarum* cultures	**Firmness (N)**	**Consistency (N.s)**	**Cohesiveness (N)**	**Work of cohesion (N.s)**
CRD7	2.28 ± 0.03^{a}	37.55 ± 0.25^{a}	-0.41 ± 0.01^{b}	-3.03 ± 0.28^{b}
HD48	2.20 ± 0.02^{b}	34.23 ± 0.75^{b}	-0.55 ± 0.03^{a}	-4.54 ± 0.32^{a}

Data are represented as Mean ± SE; (p<0.05); n=3.

a-b (along the column) values with different superscripts are significantly different from each others.

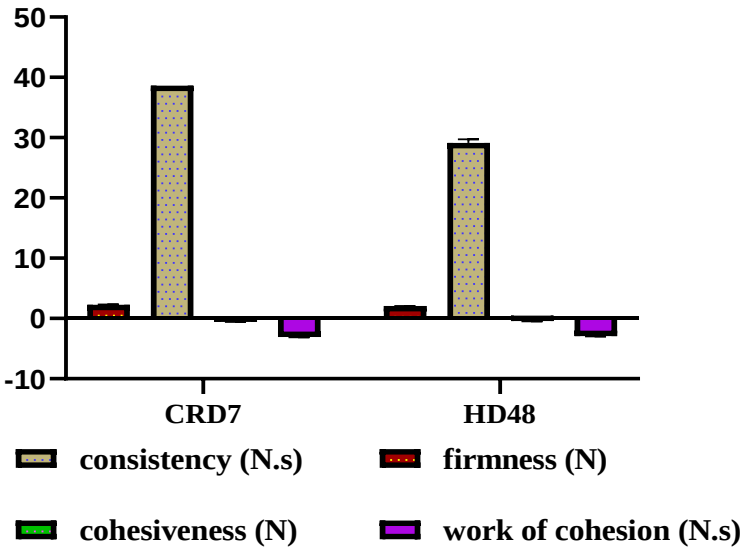


Figure 4.1.1 (c): Texture profile of *dahi* prepared by probiotic *Lactiplantibacillus plantarum* cultures.

4.2 EVALUATION OF SUITABLE PROTECTANT ON THE BASIS OF HEAT CHALLENGE EXPERIMENT

For spray drying matrix purposes, polysaccharides, proteins, and combinations thereof have been explored as carrier agents. Polysaccharides with prebiotic properties such as glucose, lactose and maltodextrin have been used to protect probiotic bacteria during spray drying and under storage conditions (Bhagwat *et al*., 2020). Maltodextrin is a polysaccharide produced by the acidic or enzymatic hydrolysis of starch. It has a nutritional value of only 4 cal/g. Maltodextrins have been widely used in the food industry as they provide different benefits such as texture improvement, reduction in floury taste, sweetness modifying agents, controlling non-enzymatic browning, decreasing the freezing point of mixtures, and as carrier materials (Behboudi-Jobbehdar *et al*., 2021). Using a protein-carbohydrate mixture result in better survival of probiotics during spray drying. Yoha *et al*., (2020) found that *Lactobacillus plantarum* microencapsulated with fructo-oligosaccharides (FOS) by spray drying, improved the encapsulation efficiency and preserved 96% of viability.

The thermal tolerance of *L. rhamnosus* GG, *L. rhamnosus* E800 and *L. salivarius* UCC 500 were compared in reconstituted skim milk (RSM, 20% w/v), supplemented with yeast extract (0.5% w/v). Sucrose was included for studies involving *L. rhamnosus* GG, as

it utilizes lactose poorly (Goldin *et al*., 1992). Two 50-ml volumes of RSM, contained in 100-mL bottles and agitated by magnetic stirrer bars, were placed in a water bath at the appropriate test temperatures of 37ºC (control and to obtain initial counts), 55, 58, 59, 60 and 61ºC. One bottle was used to monitor the temperature, while, after temperature equilibration, a 1% (v/v) inoculum of an overnight culture (~17 h) of either *L. salivarius* UCC 500, *L. rhamnosus* E800 or *L. rhamnosus* GG was added to the second bottle. At appropriate intervals (between 30 s and 4 min), 1-mL aliquots were removed from the test bottle, serially diluted in MRD and pour plated in MRS agar. Survivors were enumerated after 3 days of anaerobic incubation at 37 ºC. Tests were conducted in duplicate and mean log survivor counts were plotted as a function of heating time for each test temperature. At each temperature, a best fit straight line was obtained by regression analysis, and D-values, which represent the time (min) required to kill 90% of cells were determined by taking the absolute value of the inverse of the slope of this line (Stumbo, 1965).

The cell biomass of two selected probiotic *Lactiplantibacillus* strains *i.e. Lp. plantarum* CRD7 and *Lp. plantarum* HD48 was obtained individually by centrifugation at 8000 rpm/10 min at 4ºC from cultures grown under optimized cultural growth conditions (whey-based medium, pH 6.5±0.02, incubation at 37ºC/16 h) by batch fermentation. The harvested concentrated cell biomass of selected probiotic strains was re-suspended in 30% sterilized reconstituted skim milk (RSM) supplemented with varied concentrations (0, 0.5, 2.5, 5.0, 7.5 and 10% w/v) of respective protective agents *i.e.* lactose, sorbitol and maltodextrin. The re-suspended biomass in RSM (50 mL) was dispersed in 100 mL capacity bottles and agitated by magnetic stirrer bars. This was placed in water bath at appropriate test temperatures of 37 (control), 55, 60 and 65ºC. One bottle was used to monitor the temperature. After temperature equilibration, inoculum of selected culture was added to the second bottle to attained 10^{10} CFU/mL. One mL of aliquotes were withdrawn at intervals 1 min and 5 min and analysed for total viable probiotic counts.

4.2.1 EFFECT OF MALTODEXTRIN ON SURVIVABILITY DURING HEAT CHALLENGE EXPERIMENT

Results of viable counts and percent survival rate have been presented in **Table 4.2 (a)**. In heat challenge experiment of *Lp. planatum* CRD7, viable counts of the selected strains varied from 8.57±0.24 to 9.67±0.27 log CFU/ mL. After exposure to different temperatures of 37, 50, 55 & 65ºC at varied exposure time 1 and 5 min at different

concentrations (0, 0.5, 2.5, 5.0, 7.5 and 10% (w/v) of maltodextrin. Minimal unit reduction have been observed in viability of probiotic culture of *Lp. plantarum* CRD7 (0.48 Log CFU/ mL) during exposure to elevated temperatures of 55, 60 and 65ºC. The maximum viable counts have been observed in *Lp. plantarum* CRD7 (9.67±0.27 Log CFU/mL) with highest survivability rate of 95.27±0.24 percent when maltodextrin @2.5 percent (w/v) was used as protectant at 55ºC. However, least difference in the percent survivable rate was observed with increased concentrations of maltodextrin up to 10% at varied exposure i.e., 60, 65ºC and thus maltodextrin @2.5% (w/v) was optimization of spray drying parameters for the production of probiotic DVS starters. The percent survival rate of 93.43±0.94 and 91.89±0.14% was recorded at 65ºC at highest concentration (10% maltodextrin) to exposure time of 1 min and 5 min, respectively.

4.2.2 EFFECT OF LACTOSE ON HEAT TOLERANCE OF PROBIOTIC *LACTIPLANTIBACILLUS PLANTARUM* CRD7

Results of effect of sugar (lactose) on viable counts and percent survival rate have been presented in **Table 4.2 (b)** and **Figure (b)**. In heat challenge experiment of *Lp. planatum* CRD7, viable counts of the selected strains varied from 8.68±0.22 to 9.78±0.27 log CFU/ mL, after exposure temperatures of 37, 55, 60, 65 ºC at varied exposure time of 1 and 5 min at different concentrations (0, 0.5, 2.5, 5.0, 7.5, 10% w/v) of lactose. Minimal unit reduction in viability of probiotic *Lp. plantarum* CRD7 (0.66 Log CFU/ mL) has been observed during the heat challenge experiment at elevated temperatures of 55, 60 and 65ºC. The maximum viable counts have been recorded of *Lp. plantarum* CRD7 (9.78±0.27 Log CFU/mL) with highest survivability rate of 92.62±0.04 percent when lactose@2.5 percent (w/v) was used as protectant at 55ºC after exposure to 1 min time. However, not much difference in the survivability rate was noticed with increased concentrations of maltodextrin up to 10% at varied heat exposure temperatures selected for optimization of spray drying parameters for the production of probiotic DVS starters survival rate of 92.42±0.08 and 89.98±0.09% at 65ºC for exposure time of 1 min and 5 min, respectively was noticed. Hence, lactose @2.5 percent (w/v) can be used for preparation of probiotic DVS for better survivability.

Table 4.2 (a): Effect of maltodextrin on heat tolerance of probiotic *Lactiplantibacillus plantarum* CRD7.

Maltodextrin concentration (% w/v)	Exposure temperature (°C)	Exposure time (min)	Probiotic counts before heat treatment (Log CFU/mL)	Probiotic counts after heat treatment (Log CFU/mL)	Survivability rate (%)
0	37 (Control)	1	10.24±0.02[a]	10.19±0.08[a]	99.51±0.02
		5	10.18±0.04[a]	10.12±0.43[a]	99.42±0.08
	55	1	10.09±0.10[a]	9.37±0.22[a]	92.86±0.04[a]
		5	10.24±0.12[a]	9.43±0.27[a]	92.08±0.02[a]
	60	1	10.18±0.08[a]	9.36±0.15[a]	91.94±0.02[a]
		5	10.16±0.09[a]	9.25±0.25[a]	91.04±0.09[a]
	65	1	10.12±0.04[a]	8.83±0.51[b]	87.25±0.02[a]
		5	10.09±0.02[a]	8.57±0.24[c]	84.93±0.08[b]
0.5	37 (Control)	1	10.23±0.02[a]	10.19±0.20[a]	99.61±0.04[a]
		5	10.15±0.22[a]	10.10±0.29[a]	99.51±0.05[a]
	55	1	10.16±0.00[a]	9.57±0.04[a]	94.19±0.12[a]
		5	10.08±0.08[a]	9.47±0.02[a]	93.94±0.04[a]
	60	1	10.14±0.05[a]	9.59±0.22[a]	94.57±0.02[a]
		5	10.22±0.03[a]	9.57±0.14[a]	93.63±0.08[a]
	65	1	10.24±0.09[a]	9.35±0.25[a]	91.30±0.08[a]
		5	10.21±0.11[a]	9.28±0.53[a]	90.89±0.12[a]
2.5	37 (Control)	1	10.17±0.15[a]	10.14±0.30[a]	99.71±0.02[a]
		5	10.15±0.02[a]	10.11±0.31[a]	99.61±0.03[a]
	55	1	**10.15±0.10[a]**	**9.67±0.27[a]**	**95.27±0.24[a]**
		5	**10.11±0.03[a]**	**9.62±0.14[a]**	**95.15±0.30[a]**
	60	1	10.24±0.04[a]	9.66±0.25[a]	94.33±0.14[a]
		5	10.19±0.05[a]	9.62±0.42[a]	94.40±0.25[a]
	65	1	10.21±0.02[a]	9.54±0.45[a]	93.43±0.12[a]
		5	10.12±0.02[a]	9.30±0.53[a]	91.89±0.14[b]

5	37 (Control)	1	10.19±0.11[a]	10.17±0.35[a]	99.80±0.18[a]
		5	10.16±0.19[a]	10.13±0.11[a]	99.70±0.22[a]
	55	1	10.21±0.04[a]	9.62±0.25[a]	94.22±0.20[a]
		5	10.23±0.02[a]	9.63±0.17[a]	94.13±0.08[a]
	60	1	10.18±0.12[a]	9.57±0.03[a]	94.00±0.04[a]
		5	10.11±0.08[a]	9.45±0.34[a]	93.47±0.22[a]
	65	1	10.09±0.02[a]	9.39±0.19[a]	93.06±0.08[a]
		5	10.05±0.00[a]	9.24±0.24[a]	91.94±0.05[b]
7.5	37 (Control)	1	10.22±0.41[a]	10.21±0.05[a]	99.90±0.12[a]
		5	10.17±0.22[a]	10.15±0.06[a]	99.80±0.08[a]
	55	1	10.14±0.12[a]	9.57±0.12[a]	94.37±0.04[a]
		5	10.06±0.04[a]	9.44±0.15[a]	93.83±0.05[a]
	60	1	10.22±0.02[a]	9.61±0.18[a]	94.03±0.12[a]
		5	10.15±0.11[a]	9.48±0.06[a]	93.39±0.08[a]
	65	1	10.12±0.12[a]	9.44±0.16[a]	93.28±0.04[a]
		5	10.23±0.08[a]	9.41±0.22[a]	91.98±0.02[b]
10	37 (Control)	1	10.24±0.10[a]	10.23±0.14[a]	99.90±0.09[a]
		5	10.14±0.08[a]	10.12±0.19[a]	99.80±0.04[a]
	55	1	10.17±0.04[a]	9.59±0.07[a]	94.29±0.00[a]
		5	10.09±0.04[a]	9.50±0.15[a]	94.15±0.12[a]
	60	1	10.19±0.19[a]	9.58±0.12[a]	94.01±0.14[a]
		5	10.14±0.02[a]	9.45±0.35[a]	93.19±0.10[a]
	65	1	10.16±0.08[a]	9.47±0.26[a]	93.20±0.08[a]
		5	10.06±0.04[a]	9.24±0.46[a]	91.84±0.04[b]

Data are represented as Mean ± SE; (p<0.05); n=3. a-b (along the column) values with different superscripts are significantly different from each other.

Table 4.2 (b): Effect of lactose on heat tolerance of probiotic *Lactiplantibacillus plantarum* CRD7.

Lactose concentration (% w/v)	Exposure temperature (°C)	Exposure time (Min)	Probiotic counts before heat treatment (LogCFU/mL)	Probiotic counts after heat treatment (LogCFU/mL)	Survivability rate (%)
0	37 (Control)	1	10.12±0.02^{a}	10.07±0.08^{a}	99.51±0.02^{a}
		5	10.10±0.01^{a}	10.04±0.43^{a}	99.41±0.03^{a}
	55	1	10.24±0.06^{b}	9.47±0.22^{a}	92.48±0.05^{a}
		5	10.11±0.03^{a}	9.31±0.27^{a}	92.08±0.08^{a}
	60	1	10.31±0.05^{b}	9.57±0.15^{a}	91.71±0.01^{a}
		5	10.29±0.02^{c}	9.37±0.25^{a}	91.05±0.01^{a}
	65	1	10.22±0.02^{a}	8.81±0.51^{b}	87.05±0.09^{b}
		5	10.12±0.01^{b}	8.68±0.22^{b}	84.93±0.04^{c}
0.5	37 (Control)	1	10.30±0.03^{c}	10.26±0.25^{a}	99.61±0.01^{a}
		5	10.19±0.01^{a}	10.14±0.25^{a}	99.51±0.08^{a}
	55	1	10.29±0.05^{a}	9.46±0.68^{a}	92.50±0.04^{a}
		5	10.27±0.04^{a}	9.63±0.34^{a}	92.18±0.02^{a}
	60	1	10.19±0.13^{a}	9.52±0.11^{a}	92.84±0.09^{a}
		5	10.00±0.12^{a}	9.28±0.04^{a}	91.25±0.10^{a}
	65	1	10.09±0.08^{a}	9.27±0.25^{a}	91.97±0.09^{a}
		5	10.04±0.02^{a}	8.88±0.16^{b}	88.14±0.02^{b}
2.5	37 (Control)	1	10.51±0.09^{c}	10.48±0.44^{a}	99.71±0.14^{a}
		5	10.41±0.31^{b}	10.37±0.13^{a}	99.61±0.08^{a}
	55	**1**	**10.39±0.19^{b}**	**9.78±0.27^{a}**	**92.62±0.04^{a}**
		5	**10.33±0.03^{b}**	**9.67±0.19^{a}**	**92.52±0.10^{a}**
	60	1	10.16±0.12^{a}	9.44±0.05^{a}	92.87±0.02^{a}
		5	10.13±0.11^{a}	9.38±0.15^{a}	91.34±0.04^{a}
	65	1	10.22±0.01^{a}	9.20±0.18^{a}	92.01±0.09^{a}
		5	10.01±0.50^{a}	8.96±0.23^{b}	89.51±0.08^{b}

5	37 (Control)	1	10.37±0.36[a]	10.35±0.19[a]	99.81±0.02[a]
		5	10.28±0.25[a]	10.25±0.50[a]	99.71±0.02[a]
	55	1	10.22±0.04[a]	9.61±0.31[a]	93.03±0.08[a]
		5	10.15±0.09[a]	9.52±0.45[a]	92.79±0.06[a]
	60	1	10.24±0.45[a]	9.55±0.36[a]	92.96±0.04[a]
		5	10.19±0.02[a]	9.47±0.45[a]	92.43±0.07[a]
	65	1	10.31±0.12[a]	9.50±0.49[a]	92.14±0.12[a]
		5	10.10±0.15[a]	9.08±0.35[b]	89.90±0.09[b]
7.5	37 (Control)	1	10.24±0.12[b]	10.23±0.45[b]	99.90±0.08[a]
		5	10.11±0.10[a]	10.09±0.37[a]	99.80±0.02[a]
	55	1	10.15±0.08[a]	9.52±0.17[a]	93.17±0.04[a]
		5	10.10±0.04[a]	9.47±0.28[a]	92.86±0.09[a]
	60	1	10.19±0.02[a]	9.52±0.03[a]	92.99±0.09[a]
		5	10.24±0.04[b]	9.50±0.66[a]	92.57±0.10[a]
	65	1	10.13±0.13[a]	9.35±0.23[a]	92.30±0.02[a]
		5	10.19±0.12[a]	9.15±0.20[a]	89.94±0.01[b]
10	37 (Control)	1	10.21±0.08[a]	9.59±0.38[c]	99.91±0.08[a]
		5	10.09±0.04[a]	9.46±0.41[c]	99.82±0.04[a]
	55	1	10.12±0.09[a]	9.54±0.04[c]	93.36±0.02[a]
		5	10.16±0.02[a]	9.53±0.51[c]	92.98±0.03[a]
	60	1	10.19±0.01[a]	9.51±0.35[c]	93.00±0.02[a]
		5	10.02±0.23[a]	9.31±0.22[b]	92.64±0.00[a]
	65	1	10.11±0.14[a]	9.32±0.53[b]	92.42±0.08[a]
		5	10.05±0.18[a]	9.04±0.18[a]	89.98±0.09[b]

Data are represented as Mean ± SE; (p<0.05); n=3. a-b (along the column) values with different superscripts are significantly different from each other.

4.2.3 EFFECT OF SORBITOL ON HEAT TOLERANCE OF PROBIOTIC *LACTIPLANTIBACILLUS PLANTARUM* CRD7

Results of sorbitol effect on viable counts and percent survival rate have been presented in **Table 4.2 (c)**. In heat challenge experiment of *Lp. planatum* CRD7, viable counts of the selected strains varied from 8.59±0.10 to 9.79±0.12 log CFU/ mL. after exposure to varied temperatures of 37, 55, 65 ^{o}C at varied exposure time 1 and 5 min at different concentrations of sorbitol (0, 0.5, 2.5, 5.0, 7.5 and 10 (%w/v). Minimal unit reduction have been observed in viability of probiotic culture of *Lp. plantarum* CRD7 (0.10 Log CFU/ mL) during the heat treatment at elevated temperatures of 55, 60 and 65 ^{o}C for exposure time of 1 and 5 min. The maximum viable counts have been observed in *Lp. plantarum* CRD7 (9.43±0.11 Log CFU/mL) with highest survivability rate of 92.45±0.07 and 92.19±0.06 percent when sorbitol @2.5 percent (w/v) was used as protectant at 55 ^{o}C for 1 and 5min exposure time respectively. However, least difference in the survivable rate was observed with increased concentrations of sorbitol up to 10% at varied heat treatment temperatures during heat challenge experiment. These results sorbitol @2.5 (%w/v) can be exposure for production of probiotic DVS of *Lp. plantarum* CRD7 by spray drying have been indicated that as it was 86.78±0.03 and 88.71±0.02% at 65 ^{o}C.

4.2.4 EFFECT OF MALTODEXTRIN ON HEAT TOLERANCE OF PROBIOTIC *LACTIPLANTIBACILLUS PLANTARUM* HD48

Observation on effect of maltodextrin on thermal tolerance of *Lp. plantarum* HD48 w.r.t. viable counts and percent survival rate of *Lp. planatarum* in presence of lactose as protectant have been presented in **Table 4.2 (d)**. In heat challenge experiment of *Lp. planatum* HD48, viable counts varied from 8.70±0.10 to 9.16±0.22 log CFU/ mL, after heat treatment temperatures of 65 ^{o}C/5 min at varied exposure time of 5 min at different concentrations (0, 0.5, 2.5, 5.0, 7.5 and 10 %w/v) of maltodextrin. Minimal unit reduction have been observed in viability of probiotic culture of *Lp. plantarum* CRD7 (0.75 Log CFU/ mL) during the heat challenge experiment at elevated temperatures of 55, 60 and 65^{o}C. The maximum viable counts have been observed of *Lp. plantarum* HD48 (9.60±0.32 Log CFU/mL) with highest survivability rate of 93.21±0.02 percent when maltodextrin @2.5 percent (w/v) was used as protectant at 55^{o}C. However, least difference in the survivability rate was recorded with increased concentrations of maltodextrin up to 10% at varied heat treatment temperatures i.e. 91.17±0.03 and 89.99±0.07 % after exposure to 65^{o}C/5 min was recorded.

Table 4.2 (c) : Effect of sorbitol on heat tolerance of probiotic *Lactiplantibacillus plantarum* CRD7.

Sorbitol concentration (% w/v)	Exposure temperature (°C)	Exposure time (Min)	Probiotic counts before heat treatment (Log CFU/mL)	Probiotic counts after heat treatment (Log CFU/mL)	Survivability rate (%)
0	37 (Control)	1	10.12±0.02^{a}	10.05±0.08^{a}	99.30±0.05^{a}
		5	10.04±0.15^{a}	9.96±0.43^{a}	99.20±0.01^{a}
	55	1	10.24±0.11^{a}	9.45±0.22^{a}	92.28±0.05^{a}
		5	10.11±0.12^{a}	9.31±0.27^{a}	92.08±0.03^{a}
	60	1	10.31±0.14^{a}	9.48±0.15^{a}	91.94±0.04^{a}
		5	10.29±0.22^{a}	9.37±0.25^{a}	91.05±0.10^{a}
	65	1	10.22±0.89^{a}	8.90±0.51^{b}	87.08±0.09^{b}
		5	10.12±0.12^{a}	8.59±0.10^{b}	84.88±0.06^{b}
0.5	37 (Control)	1	10.27±0.36^{a}	10.20±0.12^{a}	99.32±0.04^{a}
		5	10.19±0.41^{a}	10.11±0.21^{a}	99.21±0.02^{a}
	55	1	10.29±0.28^{a}	9.51±0.31^{a}	92.41±0.01^{a}
		5	10.19±0.22^{a}	9.39±0.06^{a}	92.14±0.03^{a}
	60	1	10.09±0.18^{a}	9.28±0.15	91.97±0.07^{a}
		5	10.00±0.30^{a}	9.10±0.16^{a}	91.00±0.06^{a}
	65	1	10.28±0.45^{a}	9.00±0.10^{b}	87.54±0.01^{b}
		5	10.20±0.21^{a}	8.66±0.17^{c}	84.90±0.09^{a}
2.5	37 (Control)	1	10.51±0.17^{a}	10.44±0.12^{a}	99.33±0.04^{a}
		5	10.41±0.01^{a}	10.33±0.53^{a}	99.23±0.02^{a}
	55	**1**	**10.20±0.18^{a}**	**9.43±0.11^{a}**	**92.45±0.07^{a}**
		5	**10.12±0.22^{a}**	**9.33±0.53^{a}**	**92.19±0.06^{a}**
	60	1	10.20±0.24^{a}	9.39±0.09^{a}	92.05±0.07^{a}
		5	10.16±0.27^{a}	9.25±0.22^{a}	91.04±0.04^{a}
	65	1	10.15±0.11^{a}	8.88±0.11^{b}	87.68±0.03^{b}
		5	10.09±0.09^{a}	8.67±0.27^{b}	85.92±0.01^{c}

5.0	37 (Control)	1	10.09±0.21[a]	10.03±0.20[a]	99.41±0.09[a]
		5	10.06±0.47[a]	10.02±0.31[a]	99.30±0.03[a]
	55	1	10.23±0.32[a]	9.46±0.27[a]	92.47±0.07[a]
		5	10.19±055[a]	9.39±0.29[a]	92.14±0.06[a]
	60	1	10.22±0.62[a]	9.40±0.30[a]	91.97±0.01[a]
		5	10.18±0.18[a]	9.27±0.30[a]	91.06±0.04[a]
	65	1	10.21±0.20[a]	8.94±0.17[b]	87.70±0.03[b]
		5	10.17±0.17[a]	8.75±0.01[c]	86.03±0.06[b]
7.5	37 (Control)	1	10.24±0.19[a]	10.19±0.36[a]	99.51±0.01[a]
		5	10.11±0.25[a]	9.37±0.16[a]	99.41±0.09[a]
	55	1	10.28±0.66[a]	9.51±0.07[a]	92.50±0.01[a]
		5	10.25±0.36[a]	9.44±0.35[a]	92.09±0.04[a]
	60	1	10.23±0.41[a]	9.41±0.07[a]	91.98±0.03[a]
		5	10.15±0.06[a]	9.25±0.84[a]	91.13±0.07[a]
	65	1	10.28±0.17[a]	9.10±0.43[b]	88.52±0.06[b]
		5	10.14±0.28[a]	8.88±0.01[c]	86.88±0.05[b]
10	37 (Control)	1	10.24±0.35[a]	9.55±0.47[a]	93.26±0.08[a]
		5	10.12±0.04[a]	9.39±0.05[a]	93.14±0.09[a]
	55	1	10.19±0.30[a]	9.42±0.25[a]	92.44±0.01[a]
		5	10.11±0.78[a]	9.33±0.01[a]	92.28±0.07[a]
	60	1	10.09±0.01[a]	9.28±0.06[a]	91.97±0.03[a]
		5	10.06±0.63[a]	9.18±0.22[a]	91.25±0.09[a]
	65	1	10.19±0.15[a]	9.04±0.01[b]	88.71±0.02[b]
		5	10.14±0.22[a]	8.80±0.37[c]	86.78±0.03[b]

Data are represented as Mean ± SE; (p<0.05); n=3.

a-c (along the column) values with different superscripts are significantly different from each other.

Table 4.2 (d): Effect of maltodextrin on heat tolerance of probiotic *Lactiplantibacillus plantarum* HD48.

Maltodextrin concentration (% w/v)	Exposure temperature (°C)	Exposure time (Min)	Probiotic counts before heat treatment (Log CFU/mL	Probiotic counts after heat treatment (Log CFU/mL)	Survivability rate (%)
0	37 (Control)	1	10.15±0.22[a]	9.53±0.17[a]	93.47±0.09[a]
		5	10.04±0.14[a]	9.39±0.44[a]	93.32±0.05[a]
	55	1	10.24±0.32[a]	9.49±0.19[a]	92.67±0.02[a]
		5	10.11±0.22[a]	9.31±0.38[a]	92.08±0.03[a]
	60	1	10.31±0.16[a]	9.48±0.22[a]	91.94±0.01[a]
		5	10.29±0.32[a]	9.38±0.20[a]	91.15±0.03[a]
	65	1	10.22±0.29[a]	8.97±0.05[b]	87.76±0.04[b]
		5	10.12±0.43[a]	8.70±0.10[b]	85.96±0.05[b]
0.5	37 (Control)	1	10.27±0.18[a]	9.60±0.20[a]	93.58±0.08[a]
		5	10.19±0.65[a]	9.39±0.43[a]	93.39±0.09[a]
	55	1	10.29±0.20[a]	9.59±0.37[a]	93.19±0.04[a]
		5	10.19±0.05[a]	9.42±0.79[a]	92.44±0.03[a]
	60	1	10.10±0.26[a]	9.36±0.62[a]	92.67±0.02[a]
		5	10.09±0.22[a]	9.28±0.10[a]	91.57±0.01[a]
	65	1	10.11±0.32[a]	8.92±0.05[b]	88.22±0.02[b]
		5	10.08±0.15[a]	8.67±0.20[b]	86.01±0.05[b]
2.5	37 (Control)	1	10.25±0.30[a]	9.62±0.11[a]	93.74±0.06[a]
		5	10.20±0.14[a]	9.57±0.26[a]	93.59±0.04[a]
	55	**1**	**10.19±0.22[a]**	**9.60±0.32[a]**	**93.21±0.02[a]**
		5	**1.012±0.19[a]**	**9.49±0.67[a]**	**92.57±0.01[a]**
	60	1	10.20±0.22[a]	9.51±0.56[a]	92.70±0.03[a]
		5	10.15±0.25[a]	9.38±0.50[a]	91.68±0.05[a]
	65	1	10.15±0.20[a]	9.15±0.70[a]	90.14±0.09[b]
		5	10.09±0.36[a]	9.05±0.62[a]	89.69±0.07[c]

5.0	37 (Control)	1	10.09±0.33[a]	9.48±0.27[a]	93.80±0.02[a]
		5	10.06±0.27[a]	9.39±0.15[a]	93.63±0.03[a]
	55	1	10.19±0.19[a]	9.53±0.23[a]	93.52±0.01[a]
		5	10.15±0.20[a]	9.46±0.10[a]	92.65±0.06[a]
	60	1	10.16±0.25[a]	9.47±0.15[a]	92.80±0.04[a]
		5	10.13±0.45[a]	9.41±0.64[a]	91.89±0.02[a]
	65	1	10.14±0.36[a]	9.35±0.88[a]	90.29±0.03[a]
		5	10.10±0.38[a]	9.08±0.10[b]	89.80±0.05[b]
7.5	37 (Control)	1	10.16±0.22[a]	9.55±0.22[a]	93.89±0.05[a]
		5	10.12±0.18[a]	9.46±0.06[a]	93.70±0.01[a]
	55	1	10.22±0.24[a]	9.61±0.33[a]	93.63±0.09[a]
		5	10.09±0.16[a]	9.46±4.93[a]	92.75±0.06[a]
	60	1	10.24±0.21[a]	9.57±0.35[a]	92.90±007[a]
		5	10.11±0.31[a]	9.39±0.40[a]	91.95±0.03[a]
	65	1	10.26±0.24[a]	9.46±5.08[a]	90.46±0.04[a]
		5	10.06±0.26[a]	9.03±0.90[b]	89.94±0.03[b]
10	37 (Control)	1	10.11±0.29[a]	9.50±0.30[a]	93.96±0.01[a]
		5	10.06±0.11[a]	9.44±0.20[a]	93.87±0.03[a]
	55	1	10.23±0.16[a]	9.63±0.40[a]	93.80±0.08[a]
		5	10.16±0.04[a]	9.53±0.23[a]	92.90±0.04[a]
	60	1	10.24±0.18[a]	9.57±0.52[a]	92.98±0.06[a]
		5	10.16±0.14[a]	9.44±0.28[a]	92.00±0.07[a]
	65	1	10.22±0.28[a]	9.42±0.10[a]	91.17±0.03[a]
		5	10.18±0.22[a]	9.16±0.22[b]	89.99±0.07[b]

Data are represented as Mean ± SE; (p<0.05); n=3.

a-b (along the column) values with different superscripts are significantly different from each other.

4.2.5 EFFECT OF LACTOSE ON HEAT TOLERANCE OF PROBIOTIC *LACTIPLANTIBACILLUS PLANTARUM* HD48

Results of viable counts and percent survival rate of *Lp. plantarum* in presence of lactose as protectant have been presented in **Table 4.2 (e)**. In heat challenge experiment of *Lp. planatum* HD48, viable counts of the selected strains varied from 8.68±0.10 to 9.49±0.68 log CFU/ mL. After heat treatment temperatures of 55, 60 and 65 ^{o}C at varied exposure time 1 and 5 min at different concentrations of lactose. Minimal unit reduction have been observed in viability of probiotic culture of *Lp. plantarum* CRD7 (0.88 Log CFU/ mL) during the heat treatment at elevated temperatures of 55, 60 and 65 ^{o}C. The maximum viable counts have been observed of *Lp. plantarum* CRD7 (9.40±0.25 Log CFU/mL) with highest survivability rate of 90.88±0.30 percent when lactose @5 percent (w/v) was used as protectant at 55 ^{o}C at exposure time of 1 min. However, least difference in the survivable rate was observed with increased concentrations of maltodextrin up to 10% at varied heat treatment temperatures i.e. 55, 60. 65^{o}C, thus, lactose @5% (w/v) could be used for the production of probiotic DVS starters the survival rate of 88.74±0.03 and 86.62±0.08 % at 65 ^{o}C was documented.

4.2.6 EFFECT OF SORBITOL ON HEAT TOLERANCE OF PROBIOTIC *LACTIPLANTIBACILLUS PLANTARUM* HD48

Data of viable counts and percent survival rate of *Lp. planatrum* HD48 in presence of varied conc. of sorbitol have been presented in **Table 4.2 (f)**. In heat challenge experiment of *Lp. planatum* HD48, viable counts of the selected strains varied from 8.53±0.08 to 9.39±0.38 log CFU/ mL, after heat treatment temperatures of 55, 60, 65 ^{o}C at varied exposure time 1 and 5 min at different concentrations (0, 0.5, 2.5, 5.0, 7.5 and 10% w/v) of sorbitol. Minimal unit reduction have been observed in viability of probiotic culture of *Lp. plantarum* HD48 (0.72 Log CFU/ mL) during the heat treatment at elevated temperatures of 55, 60 and 65 ^{o}C. The maximum viable counts were observed of *Lp. plantarum* HD48 (9.26±0.04 Log CFU/mL) with highest survivability rate of 91.32±0.07 percent when sorbiotol @2.5 percent (w/v) was used as protectant at 55 ^{o}C, whereas survival rate of 90.27±0.07 and 88.15±0.01% at 65 ^{o}C was documented. However, least difference in the survivable rate was observed with increased concentrations of sorbitol up to 10% at varied heat treatment temperatures thus sorbitol @2.5% could be used.

Table 4.2 (e): Effect of lactose on heat tolerance of probiotic *Lactiplantibacillus plantarum* HD48.

Lactose concentration (% w/v)	Exposure temperature (°C)	Exposure time (Min)	Probiotic counts before heat treatment (Log CFU/mL)	Probiotic counts after heat treatment (Log CFU/mL)	Survivability rate (%)
0	37	1	10.04±0.02[a]	9.14±0.17[a]	91.03±0.04[a]
		5	10.12±0.01[a]	9.06±0.44[a]	90.82±0.08[b]
	55	1	10.24±0.09[a]	9.23±0.19[a]	90.53±0.12[a]
		5	10.11±0.08[a]	9.09±0.38[a]	89.91±0.09[b]
	60	1	10.29±0.10[a]	9.17±0.22[a]	89.68±0.02[b]
		5	10.31±0.10[a]	9.08±0.21[a]	88.06±0.02[c]
	65	1	10.12±0.12[a]	8.81±0.05[b]	87.05±0.03[c]
		5	10.22±0.22[a]	8.68±0.10[c]	84.93±0.01[d]
0.5	37	1	10.19±0.08[a]	9.33±0.29[a]	91.56±0.05[a]
		5	10.27±0.04[a]	9.25±0.45[a]	91.36±0.02[a]
	55	1	10.29±0.02[a]	9.38±0.18[a]	90.75±0.12[a]
		5	10.19±0.04[a]	9.16±0.12[a]	89.98±0.08[b]
	60	1	10.00±0.02[a]	9.07±0.35[b]	89.74±0.04[a]
		5	10.09±0.03[a]	9.09±0.60[a]	88.40±0.02[b]
	65	1	10.04±0.09[a]	9.02±0.40[a]	88.04±0.04[b]
		5	10.44±0.02[a]	8.88±0.20[b]	85.05±0.10[c]
2.5	37	1	10.51±0.09[a]	9.67±0.21[a]	91.79±0.02[a]
		5	10.41±0.02[a]	9.56±0.24[a]	91.55±0.04[a]
	55	1	10.39±0.01[a]	9.49±0.68[a]	90.80±0.02[a]
		5	10.12±0.08[a]	9.21±0.51[a]	89.99±0.08[a]
	60	1	10.15±0.04[a]	9.20±0.13[a]	89.83±0.03[a]
		5	10.20±0.02[a]	9.17±0.23[a]	88.50±0.08[b]
	65	1	10.09±0.11[a]	9.08±0.10[a]	88.24±0.10[b]
		5	10.15±0.22[a]	8.75±0.41[b]	85.20±0.01[c]

5.0	37	1	10.06±0.28[a]	9.39±0.27[a]	91.95±0.02[a]
		5	10.09±0.02[a]	9.37±0.79[a]	91.76±0.08[a]
	55	**1**	**10.23±0.10[a]**	**9.40±0.25[a]**	**90.88±0.30[a]**
		5	**10.19±0.14[a]**	**9.29±0.30[a]**	**90.00±0.02[a]**
	60	1	10.06±0.32[a]	9.14±0.42[a]	89.85±0.02[b]
		5	10.22±0.02[a]	9.19±0.05[a]	88.54±0.04[c]
	65	1	10.18±0.44[a]	9.17±0.85[a]	88.30±0.10[b]
		5	10.22±0.10[a]	8.81±0.22[b]	86.00±0.12[d]
7.5	37	1	10.24±0.04[a]	9.54±0.27[a]	91.99±0.12[a]
		5	10.11±0.02[a]	9.40±0.43[a]	91.77±0.01[a]
	55	1	10.28±0.04[a]	9.44±0.02[a]	90.92±0.08[a]
		5	10.25±0.09[a]	9.34±0.26[a]	90.12±0.04[a]
	60	1	10.15±0.02[a]	9.23±0.33[a]	89.94±0.02[a]
		5	10.23±0.10[a]	9.20±0.14[a]	88.62±0.09[b]
	65	1	10.14±0.08[a]	9.13±0.18[a]	88.64±0.02[a]
		5	10.28±0.08[a]	8.97±0.11[b]	86.55±0.05[c]
10.0	37	1	10.12±0.12[a]	9.52±0.76[a]	92.01±0.12[a]
		5	10.24±0.02[a]	9.45±0.25[a]	91.97±0.04[a]
	55	1	10.19±0.01[a]	9.36±0.65[a]	91.58±0.02[a]
		5	10.11±0.02[a]	9.25±0.81[a]	90.49±0.09[a]
	60	1	10.06±0.09[a]	9.15±0.72[a]	89.98±0.05[a]
		5	10.09±0.02[a]	9.09±0.81[a]	88.78±0.01[a]
	65	1	10.14±0.02[a]	9.15±0.52[a]	88.74±0.03[a]
		5	10.19±0.05[a]	8.98±0.50[b]	86.62±0.08[b]

Data are represented as Mean ± SE; (p<0.05); n=3.

a-c (along the column) values with different superscripts are significantly different from each other.

Table 4.2 (f). Heat tolerance effect of sorbitol on probiotic *Lactiplantibacillus plantarum* HD48.

Sorbitol concentration (% w/v)	Exposure temperature (°C)	Exposure time (Min)	Probiotic counts before heat treatment (Log CFU/mL)	Probiotic counts after heat treatment (Log CFU/mL)	Survivability rate (%)
0	37 (Control)	1	10.24±0.22^{a}	9.33±0.17^{a}	91.11±0.02^{a}
		5	10.18±0.18^{a}	9.22±0.44^{a}	89.88±0.01^{b}
	55	1	10.28±0.17^{a}	9.27±0.19^{a}	90.17±0.05^{a}
		5	10.16±0.12^{a}	9.13±0.38^{a}	89.86±0.09^{b}
	60	1	10.24±0.11^{a}	9.10±0.22^{a}	88.86±0.07^{c}
		5	10.19±0.26^{a}	9.00±0.21^{b}	88.32±0.08^{c}
	65	1	10.20±0.32^{a}	8.88±0.05^{c}	87.05±0.03^{d}
		5	10.18±0.29^{a}	8.65±0.10^{a}	84.97±0.01^{e}
0.5	37 (Control)	1	10.18±0.14^{a}	9.37±0.16^{a}	91.55±0.04^{a}
		5	10.16±0.25^{a}	9.25±0.05^{a}	91.24±0.05^{a}
	55	1	10.11±0.26^{a}	9.32±0.08^{a}	91.19±0.06^{a}
		5	10.08±0.14^{a}	9.07±0.10^{a}	89.98±0.01^{b}
	60	1	10.22±0.28^{a}	9.21±0.13^{a}	90.11±0.05^{a}
		5	10.16±0.00^{a}	9.12±0.29^{a}	89.76±0.06^{b}
	65	1	10.10 ±0.32^{a}	9.07±0.06^{a}	89.80±0.04^{b}
		5	10.03±0.29^{a}	8.53±0.08^{b}	85.04±0.01^{c}
2.5	37 (Control)	1	10.21±0.18^{a}	9.40±0.16^{a}	92.06±0.02^{a}
		5	10.17±0.32^{a}	9.34±0.29^{a}	91.93±0.03^{a}
	55	**1**	**10.14±0.28^{a}**	**9.26±0.04^{a}**	**91.32±0.07^{a}**
		5	**10.10±0.19^{a}**	**9.20±0.26^{a}**	**91.08±0.06^{a}**
	60	1	10.21±0.14^{a}	9.26±0.15^{a}	90.69±0.04^{b}
		5	10.17±0.22^{a}	9.15±0.15^{a}	89.97±0.03^{c}
	65	1	10.21±0.21^{a}	9.18±0.20^{a}	89.91±0.04^{c}
		5	10.14±0.16^{a}	8.75±0.17^{b}	86.29±0.05^{d}

5.0	37 (Control)	1	10.21±0.21[a]	9.53±0.04[a]	93.33±0.06[a]
		5	10.17±0.12[a]	9.44±0.15[a]	93.01±0.07[a]
	55	1	10.13±0.17[a]	9.31±0.08[a]	91.90±0.09[a]
		5	10.08±0.45[a]	9.19±0.07[a]	91.17±0.07[a]
	60	1	10.19±0.41[a]	9.26±0.18[a]	90.87±0.00[b]
		5	10.17±0.38[a]	9.15±0.06[a]	89.99±0.01[c]
	65	1	10.11±0.33[a]	9.11±0.05[a]	90.10±0.02[b]
		5	10.07±0.27[a]	8.69±0.28[b]	86.89±0.03[d]
7.5	37 (Control)	1	10.24±0.22[a]	9.54±0.29[a]	93.46±0.05[a]
		5	10.11±0.26[a]	9.40±0.27[a]	93.20±0.01[a]
	55	1	10.22±0.33[a]	9.39±0.38[a]	91.95±0.02[a]
		5	10.19±0.21[a]	9.29±0.23[a]	91.29±0.04[a]
	60	1	10.20±0.17[a]	9.28±0.50[a]	90.98±0.09[b]
		5	10.17±0.13[a]	9.15±0.03[b]	90.00±0.07[c]
	65	1	10.18±0.21[a]	9.17±0.06[b]	90.15±0.05[b]
		5	10.13±0.36[a]	8.84±0.03[b]	87.26±0.01[d]
10.0	37 (Control)	1	10.24±0.33[a]	9.54±0.45[a]	93.56±0.05[a]
		5	10.21±0.37[a]	9.49±0.50[b]	93.37±0.04[a]
	55	1	10.19±0.31[a]	9.36±0.34[b]	92.00±0.03[a]
		5	10.11±0.26[a]	9.25±0.48[b]	91.49±0.06[a]
	60	1	10.09±0.14[a]	9.18±0.38[b]	91.02±0.09[b]
		5	10.04±0.19[a]	9.05±0.10[b]	90.43±0.08[b]
	65	1	10.08±0.15[a]	9.10±0.11[b]	90.27±0.07[b]
		5	10.05±0.11[a]	8.86±0.19[c]	88.15±0.01[c]

Data are represented as Mean ± SE; (p<0.05); n=3.

a-b (along the column) values with different superscripts are significantly different from each other.

4.3 : EFFECT OF INLET TEMPERATURE ON QUALITY PARAMETER OF SPRAY DRIED PROBIOTIC *LACTIPLANTIBACILLUS PLANTARUM* CRD7.

The moisture content (w) and water activity (a_w) of spray dried powders was directly found well corresponded with their sorption data. In addition, it may be observed that in the case of the powders with inulin, polydextrose and trehalose, their moisture content (w:1.4–2.8 % db) and water activity (a_w: 0.13–0.20) were at a similar level in respect of the obtained values of monolayer coverage. Different observations were made for the powders with Nutriose whose moisture content (w: 1.7–2.0 % db) and water activity (a_w: ~0.047) were significantly lower from the value of the monolayer coverage. a_w ranges and provides the goodness of fit of this model expressed by the coefficient of determination (r^2) and mean square error (RMSE). Assuming the values of RMSE at a level lower than 10% as a good fit of the model to sorption data, it needs to be concluded that this assumption was met by the GAB model in both ranges of a_w. (Boquet *et al.*, 1978, Lomauro *et al.,* 1985).

The water activity (a_w) ranged from 0.25±0.0 to 0.36±0.01 for the above spray dried longer shelf life of DVS starters it is noticed that as per (Domian *et al*., 2017) the water activity range from 0.05 to 0.30 for longer shelf life DVS powder it is in the acceptable range. The coliform counts not detected per Log CFU/mL. The yeast and mould counts are not detected per Log CFU/mL. Probiotic counts for all the three spray dried DVS of *Lp. plantarum* CRD7 were found as per the requirement of FAO/WHO 2002. Probiotic *dahi* prepared with these cultures also exhibited all desired techno-functional attributes w.r.t. curd setting time, titratable acidity and pH. The coliforms and yeast and mould counts, were found <1 Log CFU/mL indicative of good hygienic practices adapted during probiotic *dahi* prepared. The lowest water activity of all spray dried DVS starters of probiotic *Lactiplantibacillus plantarum* documented when sugars (lactose, maltodextrin and sorbitol) were used as cryoprotective agents.

Spray-dried probiotic powder was produced at pilot scale from 300 L of 20% (w/v) reconstituted skim milk containing a rifampicin resistant variant of the probiotic *Lactobacillus paracasei* NFBC 338. During powder manufacture, air inlet and outlet temperatures of 175^{o}C and 68 ^{o}C, respectively, were used, which yielded a probiotic survival of 84.5% as per the Gardiner *et al.,* 2002. The survivability of DVS powder produced by spray dried process at different temperature 170, 180 and 190^{o}C ranged

between 73.55±0.02 to 90.02±0.05 as mentioned in **Table 4.3 (a)** it is notice that survivability rate higher than *Lactobacillus paracasei* NFBC 338 observed that survivability depends on types of strains, varied types of protectants and different inlet and outlet temperature.

Table 4.3 (a): Effect of inlet temperature on quality parameter of spray dried probiotic *Lactiplantibacillus plantarum* CRD7 direct vat set starters.

Inlet temperature (°C)	Maltodextrin concentration (%)	Moisture Content (%)	Water activity (a_w)	Total probiotic count (Log CFU/mL or g)		Survivability rate (%)
				Probiotic counts before spray drying	Probiotic counts after spray drying	
170	0	3.57±0.06^{b}	0.35±0.01^{a}	11.54±0.01^{a}	9.10±0.07^{c}	79.98±0.00^{c}
	0.5	3.61±0.02^{a}	0.30±0.03^{b}	11.14±0.05^{d}	9.42±0.02^{b}	81.68±0.01^{b}
	2.5	3.63±0.05^{a}	0.25±0.01^{c}	11.28±0.00^{c}	10.12±0.03^{a}	89.71±0.05^{a}
	5.0	3.68±0.06^{a}	0.25±0.02^{c}	11.35±0.04^{b}	10.20±0.06^{a}	89.86±0.06^{a}
180	0	3.49±0.02^{c}	0.36±0.01^{a}	11.51±0.04^{a}	9.00±0.01^{c}	78.19±0.03^{c}
	0.5	3.58±0.04^{b}	0.35±0.04^{a}	11.12±0.08^{d}	9.59±0.04^{b}	86.24±0.00^{b}
	2.5	**3.61±0.05^{a}**	**0.27±0.02^{b}**	**11.25±0.12^{c}**	**10.15±0.05^{a}**	**90.02±0.05^{a}**
	5.0	3.65±0.08^{a}	0.25±0.01^{b}	11.33±0.03^{b}	10.19±0.12^{a}	89.93±0.07^{a}
190	0	3.47±0.01^{c}	0.32±0.04^{a}	11.42±0.00^{a}	8.40±0.03^{c}	73.55±0.02^{d}
	0.5	3.54±0.04^{b}	0.30±0.02^{a}	11.09±0.02^{d}	9.36±0.07^{b}	84.40±0.05^{c}
	2.5	3.59±0.02^{a}	0.29±0.01^{b}	11.22±0.05^{c}	9.87±0.08^{a}	87.96±0.08^{b}
	5.0	3.59±0.05^{a}	0.27±0.02^{b}	11.30±0.11^{b}	9.99±0.05^{a}	88.40±0.11^{a}

4.4 OPTIMIZATION OF INOCULUM LEVEL OF PROBIOTIC DVS OF *LACTIPLANTIBACILLUS PLANTARUM* CRD7 PREPARED WITH MALTODEXTRIN AT INLET TEMPERATURE OF 170°C BY SPRAY DRYING ON TECHNO-FUNCTIONAL, SENSORY AND TEXTURE ATTRIBUTES OF *DAHI*

4.4 (a) : TECHNO-FUNCTIONAL ATTRIBUTES

Data on techno-functional attributes of probiotic *dahi* prepared with DVS of *Lactiplantibacillus plantarum* CRD7 prepared with maltodextrin at varied levels by spray drying at inlet temperature of 170°C have been presented in **Table 4.4 (a)** and **Figure 4.2 (a).** Probiotic *dahi* was prepared with standardized milk, heated to 90°C/10min. Milk was cooled to 37°C and inoculated with different inoculum levels. This was incubated at 37°C and observed for curd setting, pH, titratable acidity and probiotic counts. Probiotic *dahi* was also analysed for sensory and textural attributes. The curd setting time of probiotic DVS of *Lp. Plantarum* CRD7 for the preparation of *dahi* ranged 9.10±0.03 -7.30±0.02 h, pH ranged between 4.18±0.09-4.30±0.02 and titratable acidity ranged (0.72±0.01-0.78±0.04 % LA) and total probiotic counts ranged from 8.69±0.08 to 9.35±0.02 (Log CFU/mL). These observations revealed their survibility to be used as probiotic DVS dairy starters. On basis of minimum curd setting time, faster reduction in pH and acidity development, and highest probiotic counts, inoculum level 0.004 % (w/v) of probiotic DVS was optimized for better techno-functional properties of probiotic *dahi*.

4.4 (b): SENSORY SCORES

Observations related to sensory parameters such as colour and appearance (10), flavour (10), body and texture (10) and overall acceptability scores of probiotic DVS prepared using probiotic *Lp plantarum* CRD7 with maltodextrin are mentioned in **Table 4.4 (b)** and **figure 4.2 (b)**.

The highest score for colour and appearance *i.e.,* 9.6±0.31 was obtained for probiotic DVS prepared with *Lp. plantarum* CRD7 at inoculum level of 0.004%. Significant difference in sensory scores was obtained for probiotic *dahi* prepared using probiotic *Lp plantarum* CRD7 DVS.

The highest score of 8.60±0.60 for flavour was obtained for DVS prepared using *Lp. plantarum* CRD7 by spray drying method at inlet temperature of 170°C. Significant

difference in the flavour score of probiotic DVS prepared using *Lp. plantarum* CRD7 was noticed. Weak and moderate diacetyl favour in DVS is more acceptable whereas, high concentration of diacetyl production is not acceptable by consumers as it may leads to off flavours. The formation of methyl aldehydes in milk by the metabolic activity of *L. lactis ssp. lactis* has been recognized as the cause of off-flavours in cheddar cheese (Urbach *et al*.,1997).

The maximum score for body and texture *i.e.,* 9.0±1.05 in probiotic *dahi* was recorded for DVS powder. There was significant difference in the scores obtained for probiotic *dahi* prepared with probiotic DVS of Lp plantarum CRD7 prepared with maltodextrin @2.5% (w/v) of inoculum level 0.004%.

The highest score for overall acceptability *i.e.,* 9.3±1.03 was obtained for probiotic *dahi* prepared from DVS powder at different inoculum levels. Significant difference in the scores was obtained for probiotic DVS powder.

4.4 (c) : TEXTURE PROFILES

Hardness or firmness, is the most important characteristics in determining texture of dairy products *vis-à-vis* fermented dairy foods. It is regarded as the force required to attain a certain deformation and is considered a measure of hardness of fermented milk products such as yoghurt. Results obtained on texture profile have been presented in **Table 4.4 (c)** and **figure 4.2 (c)**. Texture parameters such as firmness (N), consistency (N.s), cohesiveness (N) and work of cohesion (N.s) of probiotic DVS prepared by probiotic DVS *Lactiplantibacillus plantarum* CRD7 at different inoculum levels. The highest value for firmness (2.95±0.08 N) was obtained for probiotic *dahi* prepared with DVS manufactured with maltodextrin concentration @2.5% (w/v) at 0.004% (w/v) inoculum level of DVS. Significant differences in the firmness of probiotic *dahi* prepared with DVS were noticed different at varied inoculum levels of probiotic DVS powder prepared with at varied level of maltodextrin.

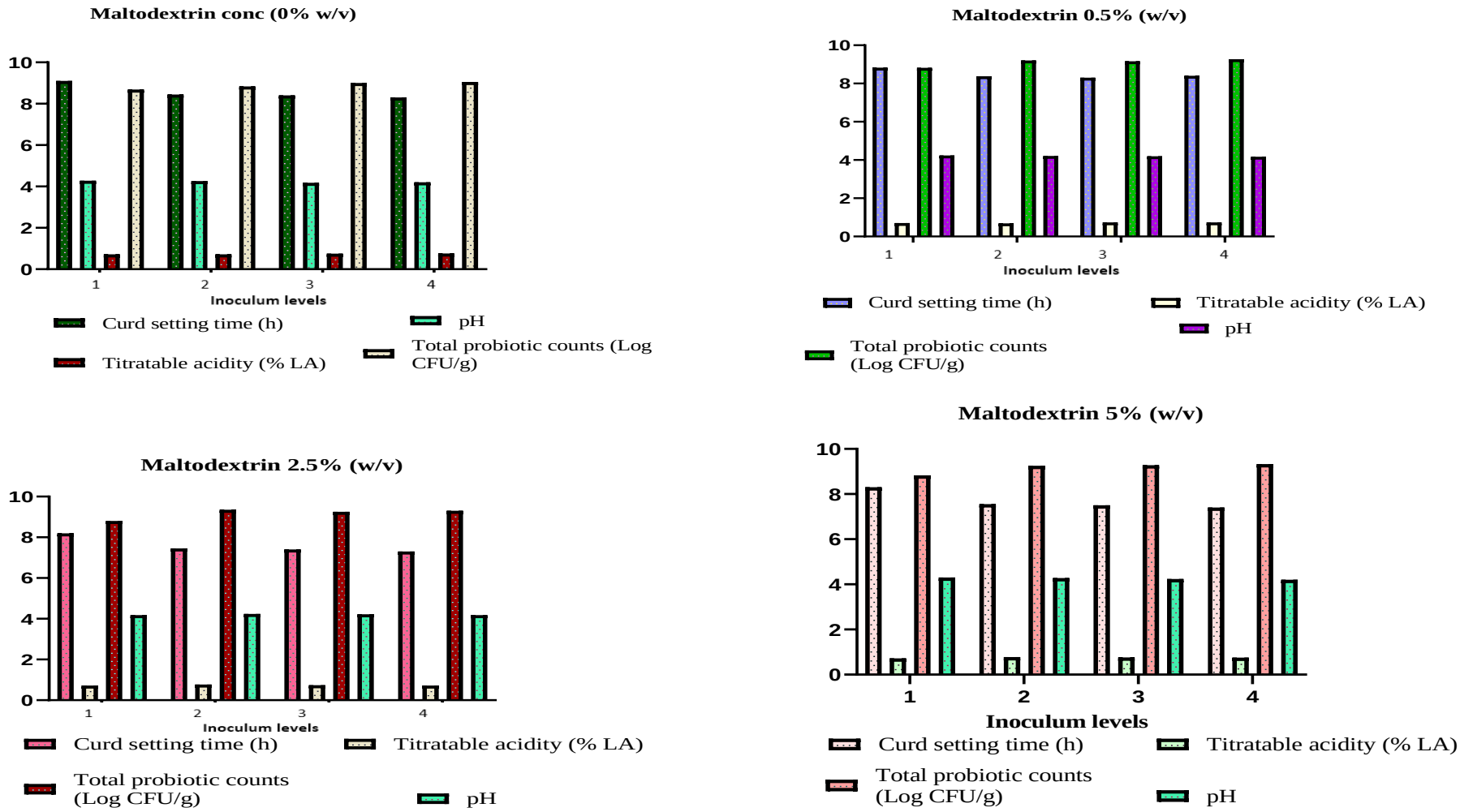
Table 4.4 (a) : Effect of inoculum levels of probiotic DVS of *Lactiplantibacillus plantarum* CRD7 prepared at 170℃ on techno-functional attributes of *dahi*.

Maltodextrin concentration (%w/v)	Inoculum levels of probiotic DVS powder (%w/v)*	Curd setting time (h)	pH	Titratable acidity (% LA)	Total probiotic counts (Log CFU/mL)
0	0.002	9.10±0.03^{a}	4.28±0.02^{a}	0.72±0.01^{b}	8.69±0.08^{a}
	0.004	8.45±0.02^{b}	4.26±0.01^{a}	0.72±0.02^{b}	8.84±0.01^{b}
	0.006	8.40±0.01^{b}	4.18±0.04^{b}	0.75±0.02^{a}	9.00±0.04^{c}
	0.010	8.30±0.02^{c}	4.20±0.01^{a}	0.76±0.06^{a}	9.05±0.03^{d}
0.5	0.002	8.10±0.07^{a}	4.26±0.02^{a}	0.72±0.08^{b}	8.76±0.02^{a}
	0.004	7.45±0.02^{b}	4.22±0.03^{a}	0.72±0.05^{b}	9.17±0.01^{b}
	0.006	7.40±0.03^{c}	4.20±0.04^{a}	0.75±0.01^{a}	9.21±0.04^{c}
	0.010	7.30±0.03^{d}	4.18±0.09^{a}	0.74±0.03^{a}	9.27±0.02^{d}
2.5	0.002	8.20±0.05^{a}	4.18±0.08^{a}	0.72±0.01^{c}	8.80±0.04^{a}
	0.004	**7.45±0.03^{b}**	**4.24±0.02^{a}**	**0.77±0.02^{a}**	**9.35±0.01^{b}**
	0.006	7.40±0.01^{c}	4.22±0.01^{a}	0.74±0.02^{b}	9.25±0.02^{c}
	0.010	7.30±0.01^{d}	4.18±0.02^{a}	0.72±0.01^{c}	9.30±0.03^{d}
5.0	0.002	8.30±0.09^{a}	4.30±0.02^{a}	0.72±0.05^{c}	8.83±0.01^{a}
	0.004	7.55±0.05^{b}	4.28±0.04^{a}	0.78±0.04^{a}	9.25±0.05^{b}
	0.006	7.50±0.04^{c}	4.24±0.01^{a}	0.76±0.02^{a}	9.28±0.01^{c}
	0.010	7.40±0.02^{d}	4.20±0.06^{a}	0.75±0.01^{b}	9.33±0.02^{d}

Data are represented as Mean ± SE; (p<0.05); n=3.

a-c (along the column) values with different superscripts are significantly different from each others.

***** Spray dried probiotic DVS of *Lp. plantarum* CRD7 prepared with maltodextrin at inlet temperature of 170˚C.



Inoculum Levels : 1=0.002%; 2 = 0.004%; 3 = 0.006%; 4 = 0.010%

Figure 4.2 (a) : Effect of inoculum levels of probiotic DVS of *Lactiplantibacillus plantarum* CRD7 prepared at 170℃ on techno-functional attributes of *dahi*.

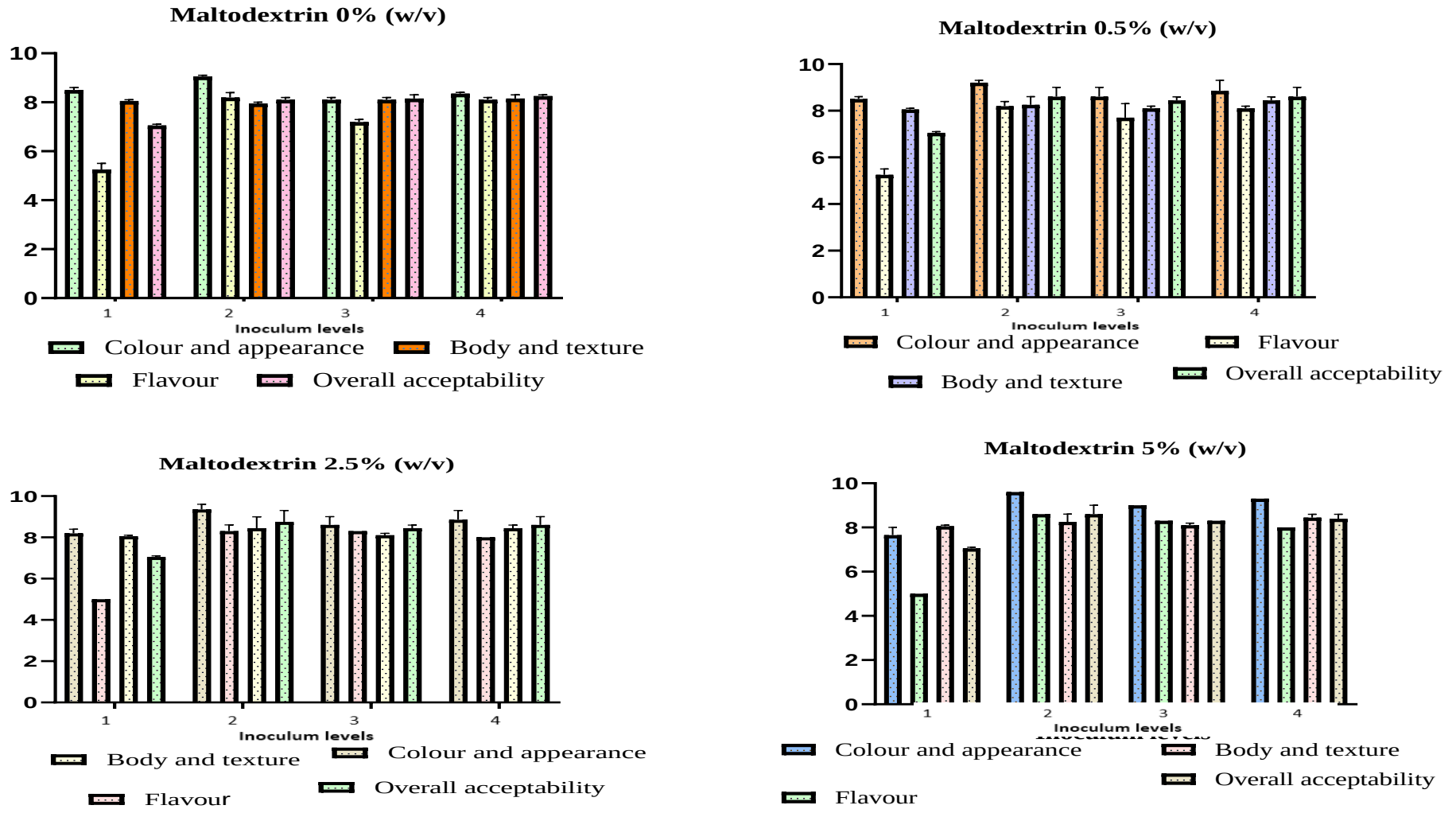
Table 4.4 (b): Effect of inoculum levels of probiotic DVS of *Lactiplantibacillus plantarum* CRD7 prepared at 170℃ on sensory attributes of *dahi*.

Maltodextrin concentration (% w/v)	Inoculum levels of probiotic DVS powder (%w/v)*	Colour and appearance	Flavour	Body and texture	Overall acceptability
0	0.002	8.6±0.28^{b}	5.0±0.33^{c}	8.0±0.51^{a}	7.0±0.78^{c}
	0.004	9.0±0.12^{a}	8.0±0.45^{a}	8.0±0.36^{a}	8.0±1.05^{b}
	0.006	8.0±0.42^{d}	7.3±0.39^{b}	8.0±0.42^{a}	8.0±0.85^{b}
	0.010	8.3±0.53^{c}	8.0±0.31^{a}	8.0±0.38^{a}	8.3±0.31^{a}
0.5	0.002	8.6±0.50^{b}	5.0±0.29^{b}	8.0±0.61^{b}	7.0±0.49^{c}
	0.004	9.3±0.25^{c}	8.0±0.25^{a}	8.6±0.49^{a}	9.0±0.56^{a}
	0.006	9.0±0.65^{a}	8.3±0.43^{a}	8.0±0.43^{b}	8.6±0.48^{b}
	0.010	9.3±0.36^{c}	8.0±0.28^{a}	8.6±0.15^{a}	9.0±0.41^{a}
2.5	0.002	8.0±0.40^{d}	5.0±0.41^{d}	8.0±0.47^{c}	7.0±0.38^{d}
	0.004	**9.6±0.31^{a}**	**8.6±0.60^{a}**	**9.0±1.05^{a}**	**9.3±1.03^{a}**
	0.006	9.0±0.56^{c}	8.3±0.33^{b}	8.0±0.36^{c}	8.6±0.85^{c}
	0.010	9.3±0.29^{b}	8.0±0.58^{c}	8.6±0.45^{b}	9.0±0.30^{b}
5.0	0.002	7.3±0.36^{d}	5.0±0.47^{d}	8.0±0.25^{b}	7.0±0.41^{d}
	0.004	9.6±0.45^{a}	8.6±0.43^{a}	8.6±0.48^{a}	9.0±0.49^{a}
	0.006	9.0±0.31^{c}	8.3±0.33^{b}	8.0±0.22^{b}	8.3±0.48^{c}
	0.010	9.3±0.29^{b}	8.0±0.42^{c}	8.6±0.31^{a}	8.6±0.36^{b}

Data are represented as Mean ± SE; (p<0.05); n=3.

a-b (along the column) values with different superscripts are significantly different from each others.

*****Spray dried probiotic DVS of *Lp. plantarum* CRD7 prepared with maltodextrin at inlet temperature 170°C.



Inoculum Levels : 1=0.002%; 2 = 0.004%; 3 = 0.006%; 4 = 0.010%

Figure 4.2 (b): Effect of inoculum levels of probiotic DVS of *Lactiplantibacillus plantarum* CRD7 prepared at 170℃on sensory attributes of *dahi*.

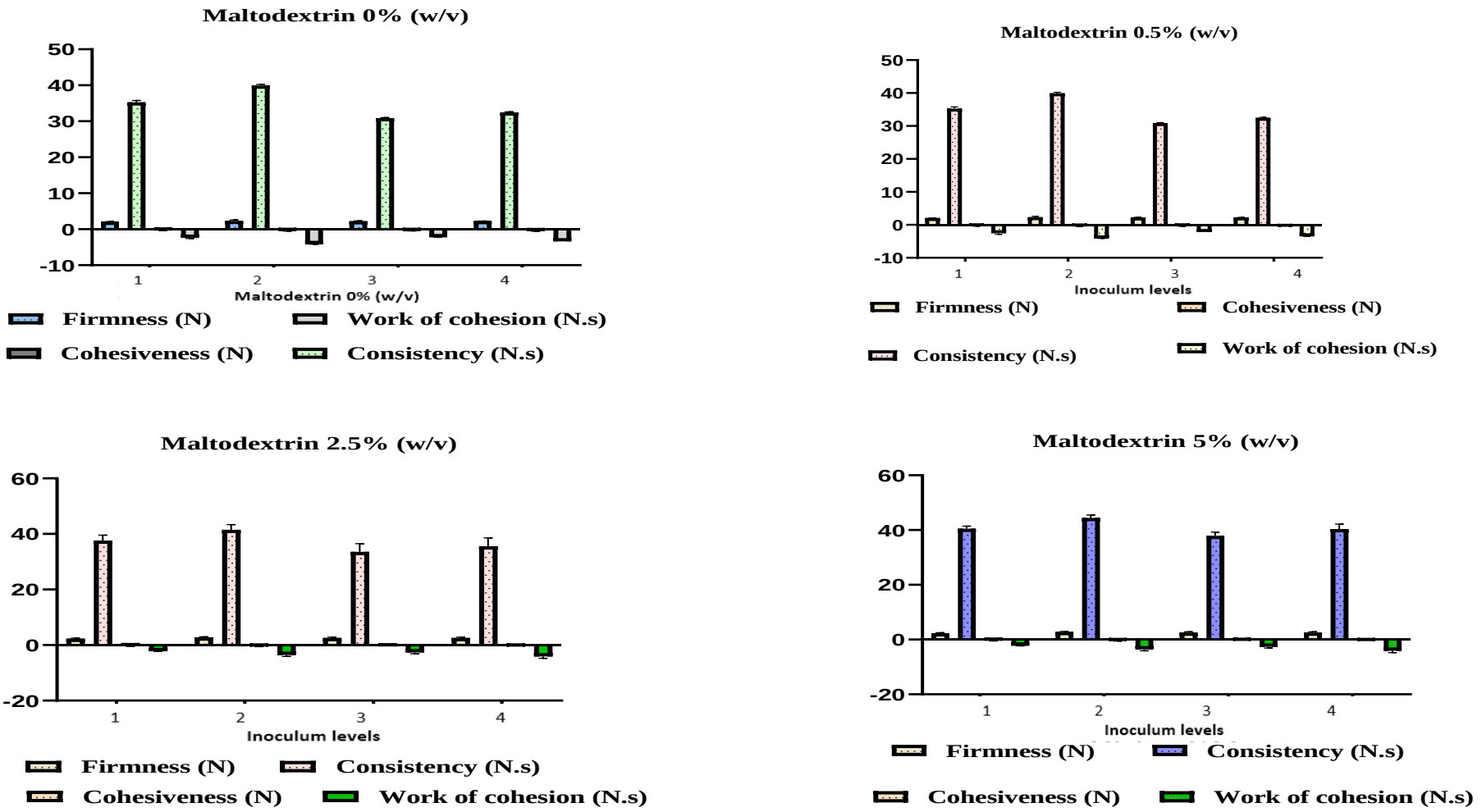
Table 4.4 (c): Effect of inoculum levels of probiotic DVS of *Lactiplantibacillus plantarum* CRD7 prepared at 170℃ on texture profile of *dahi*.

Maltodextrin concentration (% w/v)	Inoculum levels of probiotic DVS powder (%w/v)*	Firmness (N)	Consistency (N.s)	Cohesiveness (N)	Work of cohesion (N.s)
0	0.002	2.00±0.04^{c}	35.76±0.25^{b}	-0.38±0.01^{d}	-2.57±0.28^{c}
	0.004	2.14±0.10^{b}	39.62±0.49^{a}	-0.47±0.03^{b}	-4.18±0.14^{a}
	0.006	2.17±0.09^{b}	30.70±0.25^{d}	-0.44±0.02^{c}	-2.24±0.32^{d}
	0.010	2.22±0.02^{a}	32.64±0.86^{c}	-0.50±0.02^{a}	-3.38±0.18^{b}
0.5	0.002	2.14±0.04^{c}	32.76±0.75^{c}	-0.41±0.01^{d}	-2.20±0.28^{c}
	0.004	2.55±0.02^{a}	38.62±0.36^{a}	-0.51±0.01^{b}	-4.08±0.62^{a}
	0.006	2.30±0.01^{b}	31.75±0.49^{c}	-0.49±0.01^{c}	-2.18±0.14^{c}
	0.010	2.29±0.02^{b}	33.61±0.31^{b}	-0.55±0.05^{a}	-3.40±0.26^{b}
2.5	0.002	2.50±0.02^{c}	39.56±0.38^{b}	-0.49±0.03^{c}	-2.27±0.14^{c}
	0.004	**2.95±0.08^{a}**	**43.37±0.48^{a}**	**-0.57±0.02^{a}**	**-3.08±0.18^{b}**
	0.006	2.80±0.10^{b}	36.48±0.46^{c}	-0.51±0.01^{b}	-3.12±0.38^{b}
	0.010	2.80±0.08^{b}	38.55±0.25^{b}	-0.58±0.01^{a}	-4.76±0.14^{a}
5.0	0.002	2.60±0.04^{c}	41.46±0.25^{b}	-0.53±0.03^{b}	-2.49±0.14^{c}
	0.004	2.95±0.02^{a}	45.48±0.49^{a}	-0.59±0.01^{a}	-3.40±0.18^{b}
	0.006	2.90±0.01^{b}	39.26±0.86^{c}	-0.52±0.02^{b}	-3.15±0.18^{b}
	0.010	2.90±0.02^{b}	42.14±0.25^{b}	-0.61±0.05^{a}	-4.76±0.26^{a}

Data are represented as Mean ± SE; (p<0.05); n=3.

a-c (along the column) values with different superscripts are significantly different from each others.

***** Spray dried probiotic DVS of *Lp. plantarum* CRD7 prepared with maltodextrin at inlet temperature of 170°C.



Inoculum Levels : 1=0.002%; 2 = 0.004%; 3 = 0.006%; 4 = 0.010%

Figure 4.2 (c): Effect of inoculum levels of probiotic DVS of *Lactiplantibacillus plantarum* CRD7 prepared at 170℃ on texture profile of *dahi*.

4.5 OPTIMIZATION OF INOCULUM LEVELS OF PROBIOTIC DVS OF *LACTIPLANTIBACILLUS PLANTARUM* CRD7 PREPARED WITH MALTODEXTRIN AT INLET TEMPERATURE OF 180°C BY SPRAY DRYING ON TECHNO-FUNCTIONAL, SENSORY AND TEXTURE ATTRIBUTES OF *DAHI.*

4.5 (a) : TECHNO-FUNCTIONAL ATTRIBUTES

Data on techno-functional attributes of probiotic *dahi* prepared with DVS of *Lp plantarum CRD7* prepared with maltodextrin at different concentration by spray drying at inlet temperature 180°C have been presented in **Table 4.5 (a)** and **figure 4.3 (a)**. Probiotic *dahi* was prepared with standardized milk, heated to 90°C/10min. Milk was cooled to 37°C and inoculated with different inoculum levels. This was incubated at 37°C and observed for curd setting, pH, titratable acidity and probiotic counts. Probiotic *dahi* was also analysed for sensory and textural attributes. The curd setting time taken by DVS *Lp. plantarum* CRD7 for the preparation of *dahi* ranged 7.45±0.05-8.10±0.01 h, pH and titratable acidity ranged between 4.14±0.04-4.28±0.01 and 0.71±0.02-0.75±0.02 (% LA), respectively and total probiotic counts ranged from 8.70±0.04 to 9.28±0.02 (Log CFU/mL). On basis of minimum curd setting time, faster reduction in pH and acidity development, and highest probiotic counts, inoculum level 0.002% (w/v) of probiotic DVS was optimized for better techno-functional properties of probiotic *dahi*.

4.5 (b): SENSORY SCORES

Observations w.r.t to sensory parameters such as colour and appearance (10), flavour (10), body and texture (10) and overall acceptability scores of probiotic DVS prepared using probiotic *Lp plantarum* CRD7 with varied levels of maltodextrin at inlet temperature of 180^{o}C are mentioned in **Table 4.5 (b)** and **figure 4.3 (b)**.

The highest score for colour and appearance *i.e.,* 9.6±0.63 was obtained for probiotic DVS prepared with *Lp. plantarum* CRD7 at inoculum level of 0.002% (w/v) respectively. Significant difference in sensory scores was obtained for probiotic *dahi* prepared using probiotic DVS of *Lp plantarum* CRD7 at varied inoculum level.

The highest score 8.6±1.05 for flavour was obtained for probiotic *dahi* prepared using DVS of *Lp. plantarum* CRD7 by spray drying method at inlet temperature of 180°C.

Significant difference in the flavour scores of probiotic *dahi* prepared using *Lp. plantarum* CRD7 at different inoculum levels was noticed.

The maximum score for body and texture *i.e.,* 9.0±1.03 of probiotic *dahi* was recorded. There was significant difference in the scores obtained for probiotic *dahi* prepared with DVS at inoculum level of @0.002% (w/v) which was prepared with maltodextrin @2.5% (w/v).

The highest score for overall acceptability *i.e.*, 9.3±0.45 was recorded for probiotic *dahi* prepared from DVS powder at inoculum level of 0.002% (w/v). Significant difference in the overall acceptability scores was documented for probiotic *dahi* prepared with DVS of *Lp. plantarum* CRD7 prepared at inlet temperature 180°C.

4.5 (c) : TEXTURE PROFILES

Observations effect of varied inoculum levels of probiotic DVS powder of *Lp plantarum* CRD7 prepared with maltodextrin at different level at 180°C inlet temperature on texture parameters such as firmness (N), consistency (N.s), cohesiveness (N) and work of cohesion (N.s) of probiotic *dahi* have been presented in **Table 4.5 (c**) and **figure 4.3 (c)**.The highest value for firmness (2.90±0.05 N) was obtained for probiotic *dahi* prepared with DVS powder of *Lp plantarum* CRD7 manufactured with maltodextrin concentration @2.5% (w/v) at 0.002% (w/v) inoculum level of DVS. Significant differences in the firmness of probiotic *dahi* prepared with DVS were noticed at different inoculum levels of probiotic DVS powder prepared with varied level of maltodextrin. Inoculum level of 0.002% (w/v) was optimized as it resulted better quality w.r.t techno-functional attributes, sensory score and texture of probiotic *dahi* prepared thereof.

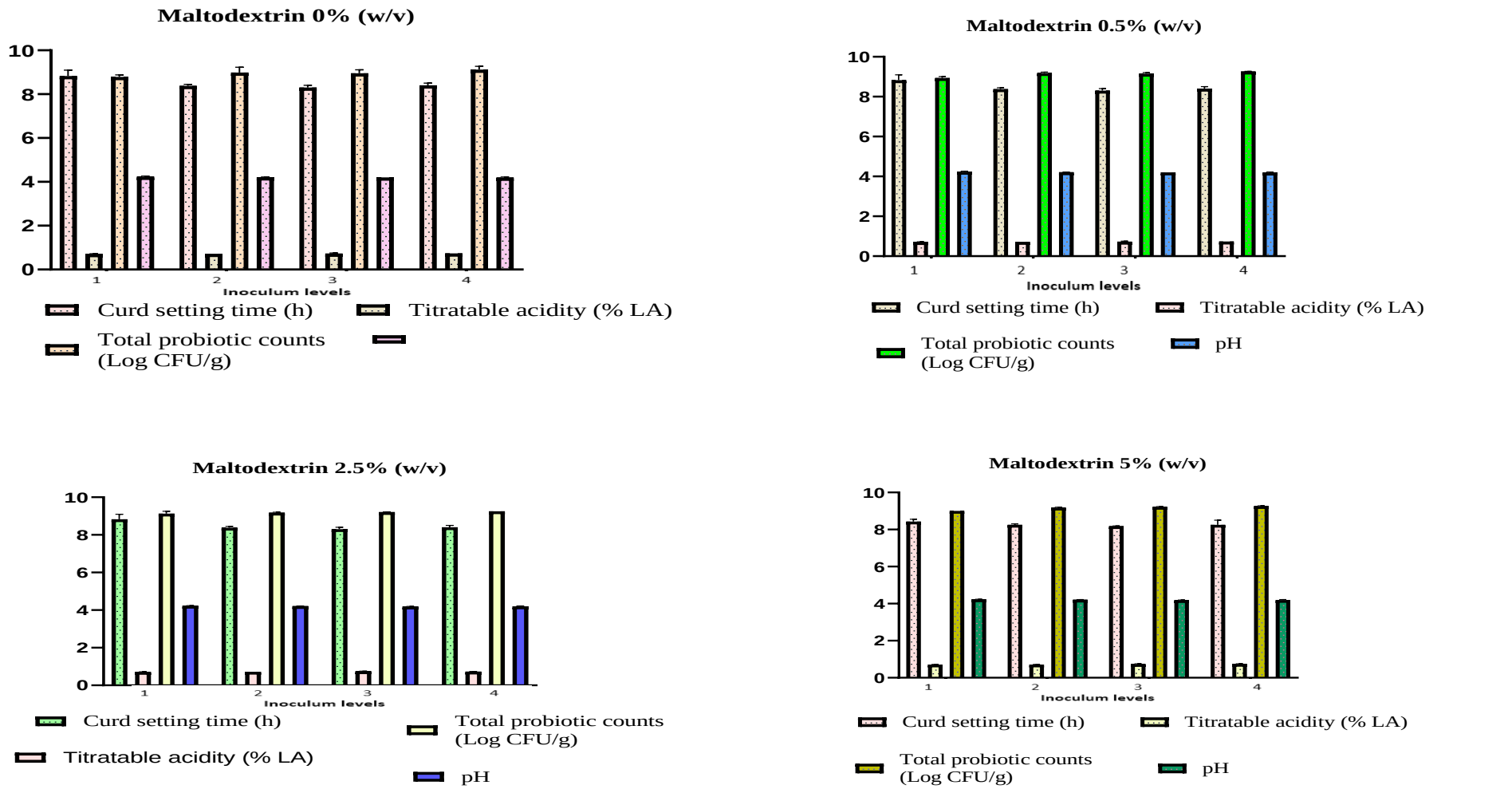
Table 4.5 (a) : Effect of inoculum levels of probiotic DVS of *Lactiplantibacillus plantarum* CRD7 prepared at 180℃ on techno-functional attributes of *dahi*.

Maltodextrin concentration (%w/v)	Inoculum levels of probiotic DVS powder (%w/v)*	Curd setting time (h)	pH	Titratable acidity (% LA)	Total probiotic counts (Log CFU/mL)
0	0.002	8.10±0.01^{d}	4.22±0.02^{a}	0.71±0.02^{c}	8.70±0.04^{d}
	0.004	8.05±0.02^{c}	4.20±0.02^{a}	0.72±0.01^{b}	8.75±0.08^{c}
	0.006	7.50±0.03^{b}	4.20±0.01^{a}	0.72±0.02^{b}	8.80±0.02^{b}
	0.010	7.45±0.05^{a}	4.22±0.02^{a}	0.74±0.01^{a}	8.97±0.03^{a}
0.5	0.002	8.10±0.01^{d}	4.22±0.03^{a}	0.72±0.05^{a}	9.00±0.01^{d}
	0.004	8.05±0.02^{c}	4.21±0.04^{a}	0.73±0.01^{a}	9.15±0.02^{c}
	0.006	7.50±0.03^{b}	4.20±0.01^{a}	0.74±0.03^{a}	9.20±0.04^{b}
	0.010	7.45±0.02^{a}	4.16±0.02^{b}	0.74±0.01^{a}	9.25±0.02^{a}
2.5	**0.002**	**8.00±0.05^{c}**	**4.24±0.03^{a}**	**0.71±0.02^{a}**	**9.25±0.02^{a}**
	0.004	7.50±0.02^{b}	4.22±0.02^{a}	0.72±0.01^{a}	9.20±0.01^{a}
	0.006	7.45±0.01^{a}	4.18±0.02^{a}	0.74±0.01^{a}	9.22±0.02^{a}
	0.010	7.45±0.06^{a}	4.18±0.01^{a}	0.72±0.02^{a}	9.25±0.03^{a}
5.0	0.002	8.30±0.02^{d}	4.28±0.01^{a}	0.70±0.03^{b}	9.00±0.04^{d}
	0.004	8.20±0.04^{c}	4.26±0.02^{a}	0.71±0.02^{a}	9.20±0.02^{c}
	0.006	8.15±0.02^{b}	4.22±0.02^{a}	0.72±0.01^{a}	9.24±0.02^{b}
	0.010	8.00±0.01^{a}	4.14±0.04^{b}	0.75±0.02^{a}	9.28±0.02^{a}

Data are represented as Mean ± SE; (p<0.05); n=3.

a-c (along the column) values with different superscripts are significantly different from each others.

***** Spray dried probiotic DVS of *Lp. plantarum* CRD7 prepared with maltodextrin at inlet temperature 180°C.



Inoculum Levels : 1=0.002%; 2 = 0.004%; 3 = 0.006%; 4 = 0.010%

Figure 4.3 (a) : Effect of inoculum levels of probiotic DVS of *Lactiplantibacillus plantarum* CRD7 prepared at 180℃ on techno-functional attributes of *dahi*.

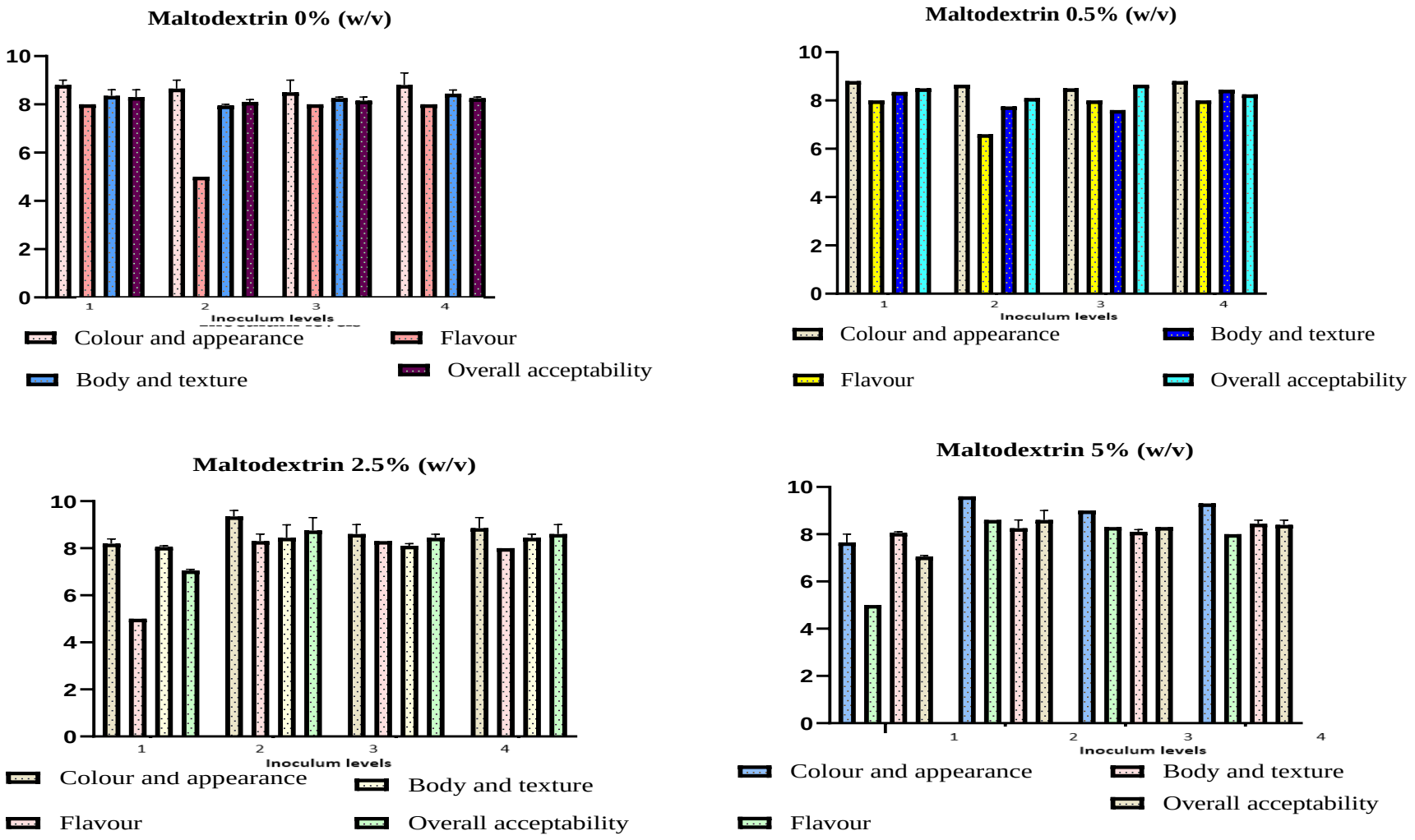
Table 4.5 (b): Effect of inoculum levels of probiotic DVS of *Lactiplantibacillus plantarum* CRD7 prepared at 180℃ on sensory attributes of *dahi*.

Maltodextrin concentration (% w/v)	Inoculum levels of probiotic DVS powder (%w/v)*	Colour and appearance	Flavour	Body and texture	Overall acceptability
0	0.002	9.0±0.28^{b}	8.0±0.75^{b}	8.6±0.25^{a}	8.6±0.27^{a}
	0.004	8.3±0.13^{c}	5.0±0.41^{c}	8.0±0.72^{c}	8.0±0.12^{c}
	0.006	9.0±0.43^{b}	8.0±0.83^{b}	8.3±0.23^{b}	8.0±0.28^{c}
	0.010	9.3±0.63^{a}	8.3±0.76^{a}	8.6±0.65^{a}	8.3±0.33^{b}
0.5	0.002	9.0±0.22^{b}	8.0±0.29^{b}	8.6±0.12^{a}	9.0±0.49^{a}
	0.004	8.3±0.36^{c}	5.0±0.12^{c}	8.0±0.19^{c}	8.0±0.41^{c}
	0.006	9.0±0.41^{b}	8.0±0.40^{b}	8.3±0.36^{b}	9.0±0.46^{a}
	0.010	9.3±0.28^{a}	8.3±0.38^{a}	8.6±0.45^{a}	8.3±0.32^{b}
2.5	**0.002**	**9.6±0.63^{a}**	**8.6±1.05^{a}**	**9.0±1.03^{a}**	**9.3±0.45^{a}**
	0.004	8.6±0.58^{d}	5.0±0.21^{d}	8.0±0.21^{c}	7.0±0.22^{c}
	0.006	9.0±0.41^{c}	8.3±0.33^{b}	8.0±0.11^{c}	8.6±0.51^{b}
	0.010	9.3±0.36^{b}	8.0±0.34^{c}	8.6±0.53^{b}	9.0±0.25^{a}
5	0.002	9.3±0.29^{a}	8.6±0.25^{a}	8.6±0.58^{a}	9.0±0.11^{a}
	0.004	8.6±0.51^{b}	8.0±0.12^{c}	8.0±0.18^{c}	8.6±0.63^{b}
	0.006	9.3±0.39^{a}	8.3±0.33^{b}	8.3±0.33^{b}	8.6±0.41^{b}
	0.010	8.3±0.42^{c}	8.0±0.14^{c}	8.6±0.25^{a}	9.0±0.18^{a}

Data are represented as Mean ± SE; (p<0.05); n=3.

a-c (along the column) values with different superscripts are significantly different from each others.

***** Spray dried probiotic DVS of *Lp. plantarum* CRD7 prepared with maltodextrin prepared at inlet temperature of 180°C.



Inoculum Levels : 1=0.002%; 2 = 0.004%; 3 = 0.006%; 4 = 0.010%

Figure 4.3 (b): Effect of inoculum levels of probiotic DVS of *Lactiplantibacillus plantarum* CRD7 prepared at 180℃ on sensory attributes of *dahi*.

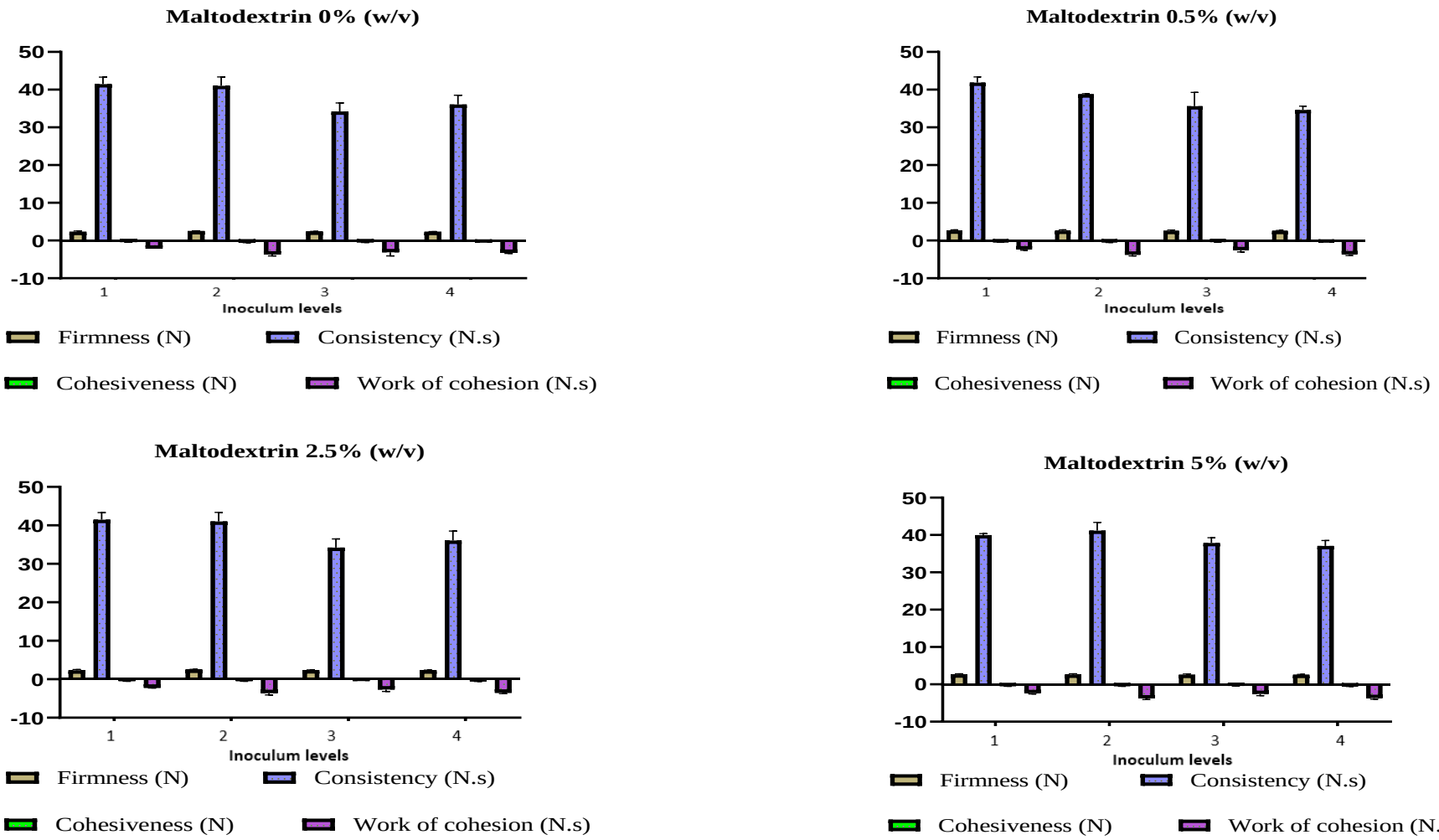
Table 4.5 (c) : Effect of inoculum levels of probiotic DVS of *Lactiplantibacillus plantarum* CRD7 prepared at 180℃ on texture profile of *dahi*.

Maltodextrin concentration (% w/v)	Inoculum levels of probiotic DVS powder (%w/v)*	Firmness (N)	Consistency (N.s)	Cohesiveness (N)	Work of cohesion (N.s)
0	0.002	2.50±0.02^{a}	43.35±0.25^{a}	-0.50±0.01^{a}	-2.18±0.28^{c}
	0.004	2.48±0.01^{a}	38.65±0.25^{a}	-0.49±0.02^{a}	-3.40±0.18^{b}
	0.006	2.43±0.02^{a}	32.01±1.18^{b}	-0.40±0.03^{a}	-4.08±0.14^{a}
	0.010	2.40±0.04^{a}	33.61±0.49^{b}	-0.55±0.05^{a}	-3.15±0.32^{b}
0.5	0.002	2.50±0.02^{a}	43.35±0.86^{a}	-0.50±0.01^{a}	-2.18±0.14^{c}
	0.004	2.48±0.01^{a}	38.65±0.49^{a}	-0.49±0.01^{a}	-3.40±0.26^{b}
	0.006	2.43±0.02^{a}	32.01±0.90^{b}	-0.40±0.05^{a}	-4.08±0.32^{a}
	0.010	2.40±0.05^{a}	33.61±0.25^{b}	-0.55±0.05^{a}	-3.15±0.38^{b}
2.5	**0.002**	**2.90±0.05^{a}**	**38.64±0.49^{a}**	**-0.53±0.01^{a}**	**-2.30±0.31^{b}**
	0.004	2.92±0.01^{a}	36.48±0.75^{a}	-0.48±0.04^{a}	-3.14±0.00^{a}
	0.006	2.90±0.01^{a}	37.01±0.25^{a}	-0.46±0.03^{a}	-3.19±0.28^{a}
	0.010	2.80±0.02^{a}	34.23±0.31^{a}	-0.52±0.03^{a}	-3.70±0.18^{a}
5.0	0.002	2.84±0.02^{a}	40.36±0.25^{a}	-0.51±0.01^{a}	-2.57±0.14^{b}
	0.004	2.80±0.01^{a}	38.95±0.18^{a}	-0.49±0.01^{a}	-3.48±0.10^{a}
	0.006	2.75±0.02^{a}	39.26±0.75^{a}	-0.41±0.02^{a}	-3.02±0.26^{a}
	0.010	2.70±0.04^{a}	35.60±0.43^{b}	-0.54±0.02^{a}	-3.96±0.28^{a}

Data are represented as Mean ± SE; (p<0.05); n=3.

a-c (along the column) values with different superscripts are significantly different from each others.

***** Spray dried probiotic DVS of *Lp. plantarum* CRD7 prepared with maltodextrin at inlet temperature of 180˚C.



Inoculum Levels : 1=0.002%; 2 = 0.004%; 3 = 0.006%; 4 = 0.010%

Figure 4.3 (c) : Effect of inoculum levels of probiotic DVS of *Lactiplantibacillus plantarum* CRD7 prepared at 180℃ on texture profile of *dahi*.

4.6 OPTIMIZATION OF INOCULUM LEVELS OF PROBIOTIC DVS OF *LACTIPLANTIBACILLUS PLANTARUM* CRD7 PREPARED WITH MALTODEXTRIN AT INLET TEMPERATURE OF 190ºC ON TECHNO-FUNCTIONAL, SENSORY AND TEXTURAL ATTRIBUTES OF *DAHI*

4.6 (a): TECHNO-FUNCTIONAL ATTRIBUTES

Data on techno-functional attributes of probiotic *dahi* prepared with DVS of *Lp plantarum CRD7* prepared with maltodextrin at different concentration by spray drying at inlet temperature 190°C have been presented in **Table 4.6 (a)** and **figure 4.4 (a)**. Probiotic *dahi* was prepared with standardized milk, heated to 90°C/10min. Milk was cooled to 37°C and inoculated with different inoculum levels. This was incubated at 37°C and observed for curd setting, pH, titratable acidity and probiotic counts. Probiotic *dahi* was also analysed for sensory and textural attributes. The curd setting time taken by DVS of *Lp. Plantarum* CRD7 for the preparation of *dahi* ranged 7.55±0.01-8.50±0.05 h, pH and titratable acidity ranged between 4.14±0.01-4.26±0.03 and 0.70±0.02-0.78±0.01 (% LA) respectively and total probiotic counts ranged from 8.60±0.05 to 9.05±0.02 (Log CFU/mL). On basis of minimum curd setting time, faster reduction in pH and acidity development, and highest probiotic counts, inoculum level 0.004% (w/v) of probiotic DVS was optimized for better techno-functional properties of probiotic *dahi*.

4.6 (b): SENSORY SCORES

Observations related to sensory parameters such as colour and appearance (10), flavour (10), body and texture (10) and overall acceptability scores of probiotic *dahi* prepared using probiotic *Lp plantarum* CRD7 DVS manufactured with maltodextrin at inlet temperature of 190ºC at varied inoculum level are mentioned in **Table 4.6 (b)** and **figure 4.4 (b)**.

The highest score for colour and appearance *i.e.,* 9.6±0.95 was obtained for probiotic *dahi* prepared with DVS of *Lp. plantarum* CRD7 at inoculum level of 0.004% (w/v). Significant difference in sensory scores was recorded for probiotic *dahi* prepared using probiotic DVS of *Lp plantarum* CRD7.

The highest score 8.6±0.83 for flavour was obtained for probiotic *dahi* prepared using DVS of *Lp. plantarum* CRD7 by spray drying method at inlet temperature (190°C). Significant differences in the flavour scores of probiotic *dahi* prepared using DVS of *Lp. plantarum* CRD7 at varied inoculum level was noticed.

The maximum score for body and texture *i.e.*, 9.0±1.03 of probiotic *dahi* was recorded for DVS powder @ 0.004% (w/v). There was significant difference in the scores obtained for probiotic *dahi* prepared with probiotic DVS of *Lp. plantarum* CRD7 at varied inoculum levels.

The highest score for overall acceptability *i.e.*, 9.3±0.81 was obtained for probiotic *dahi* prepared from DVS powder @0.004% (w/v) inoculum level. Significant difference in the scores was obtained for probiotic *dahi* prepared with DVS powder prepared with varied maltodextrin concentration at inlet temperature of 190°C.

4.6 (c) : TEXTURE PROFILES

Results obtained of probiotic *dahi* prepared with varied concentration of DVS powder prepared with different level of maltodextrin at 190^{o}C, texture parameters such as firmness (N), consistency (N.s), cohesiveness (N) and work of cohesion (N.s) of probiotic *dahi* prepared with DVS of *Lp. plantarum* CRD7 prepared at inlet temperature of 190^{o}C with different concentration of maltodextrin have been presented in **Table 4.6 (c)** and **figure 4.4 (c)**. The highest score for firmness (2.85±0.02 N) was obtained for probiotic *dahi* prepared with DVS powder @0.50% (w/v) inoculum level. Significant differences in the firmness of probiotic was noticed for *dahi* prepared with different inoculum levels of probiotic DVS powder prepared with varied level of maltodextrin at 190^{o}C inlet temperature. The observations on techno-functional, sensory and textural attributes of probiotic *dahi* prepared DVS manufactured with maltodextrin 2.5% (w/v) revealed that inoculum level of 0.004% (w/v) was found optimum.

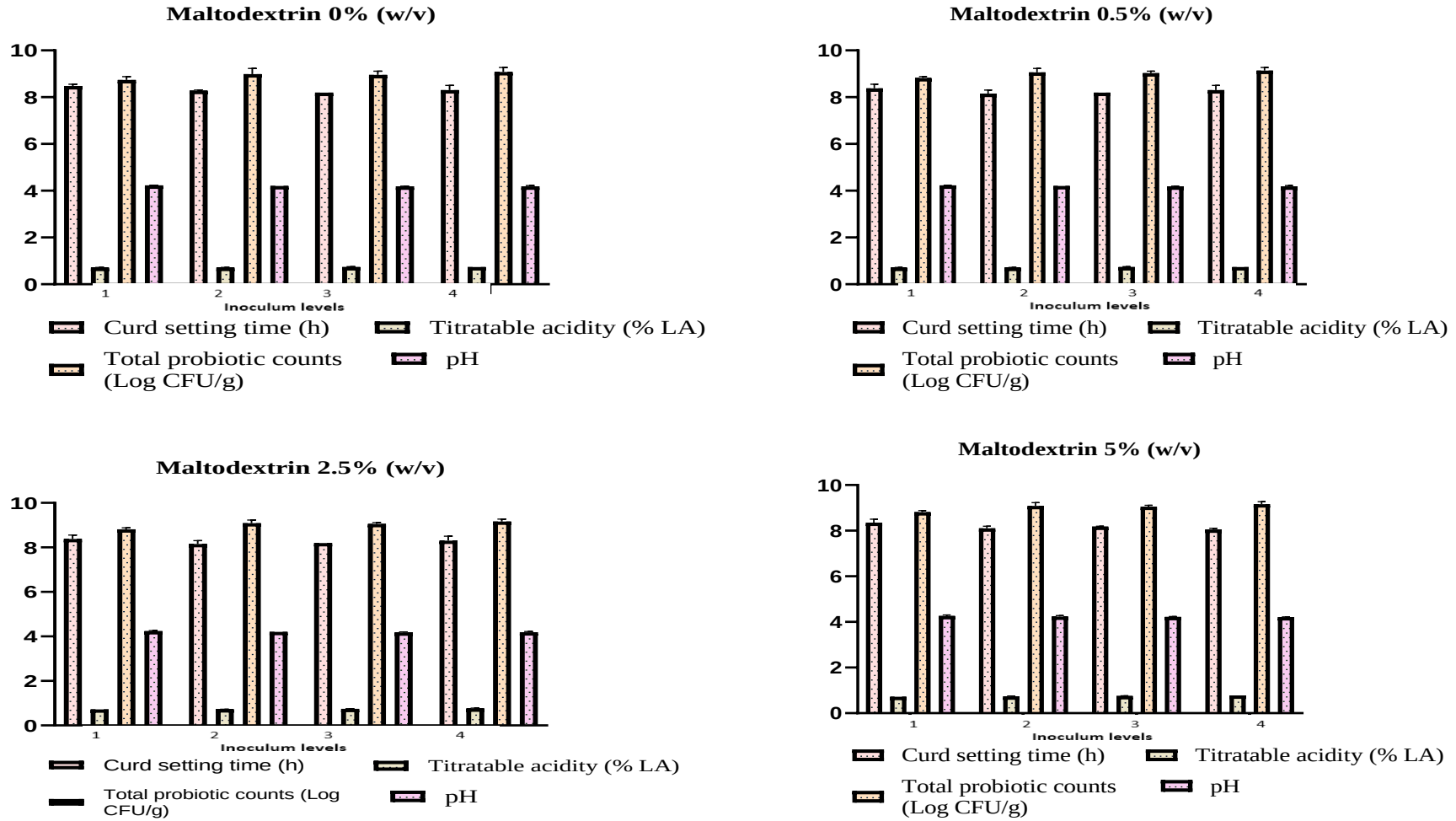
Table 4.6 (a) : Effect of inoculum levels of probiotic DVS of *Lactiplantibacillus plantarum* CRD7 prepared at 190℃ on techno-functional attributes of *dahi*.

Maltodextrin concentration (%w/v)	Inoculum levels of probiotic DVS powder (%w/v)*	Curd setting time (h)	pH	Titratable acidity (% LA)	Total probiotic counts (Log CFU/mL)
0	0.002	8.40±0.01^{a}	4.24±0.01^{a}	0.70±0.02^{a}	8.60±0.05^{d}
	0.004	8.25±0.02^{b}	4.20±0.03^{a}	0.71±0.03^{a}	8.75±0.01^{c}
	0.006	8.20±0.05^{c}	4.18±0.02^{a}	0.72±0.01^{a}	8.80±0.00^{b}
	0.010	8.10±0.07^{d}	4.16±0.02^{a}	0.74±0.04^{a}	8.89±0.08^{a}
0.5	0.002	8.40±0.01^{a}	4.22±0.01^{a}	0.71±0.01^{a}	8.76±0.07^{d}
	0.004	8.25±0.03^{b}	4.16±0.02^{a}	0.72±0.02^{a}	8.90±0.06^{c}
	0.006	8.20±0.02^{c}	4.14±0.01^{a}	0.72±0.01^{a}	8.95±0.02^{b}
	0.010	8.10±0.01^{d}	4.14±0.02^{a}	0.74±0.03^{a}	9.00±0.02^{a}
2.5	0.002	8.45±0.04^{a}	4.26±0.03^{a}	0.72±0.01^{a}	8.75±0.01^{d}
	0.004	**8.10±0.02^{b}**	**4.20±0.02^{a}**	**0.73±0.01^{b}**	**8.95±0.02^{c}**
	0.006	8.00±0.02^{c}	4.15±0.01^{b}	0.74±0.02^{a}	9.00±0.04^{b}
	0.010	7.55±0.01^{d}	4.15±0.03^{b}	0.78±0.04^{a}	9.05±0.02^{a}
5.0	0.002	8.50±0.05^{a}	4.30±0.01^{a}	0.72±0.05^{a}	8.65±0.07^{d}
	0.004	8.20±0.04^{b}	4.28±0.02^{a}	0.75±0.02^{a}	8.85±0.01^{c}
	0.006	8.15±0.04^{c}	4.24±0.01^{a}	0.76±0.03^{a}	8.97±0.02^{b}
	0.010	8.00±0.01^{d}	4.20±0.02^{a}	0.78±0.01^{a}	9.00±0.01^{a}

Data are represented as Mean ± SE; (p<0.05); n=3.

a-b (along the column) values with different superscripts are significantly different from each others.

***** Spray dried probiotic DVS of *Lp. plantarum* CRD7 prepared with maltodextrin at inlet temperature of 190˚C.



Inoculum Levels : 1=0.002%; 2 = 0.004%; 3 = 0.006%; 4 = 0.010%

Figure 4.4 (a) : Effect of inoculum levels of probiotic DVS of *Lactiplantibacillus plantarum* CRD7 prepared at 190℃ on techno-functional attributes of *dahi*.

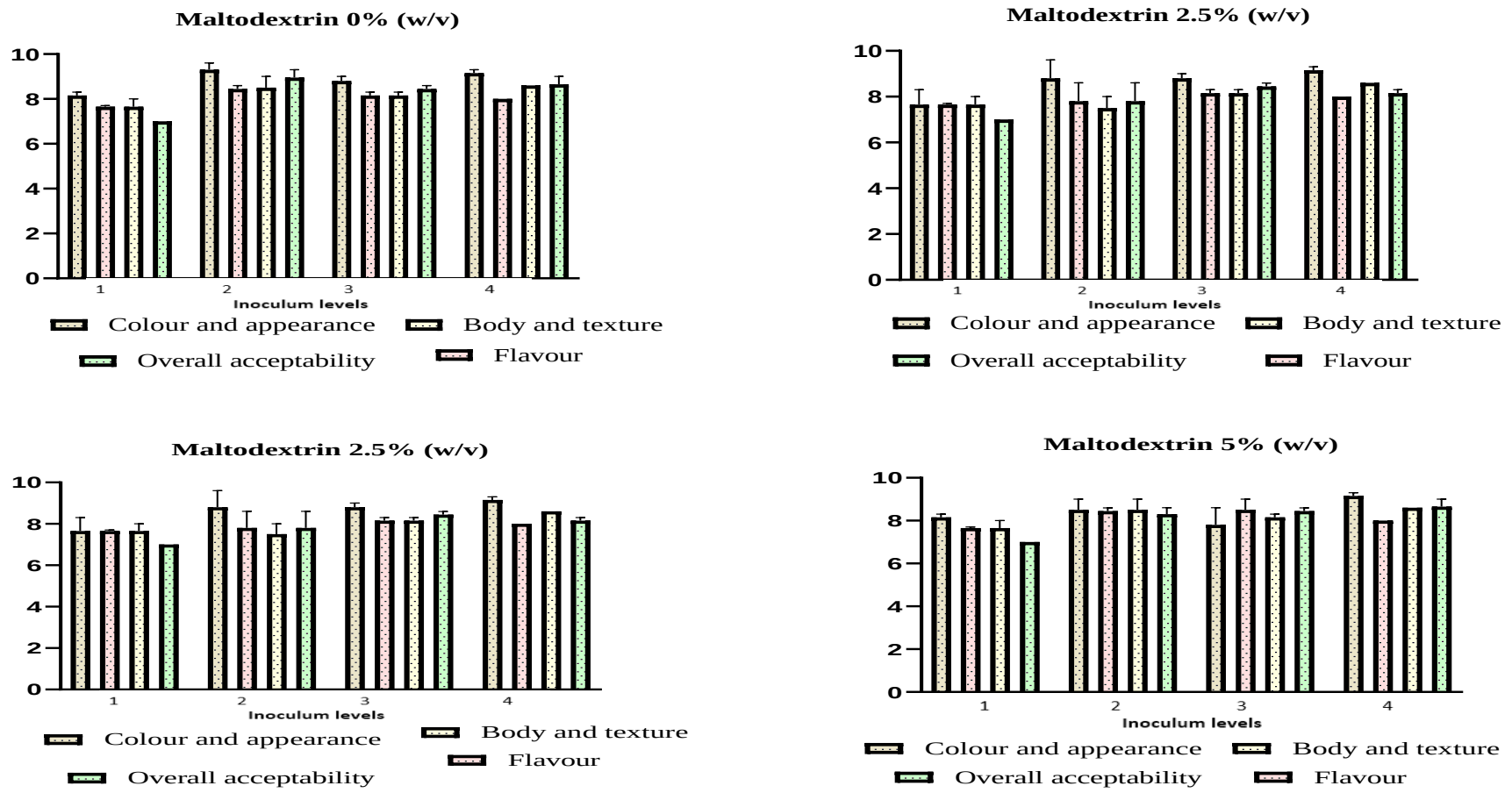
Table 4.6 (b): Effect of inoculum levels of probiotic DVS of *Lactiplantibacillus plantarum* CRD7 prepared at 190℃ on sensory attributes of *dahi*.

Maltodextrin concentration (% w/v)	Inoculum levels of probiotic DVS powder (%w/v)*	Colour and appearance	Flavour	Body and texture	Overall acceptability
0	0.002	8.3±0.28^{c}	7.6±0.47^{c}	7.3±0.70^{c}	7.0±0.35^{c}
	0.004	9.0±0.12^{a}	8.3±0.36^{a}	8.0±0.25^{b}	8.6±0.49^{a}
	0.006	8.6±0.42^{b}	8.0±0.75^{b}	8.3±0.33^{b}	8.3±0.58^{b}
	0.010	9.0±0.20^{a}	8.0±0.31^{b}	8.6±0.53^{a}	8.3±0.75^{b}
0.5	0.002	8.3±0.68^{c}	7.6±0.80^{b}	7.3±0.31^{c}	7.0±0.43^{d}
	0.004	9.0±0.55^{a}	8.3±0.72^{a}	8.6±0.49^{a}	9.0±0.37^{a}
	0.006	8.6±0.46^{b}	8.0±0.44^{a}	8.3±0.33^{b}	8.0±0.32^{c}
	0.010	9.0±0.41^{a}	8.0±0.63^{a}	8.6±0.45^{a}	8.3±0.35^{b}
2.5	0.002	8.0±0.38^{c}	5.6±0.55^{c}	7.0±0.68^{b}	7.0±0.48^{d}
	0.004	**9.6±0.95^{a}**	**8.6±0.83^{a}**	**9.0±1.03^{a}**	**9.3±0.81^{a}**
	0.006	9.0±0.71^{b}	8.3±0.61^{b}	8.6±0.81^{a}	8.0±0.60^{c}
	0.010	9.0±0.55^{b}	8.0±0.58^{b}	8.6±0.53^{a}	9.0±0.34^{b}
5	0.002	7.3±0.34^{d}	5.0±0.49^{c}	7.0±0.39^{c}	7.0±0.48^{d}
	0.004	9.6±0.32^{a}	9.0±0.43^{a}	8.6±0.45^{a}	9.0±0.39^{a}
	0.006	9.3±0.41^{c}	8.3±0.41^{b}	8.0±0.31^{b}	8.3±0.43^{c}
	0.010	9.0±0.30^{b}	8.6±0.32^{b}	8.6±0.36^{a}	8.6±0.56^{b}

Data are represented as Mean ± SE; (p<0.05); n=3.

a-b (along the column) values with different superscripts are significantly different from each others.

***** Spray dried probiotic DVS of *Lp. plantarum* CRD7 prepared with maltodextrin at inlet temperature of 190˚C.



Inoculum Levels : 1=0.002%; 2 = 0.004%; 3 = 0.006%; 4 = 0.010%

Figure 4.4 (b): Effect of inoculum levels of probiotic DVS of *Lactiplantibacillus plantarum* CRD7 prepared at 190℃ on sensory attributes of *dahi*.

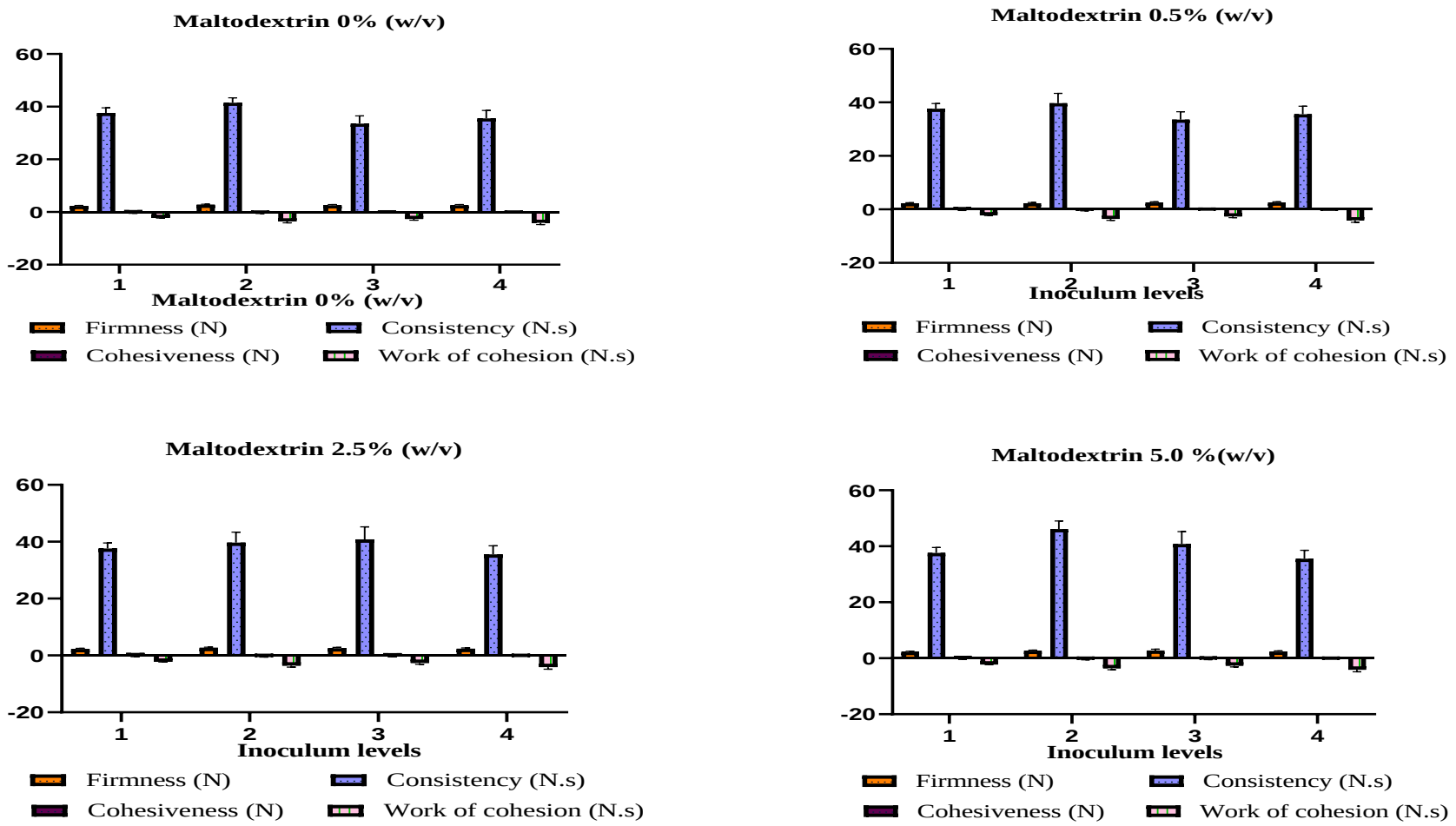
Table 4.6 (c): Effect of inoculum levels of probiotic DVS of *Lactiplantibacillus plantarum* CRD7 prepared at 190℃on texture profile of *dahi*.

Maltodextrin concentration (% w/v)	Inoculum levels of probiotic DVS powder (%w/v)*	Firmness (N)	Consistency (N.s)	Cohesiveness (N)	Work of cohesion (N.s)
0	0.002	2.00±0.01^{b}	30.56±0.25^{a}	-0.51±0.02^{a}	-3.30±0.28^{a}
	0.004	2.16±0.02^{a}	37.52±0.25^{a}	-0.47±0.04^{b}	-3.45±0.18^{a}
	0.006	2.20±0.05^{a}	30.75±0.75^{a}	-0.40±0.03^{d}	-2.16±0.14^{b}
	0.010	2.25±0.02^{a}	31.61±1.18^{a}	-0.43±0.02^{c}	-3.21±0.18^{a}
0.5	0.002	2.00±0.01^{c}	30.56±0.49^{a}	-0.51±0.04^{a}	-3.20±0.14^{a}
	0.004	2.46±0.02^{a}	37.52±0.49^{a}	-0.47±0.01^{b}	-3.48±0.32^{a}
	0.006	2.40±0.05^{a}	30.75±0.90^{a}	-0.40±0.03^{d}	-2.16±0.38^{b}
	0.010	2.28±0.04^{b}	31.61±0.25^{a}	-0.43±0.05^{c}	-3.21±0.32^{a}
2.5	0.002	2.50±0.01^{a}	37.46±0.30^{b}	-0.46±0.02^{b}	-2.18±0.62^{b}
	0.004	**2.85±0.02^{a}**	**40.35±0.18^{a}**	**-0.59±0.02^{a}**	**-3.08±0.14^{a}**
	0.006	2.82±0.03^{a}	35.41±0.25^{b}	-0.50±0.05^{a}	-3.25±0.32^{a}
	0.010	2.78±0.02^{a}	36.45±0.49^{b}	-0.55±0.01^{a}	-3.76±0.32^{a}
5.0	0.002	2.58±0.04^{a}	40.46±0.22^{a}	-0.48±0.03^{b}	-3.21±0.38^{b}
	0.004	2.75±0.02^{a}	45.48±0.40^{a}	-0.59±0.02^{a}	-3.14±0.62^{b}
	0.006	2.70±0.04^{a}	37.46±0.75^{b}	-0.42±0.08^{c}	-2.59±0.14^{c}
	0.010	2.67±0.05^{a}	42.00±0.25^{a}	-0.59±0.02^{a}	-4.14±0.14^{a}

Data are represented as Mean ± SE; (p<0.05); n=3.

a-c (along the column) values with different superscripts are significantly different from each others.

***** Spray dried probiotic DVS of *Lp. plantarum* CRD7 prepared with maltodextrin at inlet temperature (190°C).



Inoculum Levels : 1=0.002%; 2 = 0.004%; 3 = 0.006%; 4 = 0.010%

Figure 4.4 (c): Effect of inoculum levels of probiotic DVS of *Lactiplantibacillus plantarum* CRD7 prepared at 190℃ on texture profile of *dahi*.

4.7 STORAGE STUDIES

Three types of probiotic DVS prepared was packed in three type of packaging materials i.e. aluminium laminates, LDPE and EVOH and stored at two temperature i.e. -20ºC and 4ºC. The inoculum levels optimized for probiotic *dahi* preparation was used to check the performance of probiotic DVS at 30 days interval upto 60 days by preparing *dahi* and analysing quality parameters w.r.t. techno-functional, sensory, textural and probiotic counts.

4.7.1 : Effect of packaging material (aluminium laminate) and storage temperature (-20ºC) on techno-functional sensory and textural attributes of probiotic *dahi* prepared with DVS powder of *Lactiplantibacillus plantarum* CRD7

4.7.1 (a): TECHNO-FUNCTIONAL ATTRIBUTES

The curd setting time taken by probiotic *Lp. plantarum* DVS starter for the preparation of *dahi* from starter packaged in aluminium laminate and stored at -20ºC ranged from 7.45-8.15 h, respectively. pH and titratable acidity ranged from 4.05-4.26 and 0.70-0.80 %LA, respectively. Total probiotic counts ranged from 8.78±0.08 to 9.35±0.08 Log CFU/mL as mentioned in **Table 4.7 (a).** Probiotic counts of *dahi* prepared with DVS of *Lp. plantarum* CRD7 manufactured with maltodextrin @ 2.5%(w/v) as a protective agent for 0^{th} day (9.35±0.08), 30^{th} day (9.30±0.05) and 60^{th} (9.22±0.02) Log CFU/mL. There was no significant difference in techno-functional attributes of probiotic *dahi* prepared with *Lp. plantarum* DVS from 0^{th} to 60^{th} day of storage at -20ºC in techno-functional attributes. There results revealed excellent activity of probiotic spray dried DVS of *Lp. plantarum* CRD7 upto 60 days of storage at -20ºC.

4.7.1 (b) : SENSORY SCORES

Observations on sensory parameters such as colour and appearance (10), flavour (10), body and texture (10) and overall acceptability (10) of probiotic *dahi* prepared with DVS starter stored in aluminium laminates at temperature of -20ºC have been mentioned in **Table 4.7 (b)**. Overall acceptability scores for *Lp. plantarum* CRD7 *dahi* prepared DVS manufactured with maltodextrin @2.5 % (w/v) as protective agent were recorded for 0^{th} day (9.6±0.15), 30^{th} day (9.3±0.13) and 60^{th} day (9.0±0.12). No-significant difference in sensory scores of probiotic *dahi* were observed from DVS and stored from 0^{th} to 60^{th} day of storage at -20ºC in aluminium laminates. These observations indicate excellent performance of probiotic *Lp. plantarum* CRD7 DVS upto 60 days of storage at -20ºC.

4.7.1 (c) : TEXTURE PROFILES

Data on texture parameters such as firmness (N), consistency (N.s), cohesiveness (N) and work of cohesion (N.s) of probiotic *dahi* prepared with spray dried DVS starter stored in aluminium laminates at temperature of -20^{o}C have been recorded in **Table 4.7 (c**). Firmness of probiotic *dahi* prepared with DVS of *Lp. plantarum* CRD7 DVS manufactured with maltodextrin @2.5% as protective agent for 0th day in firmness (2.95±0.04 N), 30th day (2.89±0.03 N)and 60th day (2.80±0.02N). A similar trend was noticed for other textural profile viz., consistency, cohesiveness and work of cohesion. There was no significant difference observed in textural profile of probiotic *dahi* prepared with spray dried DVS of *Lp. plantarum* CRD7 stored at -20^{o}C in aluminium laminates. These results indicate storage stability of probiotic DVS of *Lp. plantarum* CRD7 without any adverse effect on starter activity.

Table 4.7 (a) : Effect of packaging material (aluminium laminate) and storage temperature (-20^{o}C) on techno-functional attributes of probiotic *dahi* prepared with DVS powder of *Lactiplantibacillus plantarum* CRD7.

Inlet temperature (^{o} C)	Inoculum levels of probiotic DVS powder (%w/v)*	Storage period (days)	Curd setting time (h)	pH	Titratable acidity (%LA)	Total probiotic counts (Log CFU/mL)
170	0.004	0	7.45±0.02^{a}	4.20±0.01^{a}	0.77±0.03^{a}	9.35±0.08^{a}
		30	7.50±0.04^{a}	4.24±0.04^{a}	0.77±0.01^{a}	9.30±0.05^{a}
		60	7.65±0.08^{a}	4.24±0.02^{a}	0.70±0.02^{a}	9.22±0.02^{a}
180	0.002	0	8.00±0.04^{a}	4.18±0.03^{a}	0.78±0.01	9.15±0.06^{a}
		30	8.10±0.03^{a}	4.20±0.05^{a}	0.74±0.03^{a}	9.10±0.07^{a}
		60	8.15±0.07^{a}	4.24±0.03^{a}	0.72±0.02^{b}	9.04±0.04^{a}
190	0.004	0	8.10±0.05^{a}	4.05±0.04^{a}	0.80±0.01^{a}	8.95±0.05^{a}
		30	8.15±0.04^{a}	4.24±0.02^{a}	0.72±0.01^{b}	8.80±0.07^{a}
		60	8.15±0.09^{a}	4.26±0.01^{a}	0.72±0.03^{b}	8.78±0.08^{a}

***** *Lp. plantarum* CRD7 probiotic spray dried DVS powder prepared with 0.25% maltodextrin.

Data are represented as Mean ± SE; (p<0.05); n=3.

a-b (along the column) values with different superscripts are significantly different from each other.

Table 4.7 (b) : Effect of packaging material (aluminium laminate) and storage temperature (-20°C) on sensory attributes of probiotic *dahi* prepared with DVS powder of *Lactiplantibacillus plantarum* CRD7.

Inlet temperature (° C)	Inoculum levels of probiotic DVS powder (%w/v)*	Storage period (days)	Colour and appearance	Flavour	Body and texture	Overall acceptability
170	0.004	0	9.6±0.15[a]	8.6±0.35[a]	9.0±0.33[a]	9.3±0.33[a]
		30	9.3±0.13[a]	8.6±0.45[a]	8.6±0.55[a]	9.0±0.11[a]
		60	9.0±0.12[a]	8.3±0.43[a]	8.6±0.65[a]	9.0±0.43[a]
180	0.002	0	9.6±0.15[a]	8.6±0.35[a]	9.0±0.41[a]	9.3±0.22[a]
		30	9.0±0.13[a]	8.3±0.65[a]	8.6±0.55[a]	9.0±0.11[a]
		60	9.0±0.11[a]	8.3±0.63[a]	8,6±0.35[a]	8.6±0.43[a]
190	0.004	0	9.6±0.25[a]	8.6±0.74[a]	9.0±0.44[a]	9.3±0.50[a]
		30	9.3±0.33[a]	8.6±0.33[a]	8.6±0.35[a]	9.0±0.62[a]
		60	9.0±0.12[a]	8.0±0.11[a]	8.3±0.33[a]	8.6±0.45[b]

***** *Lp. plantarum* CRD7 probiotic spray dried DVS powder prepared with 0.25% maltodextrin.

Data are represented as Mean ± SE; (p<0.05); n=3. a-b (along the column) values with different superscripts are significantly different from each other.

Table 4.7 (c) : Effect of packaging material (aluminium laminate) and storage temperature (-20°C) on texture profile of probiotic *dahi* prepared with DVS powder of *Lactiplantibacillus plantarum* CRD7.

Inlet temperature (° C)	Inoculum levels of probiotic DVS powder (%w/v)*	Storage period (days)	Firmness (N)	Consistency (N.s)	Cohesiveness (N)	Work of cohesion (N.s)
170	0.004	0	2.95±0.04[a]	43.37±0.25[a]	-0.57±0.01[a]	-3.15±0.14[a]
		30	2.89±0.03[a]	38.65±0.86[a]	-0.51±0.03[b]	-3.05±0.32[a]
		60	2.80±0.02[a]	32.01±0.78[b]	-0.49±0.01[c]	-2.32±0.28[b]
180	0.002	0	2.90±0.01[a]	38.64±0.49[a]	-0.53±0.02[a]	-2.30±0.18[a]
		30	2.80±0.03[a]	32.01±0.90[b]	-0.44±0.02[b]	-2.18±0.26[a]
		60	2.74±0.05[a]	30.19±0.75[c]	-0.34±0.03[c]	-2.05±0.62[a]
190	0.004	0	2.85±0.04[a]	40.35±0.45[a]	-0.59±0.02[a]	-3.08±0.58[a]
		30	2.81±0.03[a]	34.28±0.49[b]	-0.50±0.04[a]	-3.07±0.65[a]
		60	2.78±0.01[a]	31.20±0.80[c]	-0.37±0.01[b]	-3.01±0.21[a]

***** *Lp. plantarum* CRD7 probiotic spray dried DVS powder prepared with 0.25% maltodextrin.

Data are represented as Mean ± SE; (p<0.05); n=3. a-c (along the column) values with different superscripts are significantly different from each other.

4.7.2 : Effect of packaging material (LDPE) and storage temperature (-20ºC) on techno-functional attributes of probiotic *dahi* prepared with DVS powder of *Lactiplantibacillus plantarum* CRD7.

4.7.2 (a): TECHNO-FUNCTIONAL ATTRIBUTES

The curd setting time taken by probiotic *Lp. plantarum* DVS used for preparation of *dahi* starter powder packaged in LDPE and stored at temperature -20ºC for the preparation of *dahi* ranged from 7.45-8.30 h, respectively. pH and titratable acidity ranged from 4.05-4.26 and 0.72-0.80 %LA, respectively and total probiotic counts ranged from 8.60±0.01 to 9.35±0.05 Log CFU/mL. The results on techno-functional attributes have been mentioned in **Table 4.7 (d).** Probiotic counts of *dahi* prepared with DVS *Lp. plantarum* CRD7 DVS which was manufactured with maltodextrin @2.5%(w/v) as protective agents after storage for 0^{th} day (9.35±0.05) 30^{th} day (9.20±0.06) and 60^{th} (9.16±0.04). There was no significant differences in techno-functional attributes of probiotic *dahi* prepared with DVS stored in LDPE from 0^{th} to 60^{th} day at -20ºC.

4.7.2 (b) : SENSORY SCORES

Observations on sensory parameters such as colour and appearance (10), flavour (10), body and texture (10) and overall acceptability (10) of probiotic *dahi* prepared with DVS starter stored in LDPE at temperature of -20ºC have been documented in **Table 4.7 (e)**. Overall acceptability score for *Lp. plantarum* CRD7 probiotic *dahi* prepared with DVS manufactured with maltodextrin @2.5 %(w/v) as protective agent and stored at -20ºC for 0^{th} day (9.6±0.45), 30^{th} day (9.3±0.53) and 60^{th} day (9.0±0.41). No-significant difference was observed in sensory scores of probiotic *dahi* were noticed prepared from 0^{th} to 60^{th} day of storage at -20ºC in LDPE. These results indicate no adverse effect of activity of DVS of *Lp. plantarum* CRD7 of packaging material and storage temperature of -20ºC in LDPE.

4.7.2 (c) : TEXTURE PROFILES

Data on texture parameters such as firmness (N), consistency (N.s), cohesiveness (N) and work of cohesion (N.s) of probiotic *dahi* prepared with DVS starter stored in LDPE at -20ºC have been recorded in **Table 4.7 (f)**. Firmness of probiotic *dahi* prepared with DVS of *Lp. plantarum* CRD7 DVS manufactured with maltodextrin @2.5%(w/v) as protective agent and stored at -20ºC for 0^{th} day (2.95±0.03), 30^{th} day (2.80±0.01)and 60^{th} day (2.69±0.02). Almost a similar treds in values of consistency, cohesiveness and work of cohesion was noticed in probiotic *dahi* prepared with DVS. There was no significant difference observed from 0^{th} to 60^{th} of storage at -20ºC in acticity of probiotic DVS of *Lp. plantarum* CRD7 .

Table 4.7 (d) : Effect of packaging material (LDPE) and storage temperature (-20ºC) on techno-functional attributes of probiotic *dahi* prepared by DVS powder of *Lactiplantibacillus plantarum* CRD7.

Inlet temperature (º C)	Inoculum levels of probiotic DVS powder (%w/v)*	Storage period (days)	Curd setting time (h)	pH	Titratable acidity (%LA)	Total probiotic counts (Log CFU/mL)
170	0.004	0	7.45±0.02^{a}	4.24±0.03^{a}	0.77±0.02^{a}	9.35±0.05^{a}
		30	7.55±0.05^{a}	4.24±0.01^{a}	0.77±0.01^{a}	9.20±0.06^{a}
		60	8.00±0.01^{a}	4.26±0.03^{a}	0.72±0.02^{a}	9.16±0.04^{b}
180	0.002	0	8.00±0.03^{a}	4.18±0.02^{a}	0.78±0.01^{a}	9.15±0.07^{a}
		30	8.15±0.05^{a}	4.22±0.01^{a}	0.77±0.02^{a}	9.00±0.03^{a}
		60	8.25±0.02^{a}	4.24±0.03^{a}	0.76±0.03^{a}	8.94±0.06^{b}
190	0.004	0	8.10±0.04^{a}	4.05±0.02^{a}	0.80±0.01^{a}	8.95±0.05^{a}
		30	8.15±0.03^{a}	4.24±0.04^{a}	0.72±0.03^{b}	8.70±0.04^{a}
		60	8.30±0.01^{a}	4.26±0.02^{a}	0.72±0.02^{b}	8.60±0.01^{a}

***** *Lp. plantarum* CRD7 probiotic spray dried DVS powder prepared with 0.25% maltodextrin.

Data are represented as Mean ± SE; (p<0.05); n=3. a-b (along the column) values with different superscripts are significantly different from each other.

Table 4.7 (e) : Effect of packaging material (LDPE) and storage temperature (-20ºC) on sensory attributes of probiotic *dahi* prepared with DVS powder of *Lactiplantibacillus plantarum* CRD7.

Inlet temperature (º C)	Inoculum levels of probiotic DVS powder (%w/v)*	Storage period (days)	Colour and appearance	Flavour	Body and texture	Overall acceptability
170	0.004	0	9.6±0.45^{a}	8.6±0.36^{a}	9.0±0.50^{a}	9.3±0.62^{a}
		30	9.3±0.53^{a}	8.3±0.42^{a}	8.6±0.27^{a}	9.0±0.51^{a}
		60	9.0±0.41^{a}	8.0±0.44^{a}	8.3±0.63^{a}	9.0±0.36^{a}
180	0.002	0	9.6±0.35^{a}	8.6±0.51^{a}	9.0±0.48^{a}	9.3±0.41^{a}
		30	9.0±0.38^{a}	8.3±0.37^{a}	8.6±0.41^{a}	9.0±0.28^{a}
		60	8.6±0.45^{a}	8.0±0.41^{a}	8.0±0.43^{a}	8.0±0.36^{a}
190	0.004	0	9.6±0.56^{a}	8.6±0.36^{a}	9.0±0.33^{a}	9.3±0.35^{a}
		30	9.3±0.58^{a}	8.6±0.23^{a}	8.6±0.55^{a}	8.6±0.38^{a}
		60	8.0±0.41^{a}	8.0±0.33^{a}	8.3±0.41^{a}	8.0±0.22^{b}

***** *Lp. plantarum* CRD7 probiotic spray dried DVS powder prepared with 0.25% maltodextrin.

Data are represented as Mean ± SE; (p<0.05); n=3. a-b (along the column) values with different superscripts are significantly different from each other.

Table 4.7 (f) : Effect of packaging material (LDPE) and storage temperature (-20ºC) on texture profile of probiotic *dahi* prepared with DVS powder of *Lactiplantibacillus plantarum* CRD7.

Inlet temperature (º C)	Inoculum levels of probiotic DVS powder (%w/v)*	Storage period (days)	Firmness (N)	Consistency (N.s)	Cohesiveness (N)	Work of cohesion (N.s)
170	0.004	0	2.95±0.03^{a}	43.37±0.25^{a}	-0.57±0.02^{a}	-3.15±0.18^{a}
		30	2.80±0.01^{a}	37.65±0.90^{a}	-0.51±0.03^{a}	-3.05±0.28^{a}
		60	2.69±0.02^{a}	30.14±0.86^{b}	-0.46±0.04^{a}	-2.20±0.14^{b}
180	0.002	0	2.90±0.04^{a}	38.64±0.48^{a}	-0.53±0.02^{a}	-2.30±0.32^{a}
		30	2.74±0.05^{a}	32.01±0.78^{a}	-0.40±0.01^{a}	-2.15±0.26^{a}
		60	2.60±0.04^{a}	30.19±0.36^{a}	-0.37±0.02^{b}	-2.02±0.62^{a}
190	0.004	0	2.85±0.03^{a}	40.35±0.45^{a}	-0.59±0.03^{a}	-3.08±0.38^{a}
		30	2.74±0.01^{a}	34.28±0.36^{a}	-0.50±0.01^{a}	-3.07±0.18^{a}
		60	2.60±0.03^{a}	30.19±0.45^{b}	-0.37±0.03^{b}	-2.01±0.26^{b}

***** *Lp. plantarum* CRD7 probiotic spray dried DVS powder prepared with 0.25% maltodextrin.

Data are represented as Mean ± SE; (p<0.05); n=3. a-b (along the column) values with different superscripts are significantly different from each other.

4.7.3 : Effect of packaging material (EVOH) and storage temperature (-20ºC) on techno-functional attributes of probiotic *dahi* prepared with DVS powder of *Lactiplantibacillus plantarum* CRD7

4.7.3 (a): TECHNO-FUNCTIONAL ATTRIBUTES

Observations on techno-functional attributes of probiotic *dahi* prepared with optimized inoculum level of spray dried DVS have been documented in Table 4.7 (i). The curd setting time of probiotic *Lp. plantarum* DVS for the preparation of *dahi* with packaged in EVOH and stored at -20ºC ranged from 7.45-8.35 h, respectively. pH and titratable acidity ranged from 4.01-4.26 and 0.72-0.82 %LA, respectively and total probiotic counts ranged from 8.62±0.03 to 9.35±0.03 Log CFU/mL as mentioned in **Table 4.7 (i).** Probiotic counts of *dahi* prepared with *Lp. plantarum* CRD7 DVS manufactured with maltodextrin @2.5 %(w/v) as protective agent were recorded for 0th day (9.35±0.03) 30th day (9.20±0.04) and 60th (9.00±0.02). There was no significant difference observed from 0th to 60th day of storage at -20ºC in techno-functional attributes of probiotic *dahi*

prepared with spray dried DVS. These results revealed no adverse effect of packaging material (EVOH) and storage temperature of -20°C upto 60 days of storage of spray dried probiotic DVS.

4.7.3 (b) : SENSORY SCORES

Observations on sensory parameters such as colour and appearance (10), flavour (10), body and texture (10) and overall acceptability (10) of probiotic *dahi* prepared with DVS starter stored in EVOH at temperature of -20°C have been recorded in **Table 4.7 (j)**. Overall acceptability score for *Lp. plantarum* CRD7 *dahi* prepared with DVS manufactured with maltodextrin @2.5 %(w/v) as protective agent for 0^{th} day (9.6±0.44), 30^{th} day (9.3±0.31) and 60^{th} day (8.6±0.29). Almost a similar treds was noticed in sensory scores w.r.t colour and appearance, flavour, body and texture. No-significant difference in sensory scores of probiotic *dahi* prepared from DVS and stored at -20°C in EVOH from 0^{th} to 60^{th} day.

4.7.3 (c) : TEXTURE PROFILES

Data on texture parameters such as firmness (N), consistency (N.s), cohesiveness (N) and work of cohesion (N.s) of probiotic *dahi* prepared with DVS starter stored in EVOH at -20°C have been recorded in **Table 4.7 (k**). Firmness of probiotic *dahi* prepared with DVS of *Lp. plantarum* CRD7 manufactured with maltodextrin @2.5% (w/v) as protective agent was documented for 0^{th} day (2.95±0.04 N), 30^{th} day (2.70±0.02 N) and 60^{th} day (2.57±0.04 N). Almost similar observations w.r.t consistency, cohesiveness and work of cohesion were noticed for *dahi* prepared with spray dried probiotic DVS. There was no significant difference from 0^{th} to 60^{th} of storage of probiotic spray dried DVS of *Lp. plantarum* CRD7 as revealed by texture parameters. It can be calculated from results of storage of probiotic DVS of *Lp. plantarum* CRD7 in EVOH upto 60 days have no adverse effect on starter activity performance.

Table 4.7 (i) : Effect of packaging material (EVOH) and storage temperature (-20ºC) on techno-functional attributes of probiotic *dahi* prepared by DVS powder of *Lactiplantibacillus plantarum* CRD7.

Inlet temperature(º C)	Inoculum levels of probiotic DVS powder (%w/v)*	Storage period (days)	Curd setting time (h)	pH	Titratable acidity (%LA)	Total probiotic counts (Log CFU/mL)
170	0.004	0	7.45±0.01^{a}	4.26±0.01^{a}	0.77±0.02^{b}	9.35±0.03^{a}
		30	7.55±0.05^{a}	4.19±0.03^{a}	0.72±0.01^{b}	9.20±0.04^{a}
		60	8.15±002^{a}	4.24±0.01^{a}	0.78±0.01^{a}	9.00±0.02^{a}
180	0.002	0	8.00±0.01^{a}	4.18±0.02^{a}	0.78±0.03^{a}	9.15±0.01^{a}
		30	8.20±0.05^{a}	4.24±0.01^{a}	0.76±0.00^{a}	9.00±0.03^{a}
		60	8.30±0.02^{a}	4.22±0.02^{a}	0.77±0.01^{a}	8.89±0.02^{b}
190	0.004	0	8.10±0.05^{a}	4.26±0.05^{a}	0.72±0.03^{b}	8.95±0.01^{a}
		30	8.15±0.01^{a}	4.25±0.03^{a}	0.72±0.02^{b}	8.70±0.01^{a}
		60	8.35±0.04^{a}	4.01±0.04^{a}	0.82±0.01^{a}	8.62±0.03^{b}

*****_Lp. plantarum_ CRD7 probiotic spray dried DVS powder prepared with 0.25% maltodextrin.

Data are represented as Mean ± SE; (p<0.05); n=3. a-b (along the column) values with different superscripts are significantly different from each other.

Table 4.7 (j) : Effect of packaging material (EVOH) and storage temperature (-20ºC) on sensory attributes of probiotic *dahi* prepared with DVS powder of *Lactiplantibacillus plantarum* CRD7.

Inlet temperature (º C)	Inoculum levels of probiotic DVS powder (%w/v)*	Storage period (days)	Colour and appearance	Flavour	Body and texture	Overall acceptability
170	0.004	0	9.6±0.44^{a}	8.6±0.87^{a}	9.0±0.29^{a}	9.3±0.61^{a}
		30	9.3±0.31^{a}	8.3±0.63^{a}	8.6±0.36^{a}	9.0±0.47^{a}
		60	8.6±0.29^{a}	8.0±0.70^{a}	8.3±0.41^{a}	8.6±0.23^{a}
180	0.002	0	9.6±0.21^{a}	8.6±0.61^{a}	9.0±0.39^{a}	9.3±0.43^{a}
		30	8.6±0.36^{a}	8.3±0.43^{a}	8.6±0.41^{a}	8.3±0.31^{a}
		60	8.0±0.43^{a}	8.0±0.51^{a}	8.0±0.38^{a}	8.0±0.33^{b}
190	0.004	0	9.6±0.47^{a}	8.6±0.50^{a}	9.0±0.42^{a}	9.3±0.38^{a}
		30	8.6±0.36^{a}	8.3±0.42^{a}	8.3±0.33^{a}	8.3±0.49^{a}
		60	8.0±0.28^{a}	8.0±0.31^{a}	8.3±0.23^{a}	8.0±0.74^{b}

***** _Lp. plantarum_ CRD7 probiotic spray dried DVS powder prepared with 0.25% maltodextrin.

Data are represented as Mean ± SE; (p<0.05); n=3. a-b (along the column) values with different superscripts are significantly different from each other.

Table 4.7 (k) : Effect of packaging material (EVOH) and storage temperature (-20ºC) on texture profile of probiotic *dahi* prepared by DVS powder of *Lactiplantibacillus plantarum* CRD7.

Inlet temperature (º C)	Inoculum levels of probiotic DVS powder (%w/v)*	Storage period (days)	Firmness (N)	Consistency (N.s)	Cohesiveness (N)	Work of cohesion (N.s)
170	0.004	0	2.95±0.04^{a}	43.37±0.25^{a}	-0.57±0.03^{a}	-3.15±0.28^{a}
		30	2.70±0.02^{a}	37.65±0.75^{a}	-0.51±0.01^{a}	-3.02±0.14^{a}
		60	2.57±0.04^{b}	28.10±0.86^{b}	-0.40±0.01^{a}	-2.17±0.18^{b}
180	0.002	0	2.90±0.01^{a}	38.64±0.49^{a}	-0.53±0.01^{a}	-2.30±0.32^{a}
		30	2.74±0.02^{a}	32.01±0.86^{a}	-0.40±0.03^{a}	-2.15±0.38^{a}
		60	2.69±0.04^{a}	30.19±0.25^{a}	-0.37±0.01^{a}	-2.02±0.62^{a}
190	0.004	0	2.85±0.05^{a}	40.35±0.49^{a}	-0.59±0.03^{a}	-3.49±0.26^{a}
		30	2.74±0.02^{a}	34.28±0.25^{a}	-0.50±0.02^{a}	-3.07±0.38^{a}
		60	2.58±0.04^{a}	35.29±0.75^{a}	-0.42±0.03^{a}	-3.02±0.32^{a}

***** *Lp. plantarum* CRD7 probiotic spray dried DVS powder prepared with 0.25% maltodextrin.

Data are represented as Mean ± SE; (p<0.05); n=3. a-b (along the column) values with different superscripts are significantly different from each other.

4.7.4 : Effect of packaging material (aluminium laminate) and storage temperature (4ºC) on techno-functional attributes of probiotic *dahi* prepared by DVS powder of *Lactiplantibacillus plantarum* CRD7

4.7 (a): TECHNO-FUNCTIONAL ATTRIBUTES

The curd setting time taken by probiotic *Lp. plantarum* DVS starter for the preparation of probiotic *dahi* from DVS starter packaged in aluminium laminate and stored at -4^{o}C ranged from 7.45-8.00 h, respectively. pH and titratable acidity ranged from 4.18-4.26 and 0.71-0.78 %LA, respectively and total probiotic counts ranged from 8.64±0.04 to 9.35±0.01 Log CFU/mL as recorded in **Table 4.7 (l).** Probiotic counts of *dahi* prepared with *Lp. plantarum* CRD7 DVS manufactured with maltodextrin @2.5 %(w/v) as protective 0th

day (9.35±0.01) 30th day (9.20±0.03) and 60th (9.05±0.06) were found in *dahi* prepared with *Lp. plantarum* CRD7 DVS of agent. There was no significant difference from 0th to 60th day of storage at 4^{o}C in techno-functional attributes of *dahi* prepared from probiotic spray dried DVS of *Lp. plantarum* CRD7 stored at 4^{o}C in aluminium laminates upto 60 days indicative of no adverse effect on starter activity.

4.7.4 (b) : SENSORY SCORES

Observations on sensory parameters such as colour and appearance (10), flavour (10), body and texture (10) and overall acceptability (10) of probiotic *dahi* prepared with DVS starter stored in aluminium laminates at temperature 4^{o}C have been presented in **Table 4.7 (m)**. Overall acceptability score for *Lp plantarum* CRD7 prepared with *dahi* with maltodextrin @2.5 %(w/v) as protective agent for 0th day (9.3±0.36), 30th day (9.0±0.44) and 60th day (8.6±0.75). Almost a similar treds for sensory scores w.r.t color and appearance, flavour, body and texture. No-significant difference in sensory scores was observed of probiotic *dahi* prepared from spray dried from stored at 4^{o}C for 0th to 60th day in aluminium laminates.

4.7.4 (c) : TEXTURE PROFILES

Data on texture parameters such as firmness (N), consistency (N.s), cohesiveness (N) and work of cohesion (N.s) of probiotic *dahi* prepared with spray dried DVS starter stored in aluminium laminates at 4^{o}C have been recorded in **Table 4.7 (m)**. Firmness of probiotic *dahi* prepared with DVS of *Lp. plantarum* CRD7 manufactured with maltodextrin @2.5% (w/v) as protective agent for 0th day (2.95±0.04 N), 30th day (2.80±0.02 N)and 60th day (2.75±0.01 N). A similar trend w.r.t consistency, cohesiveness and work of cohesion was observed in probiotic dahi prepared with *Lp. plantarum* CRD7 DVS. There was no significant difference in textured parameter of probiotic *dahi* prepared with DVS *Lp. plantarum* CRD7 stored upto 60 days at 4^{o}C in aluminium laminates indicative no adverse effect on activity of starters.

Table 4.7 (l) : Effect of packaging material (aluminium laminate) and storage temperature (4°C) on techno-functional attributes of probiotic *dahi* prepared by DVS powder of *Lactiplantibacillus plantarum* CRD7.

Inlet temperature (° C)	Inoculum levels of probiotic DVS powder (%w/v)*	Storage period (days)	Curd setting time (h)	pH	Titratable acidity (%LA)	Total probiotic counts (Log CFU/mL)
170	0.004	0	7.45±0.02^{a}	4.22±0.02^{a}	0.77±0.05^{a}	9.35±0.01^{a}
		30	7.50±0.05^{a}	4.24±0.01^{a}	0.77±0.02^{a}	9.20±0.03^{a}
		60	8.00±0.01^{a}	4.24±0.03^{a}	0.71±0.03^{a}	9.05±0.06^{a}
180	0.002	0	8.00±0.03^{a}	4.18±0.02^{a}	0.78±0.01^{a}	9.15±0.07^{a}
		30	8.10±0.02^{a}	4.20±0.04^{a}	0.74±005^{a}	9.02±0.03^{a}
		60	8.25±0.06^{b}	4.20±0.05^{a}	0.74±0.04^{a}	8.92±0.01^{b}
190	0.004	0	8.10±0.04^{a}	4.20±0.03^{a}	0.74±0.01^{a}	8.95±0.05^{a}
		30	8.25±0.02^{a}	4.24±0.09^{a}	0.72±0.06^{a}	8.70±0.03^{a}
		60	8.35±0.06^{a}	4.26±0.04^{a}	0.72±0.03^{a}	8.64±0.04^{b}

***** *Lp. plantarum* CRD7 probiotic spray dried DVS powder prepared with 0.25% maltodextrin.

Data are represented as Mean ± SE; (p<0.05); n=3. a-b (along the column) values with different superscripts are significantly different from each other.

Table 4.7 (m) : Effect of packaging material (aluminium laminate) and storage temperature (4°C) on sensory attributes of probiotic *dahi* prepared by DVS powder of *Lactiplantibacillus plantarum* CRD7.

Inlet temperature (° C)	Inoculum level of probiotic DVS powder (%w/v)*	Storage period (days)	Colour and appearance	Flavour	Body and texture	Overall acceptability
170	0.004	0	9.6±0.41^{a}	8.6±0.28^{a}	9.0±0.29^{a}	9.3±0.36^{a}
		30	9.3±0.33^{a}	8.6±0.41^{a}	8.6±0.57^{a}	9.0±0.44^{a}
		60	8.6±0.56^{b}	8.0±0.19^{a}	8.0±0.41^{b}	8.6±0.75^{a}
180	0.002	0	9.6±0.41^{a}	8.6±0.27^{a}	9.0±0.38^{a}	9.3±0.81^{a}
		30	9.0±0.23^{a}	8.3±0.50^{a}	8.6±0.57^{a}	9.0±0.70^{a}
		60	9.0±0.39^{a}	8.3±0.61^{a}	8.6±0.49^{a}	8.0±0.52^{b}
190	0.004	0	9.6±0.45^{a}	8.6±0.53^{a}	9.0±0.23^{a}	9.3±0.38^{a}
		30	9.0±0.39^{a}	8.6±0.50^{a}	8.6±0.45^{a}	9.0±0.71^{a}
		60	8.6±0.41^{b}	8.0±0.42^{a}	8.0±0.33^{b}	8.0±0.47^{b}

***** *Lp. plantarum* CRD7 probiotic spray dried DVS powder prepared with 0.25% maltodextrin.

Data are represented as Mean ± SE; (p<0.05); n=3. a-b (along the column) values with different superscripts are significantly different from each other.

Table 4.7 (n) : Effect of packaging material (aluminium laminate) and storage temperature (4ºC) on texture profile of probiotic *dahi* prepared by DVS powder of *Lactiplantibacillus plantarum* CRD7.

Inlet temperature (º C)	Inoculum levels of probiotic DVS powder (%w/v)*	Storage period (days)	Firmness (N)	Consistency (N.s)	Cohesiveness (N)	Work of cohesion (N.s)
170	0.004	0	2.95±0.04[a]	43.37±0.25[a]	-0.57±0.01[a]	-3.15±0.18[a]
		30	2.80±0.02[a]	38.65±0.86[a]	-0.51±0.02[a]	-3.02±0.28[a]
		60	2.75±0.01[a]	30.04±0.75[b]	-0.42±0.03[b]	-2.12±0.14[b]
180	0.002	0	2.90±0.05[a]	38.64±0.18[a]	-0.53±0.01[a]	-2.30±0.32[a]
		30	2.76±0.03[a]	34.01±0.49[a]	-0.42±0.02[a]	-2.18±0.14[a]
		60	2.64±0.02[a]	31.19±0.25[b]	-0.34±0.02[b]	-2.04±0.14[a]
190	0.004	0	2.85±0.04[a]	40.35±0.18[a]	-0.59±0.01[a]	-3.08±0.38[a]
		30	2.82±0.01[a]	34.27±0.49[a]	-0.49±0.03[a]	-3.07±0.62[a]
		60	2.70±0.04[a]	30.20±0.18[b]	-0.37±0.02[b]	-2.49±0.28[b]

***** *Lp. plantarum* CRD7 probiotic spray dried DVS powder prepared with 0.25% maltodextrin.

Data are represented as Mean ± SE; (p<0.05); n=3. a-b (along the column) values with different superscripts are significantly different from each other.

4.7.5 : Effect of packaging material (LDPE) and storage temperature (4ºC) on techno-functional attributes of probiotic *dahi* prepared by DVS powder of *Lactiplantibacillus plantarum* CRD7

4.7.5 (a): TECHNO-FUNCTIONAL ATTRIBUTES

The curd setting time taken by probiotic *Lp. plantarum* DVS starter for the preparation of *dahi* starter packaged in LDPE and stored at temperature 4ºC ranged from 7.45-8.45 h, respectively. pH and titratable acidity ranged from 4.18-4.26 and 0.72-0.78 % LA and total probiotic counts ranged from 8.54±0.05 to 9.35±0.02 Log CFU/mL as recorded in **Table 4.7 (o).** Probiotic counts of *dahi* prepared with *Lp. plantarum* CRD7 starter manufactured with maltodextrin @2.5 %(w/v) for 0th day (9.35±0.02) 30th day (9.10±0.03) and 60th (9.00±0.06) Log CFU/mL. There was no significant difference observed in techno-functional attributes of *dahi* prepared from spray dried DVS of *Lp. plantarum* CRD7 stored

at 4°C upto 60 days revealing no adverse effect on activity of starter in LDPE packaging material.

4.7.5 (b) : SENSORY SCORES

Observations on sensory parameters such as colour and appearance (10), flavour (10), body and texture (10) and overall acceptability (10) of probiotic *dahi* prepared with DVS starter stored in LDPE at temperature of 4°C have been presented in **Table 4.7 (p)**. Overall acceptability score for probiotic *dahi* prepared with DVS powder of *Lp. plantarum* CRD7 prepared with maltodextrin @2.5 %(w/v) as protective agent for 0^{th} day (9.3±0.32), 30^{th} day (8.6±0.35) and 60^{th} day (8.3±0.49). Almost similar observations were recorded for other sensory parameters such as color and appearance, flavour, body and texture. No-significant difference was observed in sensory scores of probiotic *dahi* was noticed prepared with DVS stored at 4°C in LDPE indicative of no adverse effect on starter activity upto 60 days.

4.7.5 (c) : Texture profiles

Data on texture parameters such as firmness (N), consistency (N.s), cohesiveness (N) and work of cohesion (N.s) of probiotic *dahi* prepared with DVS starter stored in LDPE at 4°C have been recorded in **Table 4.7 (q**). Firmness of probiotic *dahi* prepared with DVS starter of *Lp. plantarum* CRD7 manufactured with maltodextrin @2.5% (w/v) as protective agent for 0^{th} day (2.95±0.01 N), 30^{th} day (2.70±0.04 N)and 60^{th} day (2.60±0.02 N). There was no significant difference in *dahi* prepared with spray dried DVS *Lp. plantarum* CRD7. Textured profile parameter indicates no adverse effect of storage at 4°C in LDPE on activity of starter.

Table 4.7 (o) : Effect of packaging material (LDPE) and storage temperature (4ºC) on techno-functional attributes of probiotic *dahi* prepared with DVS powder of *Lactiplantibacillus plantarum* CRD7.

Inlet temperature (º C)	Inoculum levels of probiotic DVS powder (%w/v)*	Storage period (days)	Curd setting time (h)	pH	Titratable acidity (%LA)	Total probiotic counts (Log CFU/mL)
170	0.004	0	7.45±0.02^{a}	4.20±0.02^{a}	0.77±0.02^{a}	9.35±0.02^{a}
		30	8.00±0.01^{a}	4.24±0.01^{a}	0.77±0.05^{a}	9.10±0.03^{a}
		60	8.05±0.04^{a}	4.24±0.03^{a}	0.74±0.03^{a}	9.00±0.06^{a}
180	0.002	0	8.00±0.06^{b}	4.18±0.02^{b}	0.78±0.01^{a}	9.15±0.01^{a}
		30	8.10±0.01^{a}	4.20±0.01^{a}	0.77±0.01^{a}	9.00±0.07^{a}
		60	8.35±0.05^{a}	4.24±0.04^{a}	0.74±0.02^{b}	8.87±0.04^{b}
190	0.004	0	8.10±0.01^{b}	4.20±0.02^{a}	0.74±0.05^{a}	8.95±0.05^{a}
		30	8.25±0.06^{a}	4.24±0.01^{a}	0.72±0.02^{a}	8.70±0.01^{a}
		60	8.45±0.04^{a}	4.26±0.04^{a}	0.72±0.01^{a}	8.54±0.05^{b}

***** *Lp. plantarum* CRD7 probiotic spray dried DVS powder prepared with 0.25% maltodextrin.

Data are represented as Mean ± SE; (p<0.05); n=3. a-b (along the column) values with different superscripts are significantly different from each other.

Table 4.7 (p) : Effect of packaging material (LDPE) and storage temperature (4ºC) on sensory attributes of probiotic *dahi* prepared by DVS powder of *Lactiplantibacillus plantarum* CRD7.

Inlet temperature (º C)	Inoculum levels of probiotic DVS powder (%w/v)*	Storage period (days)	Colour and appearance	Flavour	Body and texture	Overall acceptability
170	0.004	0	9.6±0.45^{a}	8.6±0.51^{a}	9.0±0.36^{a}	9.3±0.32^{a}
		30	8.3±0.33^{a}	8.3±0.49^{a}	8.0±0.45^{a}	8.6±0.35^{a}
		60	8.0±0.27^{b}	8.0±0.42^{a}	8.3±0.53^{a}	8.3±0.49^{b}
180	0.002	0	9.6±0.19^{a}	8.6±0.36^{a}	9.0±0.56^{a}	9.3±0.40^{a}
		30	8.0±0.38^{a}	8.3±0.33^{a}	8.0±0.21^{a}	8.3±0.33^{a}
		60	8.0±0.41^{b}	8.0±0.41^{a}	8.0±0.36^{a}	8.0±0.41^{b}
190	0.004	0	9.6±0.55^{a}	8.6±0.55^{a}	9.0±0.41^{a}	9.3±0.75^{a}
		30	9.3±0.63^{a}	8.6±0.63^{a}	8.6±0.63^{a}	8.6±0.27^{a}
		60	8.0±0.49^{b}	7.6±0.47^{b}	8.0±0.57^{a}	8.0±0.43^{b}

***** *Lp. plantarum* CRD7 probiotic spray dried DVS powder prepared with 0.25% maltodextrin.

Data are represented as Mean ± SE; (p<0.05); n=3. a-b (along the column) values with different superscripts are significantly different from each other.

Table 4.7 (q) : Effect of packaging material (LDPE) and storage temperature (4ºC) on texture profile of probiotic *dahi* prepared by DVS powder of *Lactiplantibacillus plantarum* CRD7.

Inlet temperature (º C)	Inoculum levels of probiotic DVS powder (%w/v)*	Storage period (days)	Firmness (N)	Consistency (N.s)	Cohesiveness (N)	Work of cohesion (N.s)
170	0.004	0	2.95±0.01^{a}	43.37±0.18^{a}	-0.57±0.01^{a}	-3.15±0.28^{a}
		30	2.70±0.04^{a}	34.21±0.25^{a}	-0.51±0.02^{a}	-3.00±0.18^{a}
		60	2.60±0.02^{a}	31.14±0.49^{b}	-0.40±0.01^{b}	-2.19±0.64^{b}
180	0.002	0	2.90±0.01^{a}	38.64±0.25^{a}	-0.53±0.02^{a}	-2.30±0.18^{a}
		30	2.84±0.02^{a}	32.04±0.18^{a}	-0.39±0.01^{a}	-2.15±0.14^{a}
		60	2.51±0.05^{a}	30.29±0.75^{b}	-0.35±0.02^{b}	-2.12±0.14^{b}
190	0.004	0	2.85±0.04^{a}	40.35±0.49^{a}	-0.59±0.03^{a}	-3.08±0.32^{a}
		30	2.64±0.08^{a}	36.28±0.37^{a}	-0.52±0.02^{a}	-3.07±0.38^{a}
		60	2.58±0.02^{a}	32.17±0.32^{b}	-0.37±0.02^{b}	-2.01±0.62^{b}

***** *Lp. plantarum* CRD7 spray dried DVS powder prepared with 0.25% maltodextrin.

Data are represented as Mean ± SE; (p<0.05); n=3. a-b (along the column) values with different superscripts are significantly different from each other.

4.7.6: Effect of packaging material (EVOH) and storage temperature (4ºC) on techno-functional attributes of probiotic *dahi* prepared with DVS powder of *Lactiplantibacillus plantarum* CRD7

4.7.6 (a): TECHNO-FUNCTIONAL ATTRIBUTES

The curd setting time of probiotic *Lp. plantarum* DVS starter for the preparation of *dahi* which was packaged in EVOH and stored at temperature 4ºC ranged from 7.45-8.45 h, respectively. pH and titratable acidity ranged from 4.18-4.26 and 0.72-0.78 % LA and total probiotic counts ranged from 8.55±0.01 to 9.35±0.06 Log CFU/mL have been mentioned in **Table 4.7 (r).** Probiotic counts of *dahi* prepared with *Lp. plantarum* CRD7 DVS with maltodextrin @2.5 %(w/v) as protective agent for 0th day (9.35±0.06) 30th day (9.05±0.07) and 60th (8.98±0.01) Log CFU/mL. There was no significant difference noticed in techno-functional attributes of *dahi* prepared with DVS *Lp. plantarum* CRD7 stored at 4ºC in EVOH upto 60 days. Indicative no adverse effect on starter activity.

4.7.6 (b) : Sensory scores

Observations on sensory parameters such as colour and appearance (10), flavour (10), body and texture (10) and overall acceptability (10) of probiotic *dahi* prepared by DVS starter stored in EVOH at 4^{o}C have been mentioned in **Table 4.7 (s)**. Overall acceptability score for probiotic *dahi* prepared with DVS of *Lp. plantarum* CRD7 manufactured with maltodextrin @2.5% (w/v) as protective agent for 0th day (9.3±0.71), 30th day (8.0±0.65) and 60th day (8.0±0.43). A similar trend for other scores *i.e.,* color and appearance, flavour, body and texture was documented for probiotic *dahi.* No-significant difference in sensory scores of probiotic *dahi* prepared from DVS *Lp. plantarum* CRD7 stored at 4^{o}C in EVOH was recorded upto 60 days of storage indicative of its stability without loss of activity.

4.7.6 (c) : TEXTURE PROFILES

Data on texture parameters such as firmness (N), consistency (N.s), cohesiveness (N) and work of cohesion (N.s) of probiotic *dahi* prepared with DVS starter stored in EVOH at 4^{o}C have been recorded in **Table 4.7 (t**). Firmness of probiotic *dahi* prepared with DVS of *Lp. plantarum* CRD7 manufactured with maltodextrin @2.5% (w/v) as protective agent have been documented for 0th day (2.95±0.04 N), 30th day (2.70±0.01 N)and 60th day (2.50±0.02 N). There was no significant difference in textural profile of probiotic *dahi* prepared with spray dried DVS stored at 4^{o}C in EVOH upto 60 days of storage. These results have indicated no adverse effect on DVS starter activity upto 60 days.

Table 4.7 (r) : Effect of packaging material (EVOH) and storage temperature (4ºC) on techno-functional attributes of probiotic *dahi* prepared by DVS powder of *Lactiplantibacillus plantarum* CRD7.

Inlet temperature (º C)	Inoculum level of probiotic DVS powder (%w/v)*	Storage period (days)	Curd setting time (h)	pH	Titratable acidity (%LA)	Total probiotic counts (Log CFU/mL)
170	0.004	0	7.45±0.05^{a}	4.20±0.03^{a}	0.77±0.02^{a}	9.35±0.06^{a}
		30	8.00±0.02$_{a}$	4.24±0.02^{a}	0.74±0.05^{a}	9.05±0.07^{a}
		60	8.20±0.01^{a}	4.26±0.01^{a}	0.72±0.01^{b}	8.98±0.01^{b}
180	0.002	0	8.00±0.06^{a}	4.18±0.05^{a}	0.78±0.03^{a}	9.15±0.05^{a}
		30	8.30±0.02^{a}	4.22±0.04^{a}	0.76±0.05^{a}	9.02±0.03^{a}
		60	8.45±0.05^{a}	4.26±0.03^{a}	0.72±0.04^{b}	8.80±0.04^{b}
190	0.004	0	8.10±0.06^{a}	4.20±0.02^{a}	0.74±0.08^{a}	8.95±0.08^{a}
		30	8.15±0.02^{a}	4.25±0.04^{a}	0.72±0.06^{a}	8.72±0.02^{a}
		60	8.45±0.04^{a}	4.26±0.03^{a}	0.72±0.05^{a}	8.55±0.01^{b}

***** *Lp. plantarum* CRD7 probiotic spray dried DVS powder prepared with 0.25% maltodextrin.

Data are represented as Mean ± SE; (p<0.05); n=3. a-b (along the column) values with different superscripts are significantly different from each other.

Table 4.7 (s) : Effect of packaging material (EVOH) and storage temperature (4ºC) on sensory attributes of probiotic *dahi* prepared by DVS powder of *Lactiplantibacillus plantarum* CRD7.

Inlet temperature (º C)	Inoculum levels of probiotic DVS powder (%w/v)*	Storage period (days)	Colour and appearance	Flavour	Body and texture	Overall acceptability
170	0.004	0	9.6±0.33^{a}	8.6±0.28^{a}	9.0±0.61^{a}	9.3±0.71^{a}
		30	8.0±0.29^{b}	7.6±0.39^{b}	8.0±0.32^{b}	8.0±0.63^{b}
		60	8.0±0.36^{b}	8.0±0.41^{a}	8.0±0.43^{b}	8.0±0.43^{b}
180	0.002	0	9.6±0.52^{a}	8.6±0.55^{a}	9.0±0.14^{a}	9.3±0.36^{a}
		30	8.0±0.49^{b}	8.3±0.61^{a}	8.3±0.52^{a}	8.0±0.43^{b}
		60	8.0±0.37^{b}	8.0±0.54^{a}	8.0±0.27^{a}	8.0±0.22^{b}
190	0.004	0	9.6±0.39^{a}	8.6±0.59^{a}	9.0±0.33^{a}	9.3±0.38^{a}
		30	9.0±0.43^{a}	8.0±0.65^{a}	8.0±0.49^{a}	8.0±0.43^{b}
		60	7.6±0.81^{b}	7.6±0.70^{b}	9.0±0.55^{a}	8.0±0.40^{b}

***** *Lp. plantarum* CRD7 probiotic spray dried DVS powder prepared with 0.25% maltodextrin.

Data are represented as Mean ± SE; (p<0.05); n=3. a-b (along the column) values with different superscripts are significantly different from each other.

Table 4.7 (t) : Effect of packaging material (EVOH) and storage temperature (4ºC) on texture profile of probiotic *dahi* prepared by DVS powder of *Lactiplantibacillus plantarum* CRD7.

Inlet temperature (º C)	Inoculum levels of probiotic DVS powder (%w/v)*	Storage period (days)	Firmness (N)	Consistency (N.s)	Cohesiveness (N)	Work of cohesion (N.s)
170	0.004	0	2.95±0.04^{a}	43.37±0.25^{a}	-0.57±0.01^{a}	-3.15±0.28^{a}
		30	2.70±0.01^{a}	37.65±0.75^{a}	-0.51±0.02^{a}	-3.02±0.18^{a}
		60	2.50±0.02^{b}	26.10±0.18^{b}	-0.42±0.06^{b}	-2.07±0.14^{b}
180	0.002	0	2.90±0.01^{a}	38.64±0.49^{a}	-0.53±0.04^{a}	-2.30±0.32^{a}
		30	2.54±0.02^{a}	30.01±0.25^{b}	-0.42±0.05^{a}	-2.09±0.14^{a}
		60	2.39±0.02^{b}	30.19±0.49^{b}	-0.31±0.07^{b}	-2.01±0.18^{b}
190	0.004	0	2.85±0.04^{a}	40.35±0.22^{a}	-0.59±0.01^{a}	-3.49±0.14^{a}
		30	2.74±0.01^{a}	34.28±0.86^{b}	-0.50±0.08^{a}	-3.27±0.32^{a}
		60	2.68±0.04^{a}	35.29±0.37^{b}	-0.42±0.03^{b}	-3.10±0.38^{a}

***** *Lp. plantarum* CRD7 probiotic spray dried DVS powder prepared with 0.25% maltodextrin.

Data are represented as Mean ± SE; (p<0.05); n=3. a-b (along the column) values with different superscripts are significantly different from each other.

CHAPTER –5

Summary and Conclusions

SUMMARY AND CONCLUSION

Optimization of spray drying conditions and selection of suitable protectants is essential prior to preparation of DVS starters, as non-optimization of these parameters may lead to low viability counts and poor performance. The result of optimization of spray drying conditions and selection of suitable protectant has been summarized here under:

- Two probiotic *Lactiplantibacillus plantarum* CRD7 and *Lp. plantarum* HD48 strains were evaluated for purity, techno-functional, sensory and textural parameters in milk system. Both strains exhibited purity and compatibility to each other in milk system by co-culturing. Thus, probiotic lactobacilli strains can be used for preparation of single and mixed strains starters for preparation of probiotic *dahi*.
- Two probiotic strains of *Lactiplantibacillus plantarum* CRD7, *Lp. plantarum* HD48. demonstrated better functionality w.r.t techno-functional, sensory and textural attributes in milk system in the form of probiotic *dahi* prepared with single cultures and combinations thereof indicative of possibility for preparation of mixed starters for preparation of fermented dairy foods.
- Among two probiotic cultures, *Lp. plantarum* CRD7 exhibited short curd setting time of 14.33±0.02 h, faster reduction of pH (4.26±0.01) and titratable acidity (0.72±0.03 % LA) development. Similarly, highest total probiotic counts 8.71±0.02 Log CFU/mL were documented.
- Heat tolerance of probiotic *Lp. plantarum* CRD7 and *Lp. plantarum* HD48 were conducted by heat challenge experiment by re-suspending respective biomass in 30% sterilized reconstituted skim milk (RSM) supplemented with varied concentrations (0, 0.5, 2.5, 5.0, 7.5 and 10% w/v) of respective protective agent *i.e.* lactose, sorbitol and maltodextrin. Among three protective agent maltodextrin @2.5% exhibited better survivability rate of 95.27±0.24 at 1 min exposure time and 95.15±0.30 at 5 min exposure time in 55^{o}C exposure temperature.
- On the basis of heat challenge experiment *Lp. plantarum* CRD7 @2.5% (w/v) maltodextrin was selected as protective agent for spray drying for preparation of probiotic DVS starter at different inlet temperatures of 170, 180 and 190^{o}C.

- Inoculum level of spray dried DVS @0.004% (w/v) was optimized as it demonstrated better results w.r.t. tecno-functional, sensory and textural profile of probiotic *dahi* prepared with probiotic spray dried DVS of *Lp. plantarum* CRD7 prepared with maltodextrin @2.5% at inlet temperature of 170^{o}C.
- Optimized inoculum level of 0.002% (w/v) showed better performance w.r.t. tecno-functional, sensory and textural profile of probiotic *dahi* prepared with probiotic spray dried DVS of *Lp. plantarum* CRD7 manufactured with maltodextrin @2.5% as protectant at inlet temperature of 180^{o}C.
- Optimized inoculum level of 0.004% (w/v) showed better results w.r.t. tecno-functional, sensory and textural profile of probiotic *dahi* prepared with probiotic spray dried DVS of *Lp. plantarum* CRD7 prepared with maltodextrin @2.5% as protectant at inlet temperature of 190^{o}C.
- Three types of probiotic DVS starters prepared at inlet temperature of 170, 180 and 190^{o}C were packed in three type of packaging materials i.e., aluminium laminates, LDPE and EVOH and stored at two temperature i.e. -20^{o}C and 4^{o}C upto two months. There was no significance difference observed in quality parameters of probiotic *dahi* prepared from stored DVS starters in three packaging materials at -20^{o}C and 4^{o}C upto 60 days at time interval of month.
- The cell survivability of selected probiotic *Lp. plantarum* CRD7 is affected by the type and concentration of the cryoprotective agent. Thus, it important to select a desired protective agent for better survivability during spray drying of probiotic DVS preparation and subsequently during storage.
- It is concluded that prepared spray dried DVS starter of probiotic *Lp. plantarum* CRD7 could be utilized for the preparation of health-promoting fermented dairy foods.

Conclusion :

On the basis of heat challenge experiments maltodextrin @2.5%(w/v) was selected for improved probiotic survivability for preparation of DVS starter of *Lp. plantarum* CRD7. The inoculum levels of 0.004, 0.002 and 0.004% (w/v) were optimized for spray dried probiotic *Lp. plantarum* CRD7 manufactured at 170, 180 and 190^{o}C inlet temperature respectively, for *dahi* preparation. Probiotic spray dried DVS of *Lp.*

planatarum CRD7 packed in all three packaging materials (aluminium laminates, LDPE and EVOH remain stable with loss of any activity upto 60 days at 4^{o}C and -20^{o}C. Thus, it is concluded that process for spray dried DVS would be utilized for large scale production *vis-à-vis*, for preparation of probiotic fermented milk for promotion of health of consumers.

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Appendix

APPENDIX

MICROBIAL GROWTH MEDIA USED

1. Media Composition

MRS Broth

Ingredients	Quantity g/(L)
Proteose peptone	10.00
Beef extract	10.00
Yeast extract	5.00
Dextrose	20.00
Polysorbate 80	1.00
Ammonium citrate	2.00
Sodium acetate	5.00
Magnesium sulphate	0.10
Manganese sulphate	0.05
Dipotassium hydrogen phosphate	2.00
Final pH (at 25°C)	6.5±0.2

Violet red bile agar(VRBA)

Ingredients	Quantity
Yeast Extract	3.0g
Enzymatic Digest of Gelatin	7.0 g
Bile salts Mixture	1.5g
Lactose	10.0 g
Sodium Chloride	5.0 g
Neutral red	0.03 g
Crystal Violet	0.002 g

Agar	15.0 g
Distilled Water	1000 ml

For the preparation of VRBA, all these ingredients were taken in distilled water, dissolved and autoclaved at 121°C for 15 min.

Potato dextrose agar (PDA)

Ingredients	**Quantity (g/L)**
Potato extract	4.0 g
Dextrose	20.0 g
Agar	15.0 g
Distilled water	1000 ml

All the ingredients were dissolved in distilled water and sterilized at 121°C for 15 min. After cooling the media to 45°C was acidified by addition of tartaric acid (10% solution) at 1% level.

Skim Milk

Constitutes	**Quantity/(mL)**
Skim milk powder	12
Distilled water	100

Glycerol Stock Medium

Constitutes	**Quantity/100 mL**
Glycerol (99 %)	20
Distilled water	80

500 µL of 20 % glycerol was added in 3 mL capacity cryovial and sterilized by autoclaving at 121°C/15 psi for 15 min. Equal volume of the overnight grown active culture was added in each cryovial aseptically and preserved with proper tagging at -20°C, at the time of preservation.

Components of Gram's staining

a) Primary stain (crystal violet stain)

Components	
Solution A	
Crystal violet	0.2 g
Ethanol (95% V/V)	20 mL
Solution B	
Ammonium oxalate	0.8 g
Distilled water	80 mL

b) Mordant (Gram's iodine)

Components	**Quantity**
Iodine	1 g
Potassium iodide	2.9 g
Distilled water	300 mL

c) Decolorizing agent

Components	**Quantity**
Acetone	50 mL
Ethanol (95 %)	50 mL

d) Counter stain (safranin)

Components	**Quantity**
Safranin O	25 g
95 % ethanol	100 mL

2. Preparation of Microbial Growth Medium

All microbiological growth media were prepared by dissolving their respective quantities of different ingredients in distilled water and pH was adjusted by using NaOH and HCl (where necessary). Agar based media were boiled for 2-3 times in microwave oven for proper dissolution of ingredients. After complete solubility of ingredients growth media were sterilized by autoclaving at 121ºC/15 psi for 15 min.

3. REAGENTS USED

a) Normal Saline Solution (0.85%)

For this. 0.85g of NaCl (Hi-Media) was dissolved in 100 mL distilled water and sterilized by autoclaving and stored at room temperature.

b) 1N NaOH

Component	Quantity
NaOH	4 g
Distilled water	100 mL

c) Phenolphthalein indicator

Component	Quantity
Phenolphthalein powder	0.5 g
Ethanol	95 mL
Distilled water	50 mL

d) Dipotassium phosphate solution

Component	Quantity
Dipotassium phosphate	10 g
Distilled water	100 mL
pH	6.7