DEVELOPMENT OF FUNCTIONAL *DAHI* BY USING BLACK CARROT (*Daucus carota L.)* AS A SOURCE OF PREBIOTICS

काशी हिन्दू विश्वविद्यालय

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THESIS

SUBMITTED IN PARTIAL FULFILMENT OF THE
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In
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Supervisor

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डेयरी विज्ञान और खाद्य प्रौद्योगिकी विभाग
कृषि विज्ञान संस्था

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CERTIFICATE

To,
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Through: The Head Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (U.P.)

Dear Sir,

I have great pleasure in forwarding the thesis entitled, **" Development of Functional *Dahi* by Incorporation of Black Carrot *(Daucus Carota L.)* as a Source of Prebiotic"** submitted by **Mr. Babu Kumar, I.D No.:20412MDT005, Enrolment No.:433974** in partial fulfilment of the requirements for the degree of **Master of Technology** in **Dairy Technology** from Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi and placing on record that he has completed the requisite residential requirements as contained in the ordinance of the University.

I certify that the entire scheme of investigation, presented here in, was planned and carried out solely by the candidate under my supervision and guidance. To the best of my knowledge, the data presented in the thesis are genuine and original and no part of the work has been submitted for any other degree or distinction.

Thanking You

Yours Faithfully

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FROM

DEPARTMENT OF DAIRY SCIENCE AND FOOD TECHNOLOGY,

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Date:

Place: *Varanasi.* ***(Babu Kumar)***

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

ANOVA	:	Analysis of variance
AOAC	:	Association of Official Analytical Chemists
F	:	F value
g	:	gram
hr	:	hour
mg	:	milligram
Min	:	Minute
ml	:	milliliter
ºC	:	Degree Celsius
HCl	:	Hydrochloric acid
H_2SO_4	:	Sulphuric Acid
Ppm	:	Parts per million
DPPH	:	2,2-diphenyl-1-picrylhydrazyl
TPC	:	Total Phenolic Content
aw	:	Water activity
GAE	:	Gallic acid equivalents
BCP	:	Black carrot pomace
TA	:	Texture Analyzer
LSD	:	Least Significant Difference

Chapter I

INTRODUCTION

For centuries, fermented foods and beverages have been an integral part of the human diet (Hutkins, 2008). Fermented food or beverages are prepared through controlled microbial growth and enzymatic degradation of macro and micro constituents of food. Fermented milk and milk products have numerous health-promoting properties like hypertensive, hypocholesterolemic, and anti-microbial effects (Ohsawa *et al.,* 2018), they played an essential role in human nutrition (Adolfsson *et al.,* 2013), and their functional and microbial properties have recently been extensively studied (Rhee et al., 2011). Lactic acid bacteria retain the nutritional quality of dairy products and increase shelf-life. It can also have beneficial effects on gut health, i.e., modulation of gut microbiota, and the prevention and treatment of inflammatory bowel disease (IBD) (Saez-Lara *et al.,* 2016), in addition to anti-carcinogenic and hypo-cholesterolemic effects (Kapila, Sinha, and Singh 2007). Fermented dairy product contains tri-peptides, i.e., valyl-prolyl-proline (VPP) and isoleucyl-prolyl-proline (IPP) investigated for their possible health effects, and it helps in the management of hypertension (Kim *et al.,* 2010). Fermentation has been used for processing foods for preventing spoilage, improving nutritional value, developing suitable physiochemical characteristics, and producing favorable sensory properties in the product. Fermented foods are known to exert a beneficial effect on gut microflora through a probiotic effect. Fermented food products or live microbial food supplements, which exert beneficial effects on the host by improving intestinal microbial balance, are generally understood to have a probiotic effect. Fermentation has long been used to preserve and enhance the shelf-life, flavor, texture, and functional properties of food (Hutkins *et al.,* 2018). More recently, the consumption of fermented foods containing live microorganisms has emerged as an important dietary strategy for improving human health (Marco *et al.,* 2017).

Dahi is a traditional Indian fermented product prepared by fermentation of milk by lactic acid bacteria. The popularity of *Dahi* is not only due to its refreshing and palatability but also due to its scientifically proven role of nutritive and therapeutic values. Besides its therapeutic values, *Dahi* is one of the most

soothing food items with a high nutritive value and as such, it is included as one of the choicest items on the Indian menu, especially during the summer season (Mohapatra *et al.*, 2019).

Goat milk is recognized as a functional food that influences biological functions in the human body, improving the state of health and well-being besides reducing the risk of developing a disease. For human nutrition, goat milk offers superior digestibility than cow milk does, due to its medium-chain Fatty acids (MCFA) content, its reduced level of αs1-casein, and, probably, its higher concentration of small-size Fat globules (Pal *et al.,* 2017). Goat milk is a good carrier for probiotic bacteria, but there is a problem to produce fermented goat milk with a consistency comparable to that of fermented cow milk. It can be improved by the addition of functional stabilizers, such as inulin, or treatment with transglutaminase. It can also be improved by adding Fibre Incarporateed sources which provide proper consistency. Recently several attempts have been made to utilize goat milk for the development of diversified indigenous milk products like Butter, Ghee, Khoa, Channa, and Cheese (Dixit *et al.,* 2014).

Probiotics are live bacteria that benefit the gut health of human beings by improving the gut microbial balance (Fuller, 1989). Probiotics bacteria possess various health benefits to human gut flora i.e. antagonistic effects, competition, and immune effects. Improved resistance to pathogens offers the most promise for the development of efficacious probiotics. Consumption of probiotics inhibits the growth of undesirable bacteria, eliminates harmful bacteria, and reinforces the body's natural defense mechanisms. Scientifically proven health benefits of probiotic bacteria have been increasingly included in yoghurts and fermented milk in recent years (Mattila-Sandholm *et al.,* 1999). The viability of probiotic bacteria, the number of viable and active cells per g or ml of probiotic food products at the moment of consumption is the most critical value for these products, as it determines their efficacy (Mortazavian and Sohrabvandi 2006; Tamime *et al.,* 2005). Therefore, to maintain consumer confidence in probiotic products, it is important to ensure a high survival rate of the bacteria both during production and over the product's shelf life. Prebiotic was described as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health. There is a problem to make Fibre Incarporateed probiotic *Dahi* because if we increase the percentage of prebiotic source powder

aggregated at the bottom an unacceptable appearance. Benefits of Fibre intake are associated with decreased risk of cardiovascular disease, type 2 diabetes, and some forms of cancer. Fiber may also have a role in lowering blood pressure and in preventing obesity by limiting weight gain (Wendy *et al.*, 2017).

Considering the importance of goat milk, fermented milk products, and probiotic cultures in the human diet, the present study is undertaken to develop probiotic *Dahi* by incorporating black carrot pulp as a prebiotic source with the following objectives.

1. Formulation of Functional *Dahi* by Using Black Carrot Pulp Powder.
2. Physico-Chemical, Textural, Microbiological analysis and Antioxidant activity of Formulated Product.
3. Shelf-life Study of Developed Product.

Chapter II

REVIEW OF LITERATURE

Curd is widely consumed by Indians along with meals or through other sources. It is a dairy product that provides animal protein along with nutrients like calcium, vitamin B-2, vitamin B-12, magnesium, and potassium (Chandan *et al.,* 2012). However, to provide health benefits, these bacteria need to survive the stomach acids and reach the intestine. Probiotics are "friendly bacteria" that are an important component of our digestive system. They are beneficial microorganisms offering several health benefits. They outnumber harmful microorganisms and maintain a healthy balance in the gut. Antibiotics are commonly used in children to treat infections. However, they can disturb the gut balance as they kill friendly bacteria along with harmful ones. This can lead to gas, cramping, or diarrhea. Probiotics are effective in restoring the balance to normal and promoting gut health. Infants and toddlers need the right amount of nutrition to become strong during their growth phase. Probiotics help in breaking down the protein and fat from the foods and making them available to the body (Yadav *et al*., 2015). Probiotics can protect the gut against such infections. Infants and toddlers need the right amount of nutrition to become strong during their growth phase

2.1 Fibre Incorporation in Food Product

Milk is not a natural source of soluble or insoluble Fibre in the human diet. Human milk and human colostrum may contain up to 12–13 and 22–24 g/l of Oligosaccharides, respectively, many of which are not digested in the small intestine, whereas bovine colostrum contains only ~1 g/l, and only trace amounts are found in mature milk (Urashima *et al*., 2009) The Fate of these human Colostrum and human milk Oligosaccharides is undoubtedly fermentation in the colon, as prebiotics to help establish a microflora rich in bifidobacteria and other beneficial species. Although this is a very interesting natural source of prebiotics in the diet of the human infant, for the general milk-consuming public any Fibres that dairy products contain would be added for their health benefit by dairy processors and sold

as such. There is increasing interest from food processors and consumers in voluntary Incarporatement or fortification of soluble Fibre in food products that are part of the human diet, to compensate for the deficiency in the diet of the general public due to the lack of sufficient natural sources of Fibre: fruits, vegetables, legumes, and whole grain.

2.1.1 Challenges of Fibre Incorporation

Most polysaccharides that are added to dairy products to modify texture are incompatible with milk proteins in solution; hence at typical concentrations used phase separation may occur, resulting in a change of functional behavior of the proteins and polysaccharides, visual separation of a clear serum ('wheying off') and a loss of desired quality in the product. In situations where the Polysaccharide concentration is high, that is, in Fibre Incorporate, this concentration will undoubtedly be above the separation threshold on a phase diagram (Doublier *et al*., 2000). Phase separation can be attributed to depletion flocculation (Tolstoguzov, 1997). In casein polysaccharide mixed systems, depletion flocculation results from the difference in chemical potential (osmotic pressure) between the solvent and the space between the casein micelles, from which the polysaccharide is excluded for steric reasons; consequently the casein micelles become Incorporate in concentration within discrete volumes. This induces the mixture to easily reach the thermodynamic incompatibility threshold, resulting in phase separation (Syrbe *et al*., 1998). A lower intrinsic viscosity or hydrodynamic molecular volume of polysaccharides leads to smaller occupied volumes, which contribute to less exclusion of polysaccharides in mixtures. Thus, depending on molecular conformation, the aggregation of milk proteins, especially casein micelles, and hence the kinetics of phase separation can be at varying rates (Dickinson and McClements, 1996; de Kruif and Tuinier, 2001). To retard this phenomenon or prevent it from occurring, κ -carrageenan can also be added to most polysaccharide blends for use with milk proteins. In the phase separating milk protein (casein micelle)–polysaccharide systems containing κ -carrageenan, the protein, and polysaccharide remain incompatible and will phase- separate at a microscopic scale into the water- in-water emulsion- structure; however, κ -carrageenan prevents this phase separation from manifesting itself into a macroscopic event in which two distinct phases become visible. Both κ -carrageenan direct adsorption to the surface of the casein micelle and κ -carrageenan helix aggregation are involved in establishing a loosely aggregated.

2.2 Probiotic Starter Culture

Oh & S. *et al*. (2015). originated the concept of probiotics. Lilly and Stillwell (1965) Probiotic is derived from the Greek word which means for life. Used the term 'for organisms and substances' with beneficial effects for animals by influencing the intestinal microflora (Kumar Singh *et al*.,2019). It is defined as 'probiotic' as 'A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance Gueimonde *et al.*,(2013). Vilà & B. *et al.,(*2010) defined probiotics as viable micro-organisms that exhibit a beneficial effect on the health of the host by improving its intestinal microbial balance. Probiotic foods to be beneficial for human health, the strain should maintain their viability during their shelf life until consumed. A range of 10^6 - 10^9 live probiotic cells is consumed for health benefits as reported by a researcher (Ozyurt *et a*l.,2014). For therapeutic effect, the fermented milk/product should contain probiotics of more than 106 cfu/ml. It was observed that consumption of acidophilus milk by volunteers suffering from gastrointestinal complaints resulted approximately 10-fold. Decrease in total counts of coliforms and a 10-fold increase in lactobacilli counts in stools. Implantation of *Lb. acidophilus* was confirmed and volunteers reported partial or good improvement in their condition and it was also beneficial in treating certain gastrointestinal disorders. The consumption of a large number of viable cells of *Lb. acidophilus* has been used to reestablish normal intestinal flora after antibiotic therapy and to treat patients infected with intestinal pathogens (Buffie *et al.,* 2013).

There are many different microorganisms currently used as probiotics. To better understand how bacteria are named and classified, the following discussion may be helpful. Genus is the first name of a bacterium (e.g., *Lactobacillu*s). It is somewhat general and refers to a grouping of organisms based on similarity of qualities, such as physical characteristics, metabolic needs, and metabolic end products. Species is a bacterium's second name (e.g., *acidophilus*). It is a much more narrow classification based on shared common characteristics that distinguish them from other species. Strain is an even more specific classification that divides members of the same species into subgroups based on several

properties that these bacteria have in common that are distinct from other members of the species (e.g., strain LA5).

2.2.1 Problem in the Development of Probiotic Dairy Products:

Many probiotic cultures cannot multiply well in pure milk, but variations occur between strains. Growth in milk is often slow or limited and this appears to be partially due to low proteolytic activities. Probiotic cultures, such as *Lb. acidophilus* or *Bifidobacterium*, generally grow faster on synthetic media than on milk but appear to be better suited for the milk substrate that are cultures of *Lb. rhamnosus*. The bifidobacteria are generally not highly proteolytic, and their growth in milk is also dependent on their *β*-galactosidase activities. In some instances, mixing nonproteolytic probiotic strains with a highly proteolytic LAB will be helpful, but if the LAB grows too fast, the probiotic strains are overwhelmed. Therefore, many groups have supplemented milk with various compounds. Supplementation of milk with yeast extracts (YE) often solves this problem, and YE can be replaced by a combination of amino acids, Minerals, and ribonucleotides or casein hydrolysates but there are instances where the strategy is ineffective, particularly with bifidobacteria. Presumably, in this last instance, factors other than amino acids were lacking in the supplements, or the redox conditions were simply inappropriate for the growth of the bifidobacteria. Redox conditions, indeed, appear to be linked to the growth-promoting properties of various supplements. Thus, in a study where different peptones, extracts, or proteins were tested for their stimulatory effect on bifidobacteria, all materials lost growth-promoting activity when their disulfide bonds were reduced. The potential of probiotic yeasts in dairy products is linked to their ability to grow at low pH values, low water activities, and grow at low temperatures and high salt concentrations. It is now admitted that many yeasts can become established in a dairy and act as a spontaneous or as a controlled starter culture.

2.2.2 Supplementation to Overcome the Problem in Probiotic Food Development

In selecting Fibre sources for Incarporatement in dairy products, the first consideration has to be the actual definition of Fibre, and this has not been easy to define for regulatory bodies (Phillips and Cui,

2011). The Institute of Medicine in the United States defined Fibre in the Dietary Reference Intakes in 2005 as: 'Dietary Fibre consists of non-digestible carbohydrates and lignin that are intrinsic and intact in plants. Functional Fibre consists of isolated, non-digestible carbohydrates that have beneficial physiological effects in humans. Total Fibre is the sum of Dietary Fibre and Functional Fibre.' In 2009, Codex agreed on the following definition: 'Dietary Fibre means carbohydrate polymers with ten or more monomeric units which are not hydrolyzed by the endogenous enzymes in the small intestine of humans and belong to the following categories: edible carbohydrate polymers naturally occurring in the food as consumed; carbohydrate polymers, which have been obtained from food raw material by physiological, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities; synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities.' They also include the following

2.2.3 Health Benefits of Probiotic Bacteria:

The immune system reduces the risk of developing infections and various strengthens diseases. Katona *et al*., (2008). It is effective in the treatment of several conditions like constipation, candidiasis, rheumatoid arthritis, and urinary tract infections. Probiotic *Dahi* can help in the treatment of infectious diarrhea and diarrhea caused by antibiotic usage. It is effective in reducing the symptoms of irritable bowel syndrome, ulcerative colitis, and Crohn's disease.

Probiotics are live microorganisms that have health benefits when consumed. Saini *et al.* (2010) Trillions of these microorganisms live in the stomach and intestines and make up the gut microbiota, which is considered a key to health. The "good" bacteria in the intestines have a symbiotic relationship with the body, getting energy from the foods people eat and providing health benefits to the entire body. Eating a variety of probiotic-rich dairy foods can enhance the good bacteria in the gut, improve health, and reduce disease risk, while also providing important vitamins, minerals, and protein.

The probiotics market has grown partly due to progress in research and new product development, and partly due to consumer-led demand for healthier products. Probiotic products have gained particular

popularity, as they are believed to garner benefits rather than merely act as a nutritional supplement. The benefits associated include: immune system stimulation, lowering of blood ammonia, reducing serum cholesterol, strengthening mucosal barriers, alleviating Lactose intolerance, and synthesis of B vitamins.

2.3 *Dahi*

Dahi, Indian curd is a traditional fermented dairy product. *Dahi* differs from yoghurt in its use of starter culture (Teles *et al*., 2007; Ares *et al.,* 2010). India ranks first in milk production an annual production to the tune of 298.16 MT (NDDB, 2019). About seven percent of the total milk produced annually in India is utilized for *Dahi* making (Prajapati, 2011). Lactic acid bacteria in *Dahi* help in arresting digestive disorders, partial splitting of protein, and greater availability of calcium. It is familiar for its therapeutic values and nutrition (Aneja *et al.*,2002).

Table- 2.1: FSSAI Standard for Probiotic *Dahi*

Characteristic	**FSSAI (2011)**
Coliform (cfu/ml)	Max 10/ g
Escherichia coli (cfu/ml)	Absent in 1 g
Salmonella (cfu/ml)	Absent in 25 g
Stephylococcus Aurius (cfu/ml)	Not more than 100 / g
Yeast and Mould (cfu/ml)	Max 100 / g
Anaerobic spore (cfu/ml)	Absent in 1 g
Listeria monocytogenes (cfu/ml)	Absent in 1 g

Table- 2.2: Chemical composition of probiotic *Dahi* prepared from different kinds of milk:

Source	Probiotic *Dahi* composition						
	Moisture	**Fat**	**Protein**	**Lactose**	**Acidity**	**Ash**	**Reference**
Cow milk *Dahi*	88.9	2.5	4.85	-	0.73	0.81	Hariom yadav *et al.*, 2007
Goat milk *Dahi*	87.9	4.4	3.34	4.48	1.12	-	Widodo et al., 2014
Buffalo milk *Dahi*	85.96	4.8	3.95	-	.0.7	0.78	Samia Afrin et al., 2016
Buffalo milk *Dahi*	85.77	2.2	2.99		0.79	0.59	An Ibrahim et al., 2021

2.4 Goat milk

Despite the significant role of goat milk in rural economy and health, the goat has remained neglected in research and development because of the misunderstood role of goat in desertification. To access and exploit it to its full potential, there is an urgent need for further research and development of goats for meat and milk, particularly the role of goat milk for its medicinal value (Kahi &A. K *et al*., 2019).

Goat milk is white and has a stronger flavor than sheep milk; it is also alkaline due to higher protein content and a different arrangement of phosphates (Pal *et al.,* 2017). Goats' milk has smaller size Fat globules compared to cow milk which provides a smoother texture. The lower amounts of the alphas1-casein present in goat milk results in softer gel products, a higher water holding capacity, and lower viscosity. However, the Flavor of goat's milk is more intense in comparison to cow's milk, which can restrict the acceptance of its derivatives by consumers (Gomes *et al.,* 2013).

Goat milk can be a great supplement for children and adults but is not appropriate for infants. In the early 1900s, infants fed primarily goat milk would commonly develop anemia from the lack of folate and B12. The problem was so prevalent that it was nicknamed 'Goat milk anemia,'" she warns. "Today we will still see children come to the hospital with goat milk anemia, typically as a result of parents giving homemade infant formulas. Even when used as part of a custom recipe to address these deficiencies, providing goat milk to infants can result in vitamin and/or Mineral deficiencies, poor growth, impaired kidney functions, and even seizures if the recipe is too diluted."

2.4.1 Composition of goat milk

Goat milk contains 3.4% Protein, 3.8% Fat, 4.1% Lactose, and Ash 0.8%. Goat milk contains a higher amount of Ca, Mg, and P than cow and human milk. Three Fatty acids viz., caproic, caprylic, and capric have great medicinal values for patients suffering from a variety of ailments. It provides 70 calories per 100 ml. The chemical composition of goat milk is influenced by several factors, which include breed, nutrition, health status, season, management, environment, and stage of lactation. Goat milk is recognized as a functional food that influences biological functions in the human body, improving the state of health and well-being besides reducing the risk of developing a disease (Mahendra et al., 2017)

2.4.2 Therapeutic and medicinal significance of goat milk

Goat milk is also very much suitable for the treatment of insomnia and neuro-digestive disorder owing to its higher level of vitamin Bl and vitamin B12 content. Non-passage of milk protein through the wall of the digestive tract in the original undigested, allergic form, accounts for the non-allergenic properties of goat milk (Verduci *et al*., 2019).

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recognized as a functional food that influences biological functions in the human body, improving the state of health and well-being besides reducing the risk of developing a disease.

Use of goat milk for feeding infants and children with IgE-mediated cow milk allergy and in other dietary therapies (Toit and Weingberg, 2002). Goat milk is recommended for feeding infants above 6 months of age but should be offered after dilution to avoid metabolic abnormalities (Hendriksz and Walter, 2004).

Goat milk is naturally homogenized and forms a softer curd that can get digested very quickly and easily. In a study on skeletal Mineralization, blood serum vitamin, Mineral, and hemoglobin level were observed for goat milk than cow milk. Medium-chain length Fatty acid or medium-chain Triglycerides (MCT) which are more in goat milk has been unique health benefits in malabsorption syndromes, chyluria, steatorrhea, hyperlipoproteinemia, and in case of intestinal resection, coronary bypass, premature infant feeding, childhood epilepsy, cystic fibrosis and gallstones (Roy and Vadodaria, 2012)

2.4.3 Allergy from goat milk:

Goat milk was also less allergenic than cow milk when introduced into the diet of rats at weaning (Lara-Villoslada, Olivares, Jiménez, Boza, & Xaus, 2004). However, there are also reports of reactions to goat milk cheese in children where there was no other evidence of allergy to cow milk (Ah-Leung *et al*., 2006; Bidat, Rancé, Baranès, & Goulamhoussen, 2003; Raghani et al., 2016). The average age of first allergic response to goat milk cheese was 5 to 6 years, which is later than when allergy to cow milk is commonly reported (Ah-Leung et al., 2006; Bidat *et al.*,2003; Raghani *et al.*, 2016). This delay in response is likely to reflect the later age of exposure to goat milk proteins.

2.5 Black Carrot:-

Black carrots are also an excellent source of anthocyanins. Anthocyanins help lower bad cholesterol (LDL) along with helping protect the arteries against oxidation. Anthocyanins also help protect us against various forms of cancer. This variety has high nutraceutical values and is rich in anthocyanins, phenols, flavonoids ß-carotene, calcium, iron, and zinc. Its Anti-oxidant activity is four times higher

than red carrot. The fresh black carrots are suitable for salad, juice, pickle, and kanji which are good appetizers.

2.5.1 Composition and functional properties of black carrot pulp powder

Drying method	**Black carrot pulp powder**						
	Moisture	**Fat**	**Protein**	**DPPH**	**TPC**	**Fiber**	**Reference**
Tray drying	4.8	0.79	8.82	-	2.30 mg/g	18.27	Evren et al.,2022
Drum Drying	-	4.4	3.34	180µgm /ml	1832mg/l	-	Widodo et al., 2014
infrared radiation	3.9	4.8	3.95	153 mg TE	54 mg GAE	12.6	Senem et al. 2016
Tray drying	5.1	-	5.5	60.3	0.79	20.9	Ibrahim et al., 2021

Carrot contains 86-90 percent of Moisture, 0.7 to 0.9 percent protein, 0.2 to 0.5 percent Fat, 6 to 10.6 percent carbohydrate, 1.2-2.4 percent crude Fibre, and 1 to 1.5 percent Ash content (Holland *et al*1991 and Gopalan *et al.,* 2010). While black carrot contains approximately 88 percent Moisture, 1 percent protein, 0.14 percent fat, 2.5 percent Fibre and 0.80 percent protein. Carrots are also a good source of minerals like Calcium, Phosphorus, Sodium, Potassium, Magnesium, Iron, and Zinc. Gopalan *et al.,* (2010) have reported 80 mg calcium, 53 mg phosphorus, and 2.2 mg iron (per/100g). Whereas, Holland *et al.*, (1991) reported 34 mg of calcium, 25 mg of phosphorus, 40 mg of sodium, 240 mg of potassium, 9 mg of magnesium, 0.02 mg of copper, 0.4 mg of iron, and 0.2 mg of zinc. Carrots are also a good source of multivitamins. It contains a very good amount of carotenes (5.33 mg/100g), however thiamine

(0.04 mg/100g), riboflavin (0.02 mg/100g), niacin (0.2 mg/100g), and ascorbic acid are also present in appreciable quantities.

2.5.2 Utilization of waste from carrot processing:

In juice extraction of black carrot, pomace is as waste which is used to produce Fibre Incorporate dairy product. In black carrots, juice yield is reported to be only 60-70% and up to 80% of the carotene may be lost with the pomace. The total carotene content of pomace may be up to 2 g per kg of dry matter, depending on processing conditions. The solid waste from carrot juice production is rich in insoluble Fibre, could reduce cholesterol levels, and should be exploited as a final ingredient. The use of carrot pomace as by-product utilization will decrease the environmental load.

Carrot juices and blends thereof are among the most popular non-alcoholic beverages. Despite considerable improvements in processing techniques including the use of depolymerizing enzymes, mAsh heating, and decanter technology, a major part of valuable compounds such as carotenes, uronic acids, and natural sugars is still retained in the pomace. The juice yield is reported to be only 60-70% and up to 80% of the carotene may be lost with the pomace. The total carotene content of pomace may be up to 2 g per kg of dry matter, depending on processing conditions. The solid waste from carrot juice production is rich in insoluble Fibre, could reduce cholesterol levels, and should be exploited as a final ingredient. The use of carrot pomace as by-product utilization will decrease the environmental load.

2.5.3 Utilization of black carrot powder in food formulation

In the development of probiotic *Dahi* by incorporation of black carrot powder as a source of prebiotic. Black Carrot is utilized in functional food because of the presence of a significant amount of bioactive compounds. The present review primarily aims at spotlighting the presence of potential phytochemicals in black carrots (*Daucus Carot*a L.) and their significant role in oxidative stress-induced major metabolic disorders. Effects of processing on polyphenols and anthocyanins of black carrot are also discussed. Black carrot anthocyanins are considered the major bioactive compound, which is

associated with its health promotion and disease prevention properties. The functional properties of these bioactive compounds call for further studies to exploit their role in the prevention of diseases.

2.5.4 Bioactive Compound in Black Carrot

In a previous study performed by our group (Kamiloglu *et al*.,2016), we showed that polyphenols initially present in black carrots are largely preserved in BCP. Furthermore, the bioaccessibility of anthocyanins from BCP was found to be higher than in black carrots. Considering the above, we suggest that BCP, a cheap and substantial source of polyphenols may be used to Incorporate food products. Bakery products including cakes are considered to be a good source of energy, however, they have low antioxidant capacity. Hence, in order to increase the nutritional value of cake and to valorize black carrot powder, in the present study we investigated the digestive stability of polyphenols from black carrot powder incorporated cakes and monitored changes in their antioxidant capacity using the standardized static in vitro digestion.

2.5.4.1 Phenolic acid:

Phenolic acids are non-flavonoids having one functional group of carboxylic acid. It can be categorized into two subgroups, based on its chemical structure namely hydroxycinnamic and hydroxybenzoic acids. In both the phenolic acids basic structure is not changed however, the difference is due to the number and position of hydroxyl groups on the aromatic ring of phenols. Hydroxybenzoic acids are polyphenolic compounds having a C6-C1 structure, a major example is Gallic acid whereas, hydroxycinnamic acids are aromatic compounds having a three-carbon side chain (C6–C3). Caffeic, ferulic, p-coumaric, and sinapic acids are the most common example of hydroxycinnamic acids (Balasundram et al. 2006, Robbins 2003). Hydroxybenzoic acids are present in high amounts in raspberry, blackberry, and tea however; blueberry and coffee are excellent sources of hydroxycinnamic acids (D'Archivio *et al.* 2007). Black carrots are also a rich source of phenolic acids apart from anthocyanins. In black carrots, mainly derivatives of hydroxycinnamic acid are identified. Kammerer et al. (2004) have reported that chlorogenic acid (5-Ocaffeoylquinic acid), an ester of caffeic and quinic acid was the major phenolic acid in black carrots. Several studies have reported that black carrot roots

exhibited a significantly higher amount of phenolic content as compared to other colored root vegetables (Leja et al. 2013 Koley *et al*. 2014). Stilbenes contain two phenyl moieties connected by a two-carbon methylene bridge (Pandey and Rizvi, 2009). Resveratrol is the main example of stilbenes, which are abundantly present in plant species, majorly in berries, grapes, and peanuts (Ignat et al. 2011). Lignans are produced by oxidative dimerization of two phenyl propane units. Linseed, containing secoisolariciresinol and low quantities of matairesinol, represents the main dietary source (Manach et al. 2004, D'Archivio *et al.*, 2007).

Phenolic compounds of black carrot extract were found to have a significant effect on reducing inflammation by inhibiting the inflammatory pathways. There have been many clinical studies, which proved the anti-inflammatory effect of black carrot extract in in-vitro and in-vivo systems. Pandey & P *et al*., (2020) have reported the positive effect of black carrot extract in the prevention of diseases characterized by metabolic disorders of Carbohydrates, Fats, and energy. However, Wright *et al.*, (2013) reported that supplementation of black carrot powder (118.5 mg/day of anthocyanins and 259.2 mg/day of phenolic acids) for 4 weeks in 16 human subjects had a non-significant effect on body mass, body composition, LDL, total cholesterol and blood pressure. On the other hand, in a rat trial, Pandey & P. et al. (2020) reported that the incorporation of black carrot juice at the 5 percent level in the diet of rats (fed with a high carbohydrate and fat diet), significantly improved glucose tolerance, and reduced the risk of reduced abdominal obesity, plasma lipids, systolic blood pressure, cardiac fibrosis, hepatic steatosis, and inflammation. Anthocyanins from black carrots are reported to be responsible for these improved metabolic effects. Black carrots also contain polyacetylene compounds, which were reported to inhibit the production of NO_2 in macrophage cells by sixty-five percent. Therefore, it can be suggested that the anti-inflammatory properties of black might also be attributed to its polyacetylene content Pandey & P *et al.* (2020).

2.5.4.2 Anthocyanins:

Anthocyanins are water-soluble pigments that exert red, purple, and blue color and are widely distributed in the plant kingdom (Prior and Wu 2009). The term anthocyanin is derived from the Greek word *anthos*- flower and *kyanos*- blue (He and Giusti 2010). These pigments are mainly distributed in

fruits although it is also present in flowers and some vegetables. Among fruits, the berry is identified to be the richest source of anthocyanin (Giampieri *et al.,* 2014 Howard *et al*2014). Anthocyanins, because of their natural coloring properties have become a very important pigment to the food industry, as they can replace synthetic colorants (Wallace and Giusti 2008). Anthocyanin pigments are considered under natural colorant by Codex Alimentarius Commission and Food and Drug administration USA. There are almost 17 anthocyanidins, which have been identified by scientists, but only six are widely distributed in the plant kingdom that are Cyanidin, Delphinidin, Malvidin, Elargonidin, Peonidin, and petunidin. Although only there are only six available anthocyanidins, however more than 600 types of anthocyanins are distributed in nature (Wu *et al.,* 2006). Sugar moieties that are attached to anthocyanidins are mainly glucose, gaLactose, arabinose, rutinose, rhamnose, and xylose. These sugar moieties are attached to anthocyanidins as mono-, di-, or trisaccharides (Fang 2014). Cyanidin-3-glucoside is the most widespread anthocyanin in plant species (Castaneda- Ovando*et al*2009). In many cases, the sugar moiety attached to anthocyanidins is acylated with coumaric, caffeic, ferulic, sinapic, *p*-hydroxybenzoic, malonic, oxalic, malic, succinic, or acetic acid (De Pascual-Teresa *et al., (*2013).

Black carrot is reported to have a significant role in human nutrition and health, as it comprises a variety of health-benefiting compounds (Suzme *et al.,* 2014). Black carrot represents a valuable source of polyphenols in addition to the presence of antioxidants such as ascorbic acid and tocopherol (Algarra *et al.,* 2014). The scientific community is attracted to it due to the unique profile of anthocyanin pigments (Olejnik *et al.,* 2016, Padayachee *et al.,* 2013 Kamiloglu *et al*2015). Along with their colorant properties, anthocyanins may serve an essential role in promoting health. Anthocyanins have been recognized as a component of the diet with established nutraceutical properties, associated particularly with their free radical scavenging capacity (Kamiloglu *et al.,* 2015a), anticancer activity (Sevimli-Gur *et al.,* 2013), and anti-inflammatory properties (Metzger *et al*2008). Anthocyanins can be directly absorbed in the intestine without any metabolic changes. They can further be converted into glucuronide, methyl, or sulFate compounds in the small intestine and liver in presence of phase II enzymes. These formulated compounds may exhibit a protective effect on endothelial cells (Kuntz *et al.,* 2015). Apart from anthocyanins as the main polyphenol, black carrots also comprise a good amount of phenolic acids, such as caffeic and hydroxycinnamates acid (Kammerer *et al.,* 2004). Black carrots

are reported to have a huge range of health-promoting compounds, notably, 12 times more antioxidants as compared to orange carrots, cholesterol-reducing properties, and rich in vitamin A, B, and C. Black carrot's anthocyanins are 28 times higher than the orange carrot (Erkon-Konsantre 2013).

Despite its significant value as a source of natural colorant, antioxidant, and dietary Fibre, black carrot is a grossly underutilized crop that is not recognized as a vegetable. These underutilized and neglected crops have the potential to significantly reduce poverty in India. Underutilized crops are not common in the industrial world. They're found to be more prevalent in the diets of rural and urban poor people for nutritional, medical, energetic, and other reasons. The promotion of neglected and underutilized crops can help not only to conserve agrobiodiversity, risk management, and farming system stability but also to promote local farmers' cultural identification and empowerment in the face of what is too frequently labeled as backward or unmodern.

Several scientists have refined anthocyanin pigment extraction from black carrots, reporting that the optimal strategy for maximal anthocyanin extraction in large-scale manufacturing is at low pH and high temperature. (Turker and Erdogdu, 2006, Guldiken *et al.*, 2016). (Kammerer *et al.*, 2004) studied the anthocyanins content in black carrots and evaluated their coloring properties. The anthocyanins contents varied greatly between and within carrot cultivars. The black carrot responded positively under different pH, which suggested that black carrot anthocyanins could be used as natural food colorants even for low acid foods.

2.5.4.4 Dietary Fibre in Black Carrot

Black carrots are extremely high in dietary Fibre which can help to stimulate peristaltic motion, improves nutrient absorption in the gut, and help smoothen bowel movements. It helps as Fibre can help in quick weight loss and lower cholesterol levels. Dietary Fibre also regulates the release of insulin and glucose in the body, which makes it an excellent choice for diabetics.

Chapter III

MATERIALS AND METHODS

The experiments were carried out in the laboratory of the Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, BHU, Varanasi. This section contains information on the materials and methods used in the current study.

3.1 Raw Material

3.1.1 Goat Milk

Fresh goat milk was procured directly from the goat's former locality of Varanasi, Uttar Pradesh. Milk samples were collected at the time of milking and transport and stored at refrigeration temperature. Further chemical analysis of goat milk was carried out after warm-up to 42°C. Goat milk containing 3.9 % Fat, 8.6 % SNF, and 0.16 % (lactic acid) acidity

3.1.2Black carrot

Fresh and matured black carrot (Daucus carota L.) was purchased from the local market, Varanasi (India).

3.1.3 Starter culture

The probiotic culture *(Lactobacillus acidophilus* La-5 and *Lactococcus lactis sub sp. Lactis biovar Diacetyl lactis*)was procured from Shree Additive (Pharma & Foods) Pvt Ltd., Kothari Estate, Gandhinagar. The culture was stored below -18°C.

3.3 Chemicals

Almost all of the chemicals used in this study were analytical-grade substances. The chemicals came from HI Media Laboratories Pvt. Ltd. (Mumbai, India), Merck Specialties Pvt. Ltd. (Mumbai, India), and Fisher Scientific (Mumbai, India).

Table 3.1 Instruments used in Manufacturing and analysis of prebiotic *Dahi* Materials used.

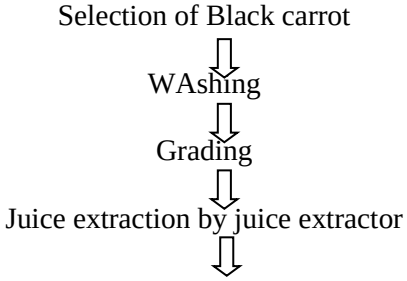
Instruments	The company, Model, and Country	Application
Electronic weighing balance	Make: Metllertoledo, Model: jbi 603 – cif act	The electronic weighing balance was used in weighing the ingredient during product formulation and preparation. In, addition it is used in weighing chemicals and agar during the different analyses of the product.
pH meter	Thermoscientific sn821899, Singapore	pH meters were used to determine of pH of *Dahi* during incubation as well as storage.
Texture profile analyser	Text Plus Texture Profile Analyser Stable Micro Systems, UK; Model:	Texture analyzers were used to determine textual properties like hardness, springiness, chewiness, gumminess, cohesiveness, and adhesiveness of *Dahi*.
Vortex shaker	Macro Scientific Marks Pvt. Ltd, Delhi	Vortex shaker used in shaking of black carrot powder in milk during *Dahi* making and in serial dilution during microbial analysis.

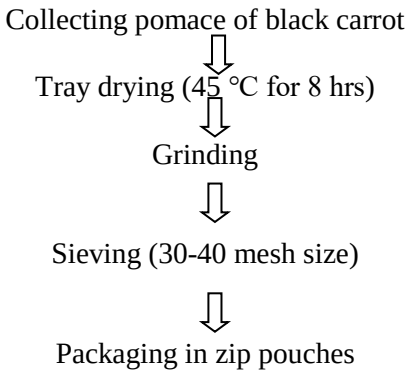
Hot air oven	Per Fit, India	Hot air oven used in Moisture and Ash analysis of carrot powder and *Dahi*.
Laminar airflow	Lab tech lcb 1201v, daihanpvt.lmt., India	Laminar airflow is used in the microbial analysis for providing sterile conditions.
Centrifuge machine	Sigma, 3-30k, Germany	Centrifuge machine used for separating supernatant in the analysis of DPPH and TPC.
Incubator	Remi, India	Incubator used for the growth of probiotic bacteria also used in the enumeration of yeast & mold, coliform.
Soxhlet Apparatus	SOCS PLUS, SCS-4, Chennai	Soxhlet Apparatus used in the detection of Fat percentage in black carrot powder.
Spectrophotometer	Shimadzu, Japan	A spectrophotometer is used in the determination of DPPH and TPC in black carrot powder as well as in *Dahi*.

3.5 Methodology

Preparation of Black carrot powder

Firstly, fresh black carrots were cleaned with water, and carrots were spread in trays evenly to remove excess water. Then black carrot pomace was removed by juice extractor and some were put in a tray drier at 50–55ºC till 5-6% Moisture level was reached. After drying, black carrot pomace was ground into powder form then the powder was sieved and packaged in polythene bags at room temperature for further preparation of herbal yogurt.





Flow diagram

Fig. 2.1

For producing black carrot pomace powder first select black carrot wAsh it and grade. After grading extract juice from the juice extractor and collect pomace and dry it in a tray dryer at 45^0 C for 8 hrs. Dried pomace collect and grind it in a grinder sieving it 30-40 mesh size sieve (Ashitha *et al*., 2014), and packaging it in Zip Pouches.

Fig. 3.2 Flowchart of preparation of Black carrot pomace powder

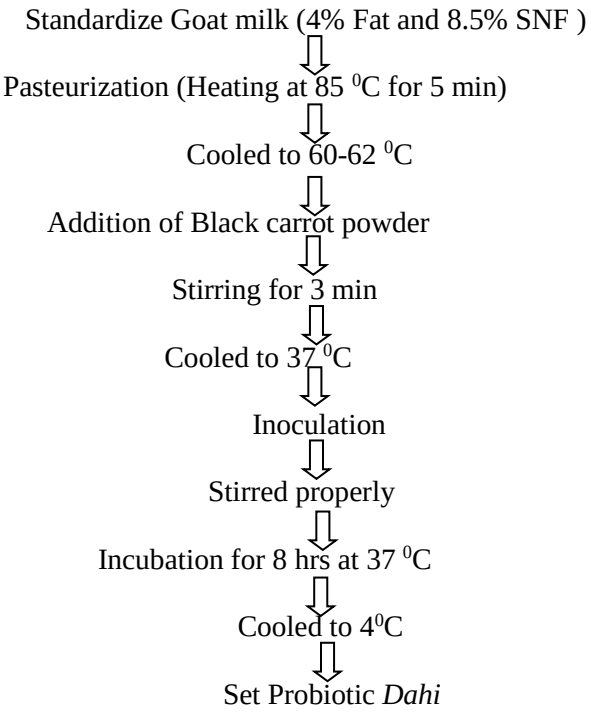


Fig 2.2 Process flow diagram of *Dahi*

Preparation of probiotic *Dahi* incorporated with black carrot powder:

For the preparation of probiotic *Dahi* by incorporation of black carrot as a source of probiotic first standardize goat milk at 4% Fat and 8.5 % SNF. Pasteurize this at 85^0 C for 5 min cool it at 60^0C (Rynne *et al*., 2004)and add black carrot powder 0, 1, 1.5, 2, 2.5, 3.0% and mix properly after maintaining the temperature up to 37˚C inoculate culture 0.2 % and incubate it for 8 hrs and keep it for setting. After setting curd stored it at 4^0 C.

Experimental Design

Table 3.2 Experimental design of probiotic *Dahi*.

Trial	**Black carrot pulp powder (%)**	**Probiotic Culture (%)**
T0	0	0.2
T1	1	0.2
T2	1.5	0.2
T3	2	0.2
T4	2.5	0.2
T5	3	0.2

Optimization of the level of black carrot pomace powder in probiotic *Dahi*

Sensory Evaluation

Black carrot powder added probiotic *Dahi* analyzed for different sensory characteristics like Flavor, Color and Appearance, Mouthfeel, Body and Texture, and Overall Acceptability. Sensory evaluation

was performed by a panel of 10 semi-trained judges from the Department of Dairy Science and Food Technology, Banaras Hindu University, Varanasi (India). Sensory evaluation was performed at Room temperature (27 ℃) and 60 % relative humidity. 9 – Point Hedonic scale rating (where, 1= dislikes extremely, 9). Lim *et al*., (2010) were used for Color & Appearance, Body and Texture, Mouthfeel, and Overall Acceptability. The sensory score card is attached as appendix-1.

3.5 Physico-chemical analysis of black carrot powder incorporated probiotic *Dahi*

1. Texture analysis (TA)

Textural parameters of the product like Fracturability, springiness, chewiness, gumminess, adhesiveness, cohesiveness, and resilience were analyzed using Texture Analyser (TA.XT plus texture profile Analyser, Stable Micro Systems, and the UK).

Table 3.3 Texture analyser setting

Mode	**Measure Force in Compression**
Option	Return to start
Pre- Test speed	1.5mm/s
Test speed	1.0mm/s
Post- test speed	10.0mm/s
Distance	5.0mm
Trigger force	Auto-5g
Tare force	Auto

In texture profile analysis of *Dahi* pre-test speed 1.5 mm/s, test speed 1.0 mm/ s, test post-test speed 10.0 mm/s, Trigger force -5 g.

Moisture

A weighed sample of finely ground material (5g) is dried in a hot air oven for 8 hours at 105℃. In the case of a wet sample, It is dried to constant weight. Crucible with dried material is transferred immediately to a desiccator, cooled, and weighed.

$$\%\ Moisture\ content\ = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Where, W_1 = Weight of empty Petri-plate

W_2 = Weight of empty petri-plate + Sample

W_3 = Weight of petri-plate after drying

Ash Content

One gram of completely homogenized sample was taken accurately in a Moisture free silica crucible. The crucibles were then placed on a hot plate at 130℃ till the smoke disappeared. The crucibles were then placed in a muffle furnace at 550℃ (6 h.). Weights of the cooled crucibles were noted down.

$$\%\ Ash\ = \frac{weight\ of\ ash}{weight\ of\ sample} \times 100$$

pH

The pH values were determined with the help of an electronic pH meter, as recommended by Ranganna (2001). The calibration of the electronic pH meter was done using7 pH and 4 pH standard buffer solutions. The function selector switch was set to pH and the reading of the digital display was allowed to get stable before it was noted.

Crude Fibre

Fibra Plus crucibles were oven-dried, cooled, weighed, and marked as W1. Five grams of sample were weighed and transferred to crucibles. The crucibles were placed in metal adapters of Fibra plus (FES, 6, Pelican equipment, India) hot extraction unit (Pelican India Limited) ensuring the proper sealing of crucibles against the adapter rubber. The acid, base, and distilled water were heated up on a hot plate. Approximately 150 ml of the 1.25% of H_2SO_4 was poured into the extractor unit of Fibra plus from the top. Equipment was switched on and the initial temperature was set at 500°C. After boiling started, the temperature was reduced to 400°C. The sample was boiled for 40 minutes in acid. After boiling, acid was drained off and rinsed with distilled water thrice, to ensure that the sample was free from acid residue. Acid washed sample was then boiled with 150 ml of 1.25 % NaOH for 40 minutes in the same process as an acid wash. The sample was rinsed with distilled water thrice or more to assure no alkali residue was left within the sample. The crucibles with digested samples were removed from the equipment and dried in a hot air oven until the samples were free of Moisture. The samples were cooled down in a desiccator and the crucibles were weighed (W1). Crucibles with dried samples were placed in the muffle furnace for 4 hours at 550°C. After ashing, the crucibles were removed from the furnace, cooled in a desiccator, and weighed (W2). The loss in weight was recorded and calculated Fibre content of the sample as:

$$\text{Crude Fibre (\%)} = \frac{W1-w2}{w} \times 100$$

Where,

W = Weight of sample

W1=Weight of residue before ignition

W2= Weight residue after ignition

Protein Determination

The protein content in the black carrot powder added *Dahi* was estimated by following the protocol (FSSR 2011).

Digestion

The system was switched ON, and the digestion unit was pre-heated up to 350 °C. 0.2g sample was weighed (W) in the filter paper and wrapped inside it, in triplicates. The sample was taken in a 250ml Macro DTL tube. Then to the sample, 10ml conc. H_2SO_4 was added, followed by the addition of 5g of catalyst mixture [(5:1) (Potassium Sulphate: Copper Sulphate). The sample tube was then loaded in the digestion unit with manifold & KEL FLOW setup. Tap water was connected with maximum pressure for KEL FLOW. The temperature was increased to 420^o C. Digestion was carried out till clear green color appeared. According to the sample, ninety minutes were taken for digestion. Then the digested mixture was cooled on the cooling rack for approximately thirty minutes

Distillation

The system was switched ON and the solutions viz. 4% Boric acid, 40% Alkali, and 0.1N HCl were prepared and kept. The Alkali, Boric acid, and $KMnO_4$ were loaded into the system through silicon hoses provided at the back of the equipment while waiting for the ready signal. In a 250ml conical flask 25ml, Boric acid was taken with an indicator and placed at the receiver end. The sample was diluted with distilled water (dilution of 10ml to 20ml). Then the sample tube was loaded on the sample side. Before starting the sample testing, the water was allowed to flow through the system for cooling purposes (check the INLET and the OUTLET). The sample testing was started after the ready signal appeared. 40ml of the 40% Alkali was added to the above (until dark brown color appears). Then the process was started. During the process, liquid ammonia was collected into Boric acid and its color changed according to the indicator used. After the completion of the process, the conical flask was removed from the receiver end and then titrated. The tube from the sample side was now removed.

Titration

0.1N HCl was taken in the burette and titrated first against blank and then against the sample. Titre value was noted down.

Calculation

$$\% \, Nitrogen \, = \frac{14.01 \times 0.1N \times (TV - BV)}{W \times 1000} \times 100$$

$$\% \, Protein = \%N \times 6.25 \; (for \; food \; samples)$$

Where,

TV = Titration reading

BV = Blank reading

W= Sample weights

5 . Fat Analysis (IS: 1011: 2002)

Procedure:

Two grams of sample were taken in a thimble and the thimble was placed in a previously weighed soxhlet beaker. The beakers were then placed in the extractor (SocsPlus). After that extractor was filled with petroleum ether and its top was covered with cotton plugs. The Soxhlet apparatus (SOCS PLUS, SCS-4, Chennai) was then switched on with a set temperature of 70 °C for 90 minutes. After completion of extraction, the temperature was increased up to 150 °C again for 90 minutes, for the complete removal of Moisture. The beakers were removed from the Soxhlet apparatus and cooled in a desiccator. The cooled beakers were then weighed.

Calculation:

$W = W_1 - W_2$

W =Weight of residue

W_1= Weight of beaker after drying

W_2=Weight of empty beaker

6. Antioxidant activity

Procedure for DPPH inhibition method:

Determination of the antioxidant activity of the sample was done by the DPPH inhibition method **(**Nishino *et al*., 2000**)**. 1 g finely ground Sample was taken in 10 ml methanol and was kept overnight in dark at room temperature. 0.1 ml of eluted extract was taken and to it 0.25 ml of DPPH solution (80 µg/ml methanol) followed by 2 ml of methanol. A control blank was set up with 2.1 ml distilled water and left at room temperature for 30 min. The sample sets were centrifuged at 6000 rpm for 20 min. A cuvette was filled with 0.5 mL of centrifuged solution and 1 mL of methanol. Using methanol as a reference, absorbance was measured at 517 nm individually for the blank and samples.

$$\text{DPPH activity (\% RSA)} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

Total Phenolic Content

The total phenolic content was determined by the Folin-Ciocalteau method **(Makasana et al., 2017).** 2g samples were homogenized in 15ml of 80 % v/v aqueous ethanol at room temperature and centrifuge in cold condition at 6,000 rpm for 20 min at 4^0C and the supernatant was poured into Petri dishes and evaporated to dryness at room temperature. The residue was dissolved in 5ml of distilled water. 100µl of this extract was diluted with 3 ml of distilled water and 0.02ml of the Folin- Ciocalteau reagent was added. After 3 minutes, 2ml of 20% sodium carbonate was added and content was mixed thoroughly and blue was developed. The absorbance was measured at 760 nm in a UV- Spectrophotometer (Shimadzu, Japan) using gallic acid as a standard. The result was expressed as mg gallic acid / g dry material.

Microbiological Analysis

The control and antioxidant-rich *Dahi* were examined for the lactic acid bacteria count, yeast, and mold count during storage for 15 days at refrigeration temperature every 2 days.

Preparation of serial dilution blank

Sterile dilution blank (0.85 %) was prepared by dissolving 8.5 g of NaCl in 1 ltr of distilled water The pH was adjusted to 7.0 followed by autoclaving 9 ml aliquots at 121 ^{0}C for 15 min.

Preparation of serial dilution

1 ml of probiotic *Dahi* sample was taken and poured into a sterile 9 ml dilution blank. The content of the test tube was properly mixed. This represents the first dilution (1:10), subsequently, dilutions were prepared by transferring 1 ml in 9 ml sterile dilution blanks

Coliform count

Violet red bile agar was used for the coliform count. The probiotic *Dahi* samples were examined for the coliform count. Enumeration of the coliform count of functional *Dahi* was done using the pour plate method described by **Hought *et al.*, (1992)** employing violet red bile agar (pH 7.4 ± 0.1). The prepared plates were incubated at 37 ˚C for 48 hr. Colonies with dark red coloration were counted and they were expressed as log cfu per g of sample.

Yeast and mould count

PDA (Potato dextrose agar) was used to determine the yeast and mould in the probiotic *Dahi* was ascertained for yeast and mould counts as per suggested by **Hought *et al*., (1992)** using Potato dextrose agar and pH of media incubated at 3.5 ± 0.1 using a tartaric acid solution. The prepared plates were incubated at 30˚C for 3 to 5 days and counts were expressed as log cfu per g of sample

Shelf-life study of probiotic *Dahi*

The functional *Dahi* was stored at 4 - 7 °C and examined for microbial analysis at 3 days intervals Up to 17 days. The probiotic *Dahi* were analyzed for change in pH, %TA, and sensory evaluation scores. The estimation of the shelf life of the product was determined by sensory, and nutritional properties. The effect of tomato pomace powder incorporated into lassi can be determined by analyzing the total phenolic content, and DPPH inhibition at a time interval during storage. The microbiological analysis of the optimized product was carried out as per the standard method for coliform and yeast and mould.

Statistical analysis

Statistical analyses were performed using the software MS Excel data were analyzed by one-way ANOVA. The level of significance was set at $P < 0.05$. The data are presented as means ± Standard error. Sensory analysis, physicochemical properties, and storage studies data of probiotic *Dahi* were analyzed by one-way ANOVA (Analysis of Variance) using SPSS software (Version 20, USA)

Chapter IV

RESULTS AND DISCUSSION

The objective of this study was to optimize the technique for making functional *Dahi* by using black carrot as a source of prebiotics. The level of the ingredients was optimized using a 9-point hedonic sensory characteristics scale and textural parameters utilizing a texture analyzer in the early stages of the study. Finally, the product's Physico-chemical and functional qualities were evaluated.

4.1 Chemical composition of black carrot powder prepared by tray drying :

The black carrot powder was prepared by the tray drying technique. For black carrot powder preparation first black carrot with tap water and peel it after that blanch the black carrot at 80^0 C for 2 min and slice in pieces. The sliced black carrot passed through a pulper extractor, the juice was collected pomace remain after juice extraction, further processed for black carrot pomace powder preparation. Black carrot pomace was dried at 65^0 C for 8- 10 hrs in a tray drier. Physico-chemical and functional property analysis of pomace powder shown in Table 4.1 The average composition of black carrot powder was 5.04 ± 0.11% Moisture, 0.70±001 % Fat , 7.60±0.16 % Protein , 5.58± 0.13 % Ash , 63.41± 0.53% DPPH , 2.43 ± 0.21 %TPC , 14.00 ±0.22 mg / g crude Fibre . The prepared powder has good Fibre content and DPPH antioxidant activity and total phenolic content (TPC) with 63.41 ±0.53 (% inhibition) DPPH activity and 2.50 mg/ g DW. Similar composition reported by (Evren *et al.* 2022), 6.06 ± 0.12 Moisture , 1.60 ± 0.21 Fat , 8.82 ± 0.28 protein , 12.26 ±0.36 crude Fibre , 58.36 ± 0.45 DPPH , 2.30 ± 0.36 total phenolic compound .

Table No. 4 1 Chemical composition of Black carrot pomace powder:

Sample	Results
Moisture	5.04±0.11
Fat	0.70 ± 0.01
Protein	7.60 ± 0.16
Ash	5.58 ± 0.13
DPPH	63.41 ± 0.53
TPC	2.43 ± 0.21
Crude Fibre	14.00 ± 0.22

Value reported as Mean ± S.D (n=3)

4.2 Physico-chemical analysis of goat milk :

Fresh goat milk was procured directly from the goat's former locality of Varanasi, Uttar Pradesh. Milk samples were collected at the time of milking and transport and stored at refrigeration temperature. The Physico-chemical analysis of goat milk is presented in Table 4.2. The Physico-chemical analysis of goat milk was 3.92± 0.12 % Fat, 8.54 ± 0.23% SNF , 12.46 ± 0.34% TS , 3.46 ± 0.1 % Protein , 0.82 ± 0.01% Ash , 6.34 ± 0.08% pH , 0.164 ± 0.003% Acidity. The present study result similar to Ceballos *et al*., (2009), 3.45 ± 0.20 % Fat, 8.32 ± 0.12 % SNF , 12.55 ± 0.29 % TS , 3.34 ± 0.21 Protein , 0.79 ± 0.03 % Ash , 5.95 ± 0.41 pH and 0.166 ± 0.003 % acidity. One more similar study is (Wang, Li, *et al*., 2020), 3.23 ± 0.22 % Fat, 2.99 ± 0.28 Protein, 0.68 ± 0.33 Ash, etc.

Table No. 4.2 The Physico-chemical analysis of goat milk:

Goat milk	Results
Fat	3.92±0.12
SNF	8.54± 0.23
TS	12.46± 0.34
Protein	3.46 ± 0.1

Ash	0.82 ± 0.01
PH	6.32 ±0.08
Acidity	0.164± 0.003

Value is reported as Mean ± S. D. (n= 3)

4.3 Sensory evaluation of functional *Dahi* incorporated with black carrot powder (BCP) as a source of prebiotic.

A panel of semi-trained and trained judges assessed the sensory quality of black carrot pomace powder incorporated probiotic *Dahi* samples were evaluated at 9 – the point Hadonic Scale on Color and Appearance, Mouthfeel, Flavor, Body and Texture, and Overall Acceptability attributes. Results of sensory of functional *Dahi* are presented in Table 4.4.

4.4 Results of sensory attributes of probiotic *Dahi* incorporated with black carrot powder

Treatment	**Color and Appearance**	**Body and Texture**	**Flavor**	**Mouthfeel**	**Overall Acceptance**
T1(1% BCP)	8.40 ± 0.43^{b}	8.62 ± 0.14^{c}	8.38 ± 0.07^{c}	8.58 ± 0.08^{c}	8.28 ± 0.08^{d}
T2(1.5% BCP)	8.62 ± 0.41^{c}	8.82 ± 0.07^{b}	8.40 ± 0.08^{c}	8.70 ± 0.01^{c}	8.54 ± 0.028^{a}
T3(2% BCP)	7.68 ± 0.31^{a}	8.08 ± 0.11^{d}	8.30 ± 0.16^{a}	8.32 ± 0.08^{c}	8.26 ± 0.15^{b}
T4 (2.5% BCP)	7.52 ± 0.37^{d}	7.84 ± 0.10^{c}	7.68 ± 0.18^{a}	7.40 ± 0.02^{b}	7.66 ± 0.18^{c}
T5(3% BCP)	6.9 ± 0.29^{a}	6.30 ± 0.20^{a}	7.34 ± 0.18^{a}	5.0 ± 0.15^{b}	5.82 ± 0.19^{c}

The values are reported as Mean ± SD (n=10); a,b,c,ddifferent superscripts in a row are significantly different at the level of $P < 0.05$.

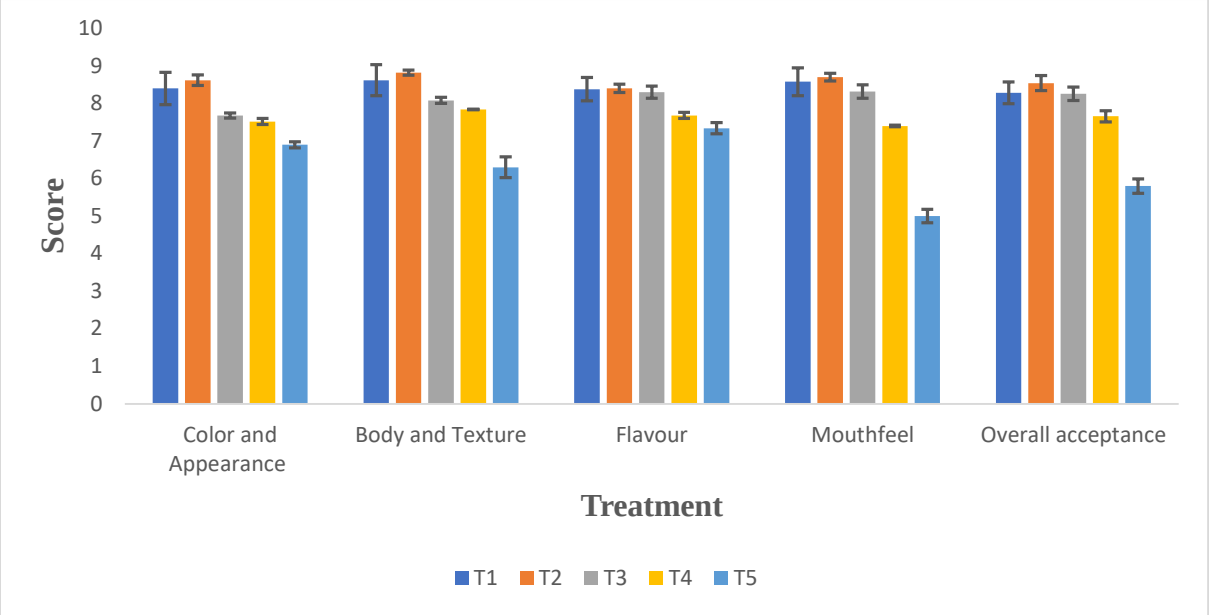


Fig. 4.1

4.4 Sensory score

The Overall Acceptability of sensory score of functional *Dahi* were 8.28±0.08%,8.54±0.028%,8.26±0.15%,7.66±0.18%,5.82±0.19 % for T0(0% BCP), T1 (1% BCP), T2 (1.5% BCP), T3 (2% BCP), T4 (2.5% BCP) and T5 (3% BCP), respectively .

The color and appearance scores varied from 6.90±0.29 to 8.62± 0.43. The minimum color and appearance score were obtained due to dark pink color in treatment T5 in which there is @ 3% black carrot powder added, while the maximum color and appearance score of 8.62 ± 0.41 was observed in T2 in which there is @ 2% addition of black carrot powder its color was light pink. The color and appearance are also good in T1 with a score of 8.4±0.43 but T2 was optimized because due overall acceptability of T2 was 8.54 ± 0.13 is higher than T1 was 8.28± 0.07.

Similar studies were carried out by Fernandez-Garcia and McGregor (2005) by incorporating oat Fibre, sugar beet Fibre, and rice Fibre. The results of this study are in line with the current study.

These sensory mean values for Body and Texture of black carrot powder added *Dahi* is recorded for T2 (8.82±0.07) is significantly higher than other trails because fine curd with. The minimum value is recorded for T5 (6.30±0.20). In T5 there is a coarse body also lumpy texture. Oat Incarporateed Fibre *Dahi* has reported that the source of dietary Fibre affects the body and textural of cultured dairy products

(Staffolo *et al.*, 2004). As well as the level of Fibre powder incorporated increased firmness and decreased smoothness.

T2 (8.70±0.10) had the highest sensory mean value for the Mouthfeel of black carrot powder added to probiotic *Dahi*, whereas T5 (5.0±0.15) had the lowest. 3% BCP addition had higher sedimentation and also contains insoluble Fibre that comes into the mouth during chewing.

From the table it can be inferred that the Flavor score of black carrot powder added there *Dahi* was highest in T4 (8.40±0.08) and the lowest value was for T5 (7.34±0.18). There is good Flavor in T1 but T2 has a slightly sweet flavor.

Trial T2 has the highest overall acceptance (8.54±0.15) while trial T5 had the lowest (5.82±0.192). All of the samples got mean scores that ranged from "like moderately" to "like a lot." No variety was felt within the dislike or neither like nor dislike categories Color & appearance and Flavor were the sensory factors that showed substantial differences and had an impact on the product's sensory scores and consumer acceptability, hence they were employed for the product optimization.

Chemical analysis of black carrot powder incorporated *Dahi*:

Fig. 4.2

Parameter	**T0**	**T1**	**T2**	**T3**	**T4**	**T5**
pH	4.90 ± 0.01^{e}	4.81 ± 0.11^{e}	5.42 ± 0.12^{d}	5.85 ± 0.07^{c}	6.36 ± 0.09^{b}	6.83 ± 0.06^{a}

Acidity	0.78 ± 0.002[a]	0.73 ± 0.001[b]	0.67 ± 0.01[c]	0.65 ± 0.002[c]	0.62 ± 0.04[cd]	0.59 ± 0.04[de]
Moisture	87.20 ± 0.51[a]	86.90 ± 0.25[b]	86.79 ± 0.11[c]	86.51 ±0.03[d]	86.25 ± 0.23e	85.95 ± 0.04[f]
Fat	3.63± 0.02[d]	3.7± 0.02[c]	3.73 ± 0.01[c]	3.81 ± 0.01[b]	3.85 ± 0.01[b]	3.90 ± 0.01[a]
Protein	3.63 ± 0.01[e]	3.71 ± 0.02[d]	3.74 ±0.005[d]	3.82 ±0.02[c]	3.88 ±0.01[b]	3.95 ±0.02[a]
Ash	0.61 ±0.01[e]	0.73 ±0.02[d]	0.78 ±0.005[c]	0.83 ± 0.01[b]	0.86 ±0.005[b]	0.89 ± 0.01[a]
Whey syneresis	76.16 ± 0.51[a]	74.81 ± 0.25[b]	71.77 ± 0.23[c]	73.59 ± 0.03[d]	72.77 ± 0.23[d]	70.44 ± 0.16[e]
DPPH	23.38 ± 0.02[e]	44.65 ± 0.04[d]	45.04 ±0.01[c]	45.45 ±0.04[b]	46.58 ± 0.03[b]	47.31 ±0.16[a]
TPC	6.45 ±0.016[e]	6.48± 0.004[e]	6.52 ± 0.02[d]	7.11 ± 0.05[c]	7.22 ± 0.01[b]	7.27 ± 0.01[a]
TS	12.75±0.15[de]	12.77±0.23[cd]	12.80± 023[c]	12.95±0.37[c]	13.05± 0.33[b]	13.22±0.25[a]
Crude Fibre	0	0.24 ± 0.001[cd]	0.26± 0.0008[bc]	0.248± 0.08[b]	0.30 ± 0.04[a]	0.31 ± 0.004[a]

Fig4.4 Black carrot pomace powder incorporated with probiotic *Dahi*

Functional *Dahi* prepared by incorporation of black carrot as a source of prebiotic by different treatments were analyzed Moisture, Fat, protein, ash, pH, acidity, DPPH, TPC whey syneresis, etc.

Fat :

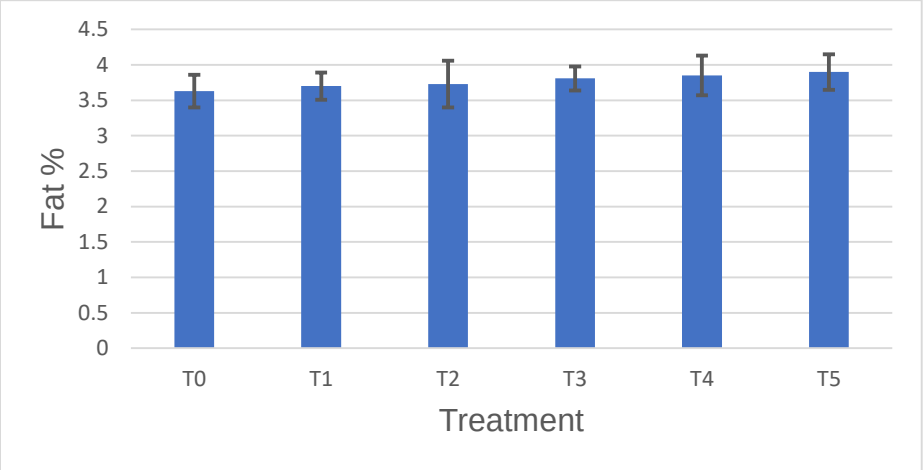


Fig 4.3

4.4.1 Effect of different levels of black carrot powder on Fat percentage of functional *Dahi*:

The data of optimized product and other treatment product Fat of functional *Dahi* is presented in table 4.4 and fig 4.2. The variation in the Fat content of functional *Dahi* is slight because the percentage in black carrot powder was 0.70 ± 0.02. In this treatment T0, T1,T2, T3, T4, T5, Fat obtained 3.63± 0.01, 3.7± 0.02, 3.73 ± 0.01, 3.80 ± 0.01, 3.85 ± 0.01, 3.90 ± 0.01 respectively . The Fat content was highest at 3.90 ± 0.01 in T5 in which there is a higher rate of addition of black carrot powder and lowest in T1, 3.7± 0.02 in which lower rate of addition. The optimization was T2 in the percentage of Fat was 3.73 ± 0.01 in this analysis we get data that are significantly different from each other.

Similarly, results **(**Oliveira *et al.*, 2009) reported that prepared of low-Fat *Dahi* by incorporation of inulin as a source of prebiotic in which treatment T1, T2, T3, @ 0.5 %, 1 %, 1.5 % and Fat varied range from 2.48 to 3.15 at a different level of addition of Fibre.

One more study sample is T4, T3, T2, and T1. The fat content of the sample showed a gradual increase as the level of oats was increased. The increase in fat content of oats added *Dahi* may be attributed to the 6 percent oil present in oats. The fat content of *Dahi* depends on the initial Fat content of milk used for curd making or raw material used to prepare the curd and the level of volume reduction of milk

during boiling (Chowdhury, et al., 2011). The fat percent of plain Misti *Dahi* varies from 4.3 to 8.8 percent with an average of 3.8 percent (Gosh and Rajorhia, 2012).

Protein:

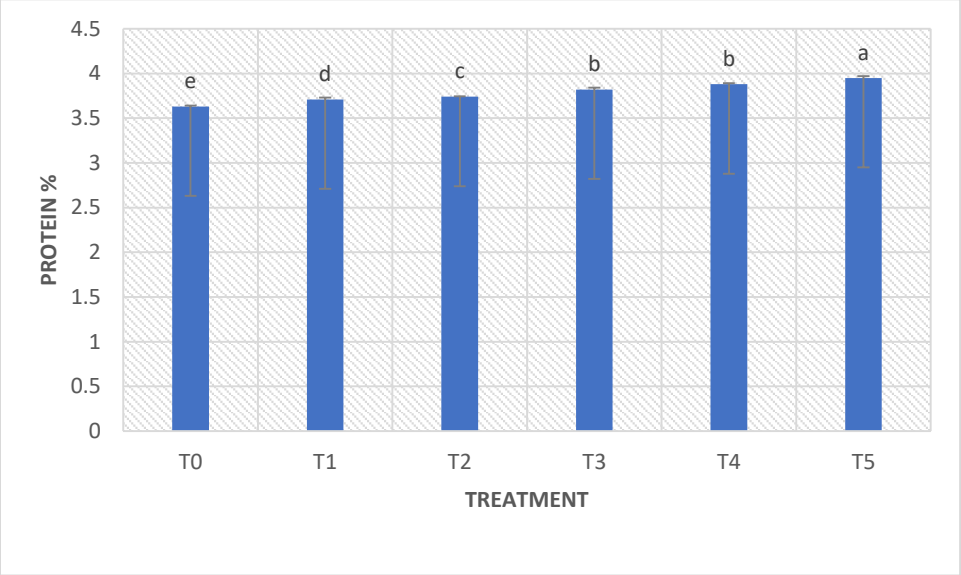


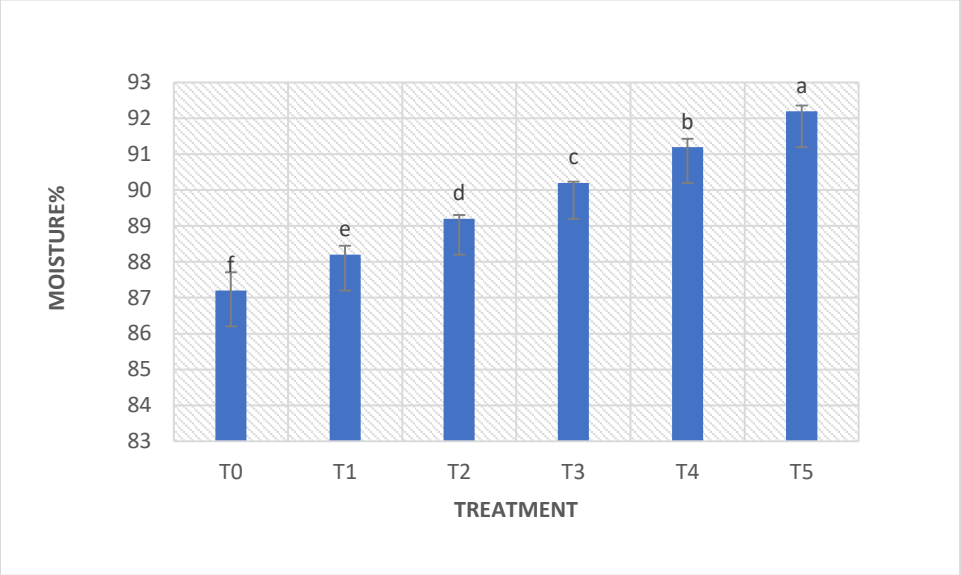
Fig 4.4

4.4.2 Effect of different levels of black carrot powder on protein percentage of functional *Dahi*:

The data of optimized product and other treatment product Fat of functional is presented in table 4.4 and fig 4.3. As presented in the table incorporation of black carrot pomace powder added at a different level significantly affects the protein content in functional *Dahi*. For control sample T0 and treated samples T1, T2, T3, T4, T5, and the value recorded were 3.63 ± 0.01, 3.71 ± 0.02, 3.74 ±0.005, 3.82 ±0.02, 3.88 ±0.01, 3.95 ±0.02 respectively T0 has lowest Protein content 93.63 ± 0.01) of *Dahi* and T5 has highest Protein 3.95 ±0.02 in which 3% of black carrot pomace powder added such as we conclude that data is significantly different to each other due to as we increase the percentage of black powder percentage there is also increase in protein content of functional *Dahi*.

Similar study - Probiotic *Dahi* fortified with Incarporateed oats Fibre at 0, 1, 2, 3, and 4 percent levels were prepared in the laboratory and were designated as T1, T2, T3, T4, and T5. Desai et al. (1994) reported that the protein percentage of fresh milk *Dahi* was within the range of 3.7 to 4.3 percent. The addition of an increased level of non-Fat dry milk increased the protein percentage of *Dahi* (Begum et al. 2011).

Moisture:



4.4.1 Effect of different levels of black carrot powder on Moisture percentage of functional *Dahi*

Table 4.4 presents that Moisture *Dahi* was significantly decreasing as we increase the rate of addition of black carrot powder due to addition at a different level of carrot powder at a constant amount of Moisture in processed milk. Moisture of functional *Dahi* T0 treated samples T1,T2,T3,T4,T5 and mean± S.D.were 87.20 ± 0.51, 86.90 ± 0.25, 86.79 ± 0.11, 86.51 ±0.03, 86.25 ± 0.23, 85.85 ± 0.16 respectively. It was observed that from the table, Moisture ranged varied from T0 to T5. Noticed that from the table T0 i.e control (0 %) was the highest Moisture (87.20 ± 0.51) while T5with addition of black carrot pomace powder (3%) was the lowest Moisture (85.85 ± 0.167).

A similar study evaluated the effect of the addition of four different types of dietary Fibres on the rheological, physicochemical, and sensory characteristics of yogurt. The four types of Fibres (inulin, pea, oat, and wheat) were added in the *Dahi* formulation in different proportions (1%–2.5%) using classical technology adapted to laboratory conditions to reduce Moisture content with the rate of addition of Fibre incorporation (Desai et al., 2005)

4.4.5 Effect of different levels of black carrot pomace powder on the total solid percentage of functional *Dahi* :

The data presented for different treatments of functional *Dahi* in table 4.4 shows that functional total solid increase significantly ($p<0.05$) by addition of black carrot pomace powder . total solid for control

sample T0 and treated sample T1,T2,T3,T4,T5 and mean ± S.D., 12.75 ± 0.23, 12.95 ± 0.31, 13.10 ± 0.22, 13.25 ± 0.21, 13.35 ± 0.33, 13.44 ± 0.17 respectively .

From the table, we obtained T0 has minimum total solid while T5 has maximum due to maximum percentage of rate of addition such as we conclude as the rate of addition increases total solid of functional *Dahi* also increases.

The present study was supported by the result obtained by **(Patel, 2011**) prepared *Dahi* blended with carrot pulp and reported that the total solid content of the finished product increased ranged from 12.65 to 13.16at different rates of addition, **(Bagal *et al.*, 2015)** reported that significantly increased in total solid content finished products ranged from (12.18 to 13.05).

4.5 Ash :

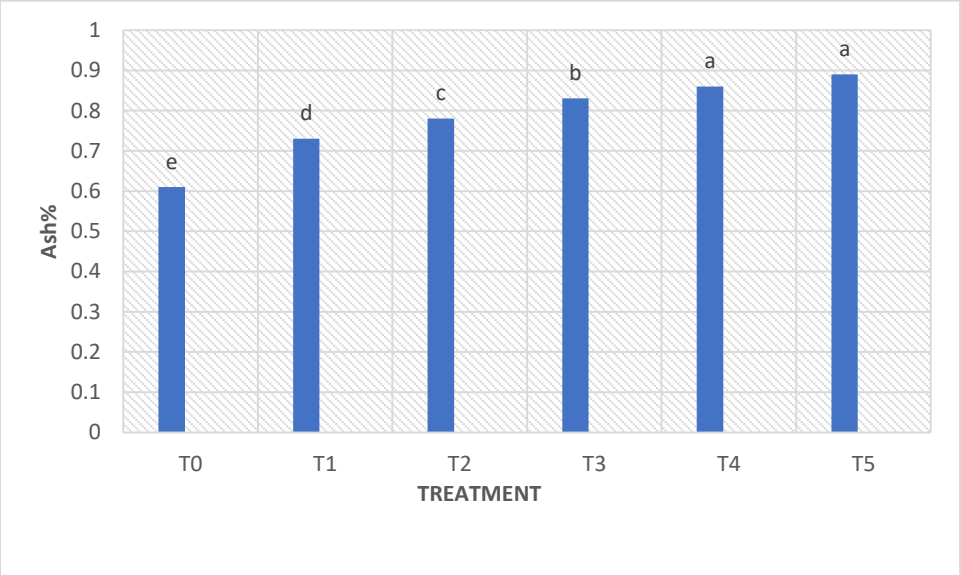


Fig.- 4.5

4.5.1 Effect of different levels of black carrot pomace powder on Ash percentage of functional *Dahi*.

Table 4.4 shows that the Ash content of functional *Dahi* was increased significantly due to the addition of black carrot pomace powder. Ash content of *Dahi* for control T0 and treated samples T1, T2, T3 and T4 ,T5 mean value were 0.61 ±0.01, 0.73 ±0.02 , 0.78 ±0.005 , 0.83 ± 0.01 , 0.86 ±0.005, 0.89 ± 0.01 respectively.It was observed that from the table, directly proportional to Ash, increased from T0 to T5.

Noticed that from the table T0 of the control sample (0 %) was the lowest Ash (0.61 ±0.01) and T5 with the addition of black carrot pomace powder (3%) was the highest Ash (0.89 ± 0.01).

Garcia-Perez *et al.* (2006) reported that low doses of orange Fibre i.e..0.6, 0.8 %, and 1.0 % increase the Ash content of functional *Dahi* due to an increase in total solid of functional *Dahi* in the textural analysis of T1, T2, T3 of the sample.

Yotkar, (2019) prepared utilization of mango pulp in cow milk and reported that the increased level of pulp resulted in the Ash content of i*Dahi* significantly increasing from 0.71 to 1.23 Similarly.

4.5.1 pH :

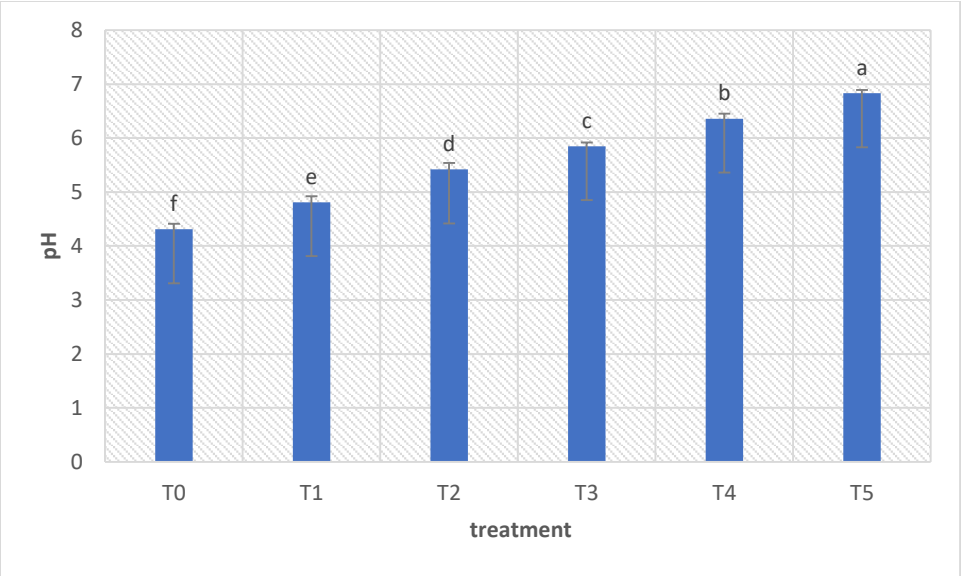


Fig .4.6

4.5.2 Effect of different levels of black carrot pomace powder on pH percentage of functional *Dahi*.

Table 4.4 shows that pH decrease with the increased rate of addition of black carrot from 4.31 to 6.83. Black carrot powder revealed that the pH of functional was increased significantly due to the addition of black carrot pomace powder. pH of functional *Dahi* for control sample T0 and treated samples T1, T2, T3 and T4mean value were 4.31 ± 0.10, 4.81 ± 0.11, 5.42 ± 0.12, 5.85 ± 0.07, 6.36 ± 0.09, 6.83 ± 0.06 respectively.

It was observed that pH, increased with the increased rate of addition of black carrot powder from T0to T5. Noticed that from table T0 i.e control (0 %) was the lowest total solid and T5with the addition of black carrot pomace powder (3%) was the highest total solid.

There was a significant difference ($p<0.01$) present in the pH value collected for black carrot powder incorporated *Dahi* . The highest pH value (6.83 ± 0.06) was found and the lowest value (4.31 ± 0.10) was observed. The result of the present findings was agreed with the work of Kamruzzaman *et al*. (2002) who reported that the pH value of *Dahi* samples in a room incorporated beetroot powder from 4.01 to 4.16.

4.5.2 Acidity :

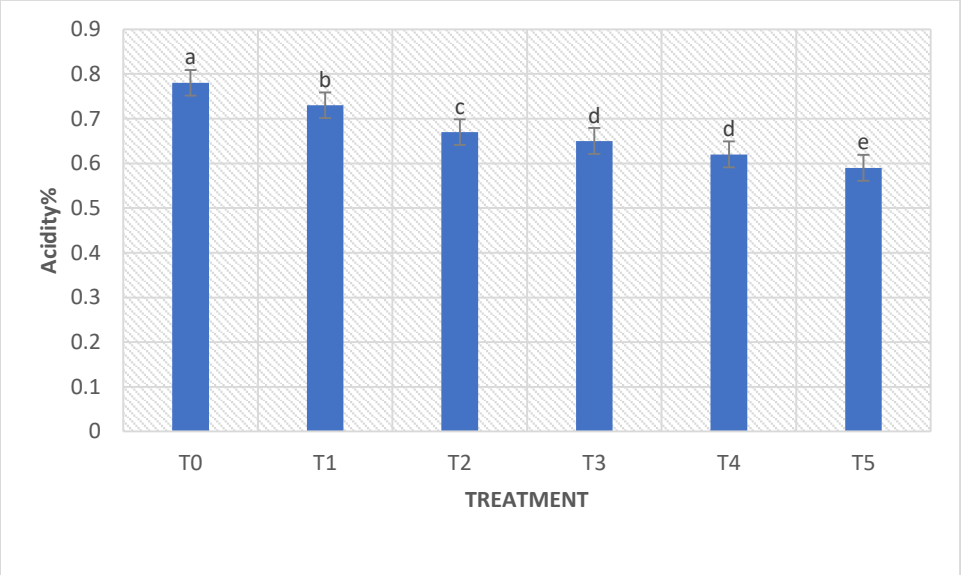


Fig. 4.7

4.5.3 Effect of different levels of black carrot pomace powder on titratable acidity percentage of functional *Dahi*.

This table presents that the Titratable content of functional *Dahi* was increased significantly due to the addition of black carrot pomace powder. Titratable acidity ranged from 0.52 to 0.78 incorporated with black carrot powder of 1%, 1.55, 2%, 2.5 %, and 3 % of black carrot powder respectively.

It was observed that from the table that as well as the rate of addition increased acidity decreased, from T 0 to T5 Noticed that from the table T0 i.e without the addition of tomato pomace powder (0 %) was the lowest total solid and T5 with the addition of black carrot pomace powder (2%) was the highest total solid.

This study was supported by (Dey et al., 2011); the titrable acidity showed a gradual increase from T1 to T5 samples as the level of oats increased *Dahi*. However, their differences were statistically significant. The acidity of *Dahi* noted in the study matches the reported value of 0.68 to 0.81 percent 0.69 to 0.75 percent (Salunkhe, 2008), and 0.80 to 0.83 percent (Younus *et al*. 2002). The titrable acidity observed in the present study may be due to the incorporation of black carrot powder.

Whey syneresis :

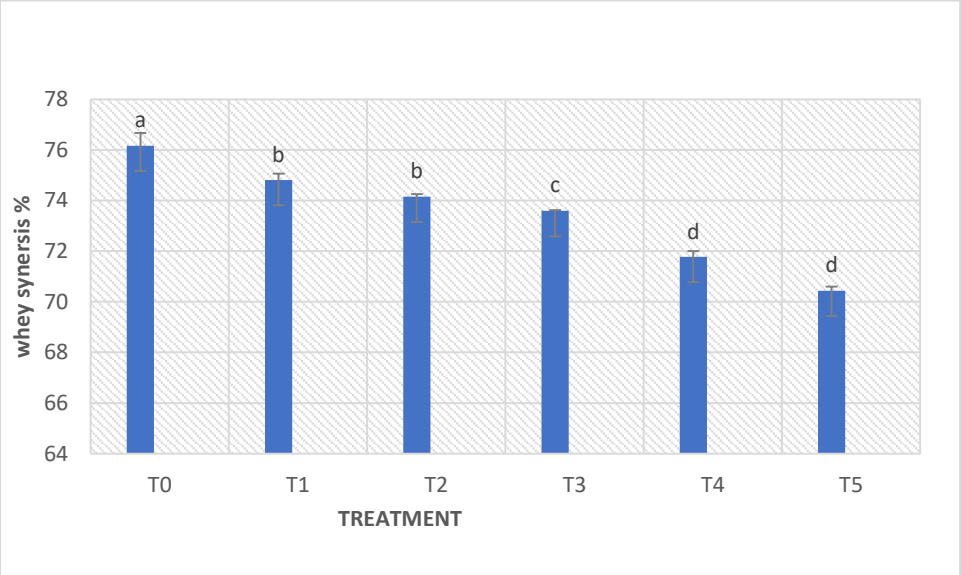


Fig 4.8

4.5.1 Effect of different levels of black carrot pomace powder on whey syneresis percentage of 4.5.2 functional *Dahi* :

Table 4.4 shows, that the black carrot pomace powder led to a reduction in the Syneresis value of functional *Dahi* due to the fact that tomato pomace powder contains a substance that helps in the water holding capacity and another function is to stabilize its capacity for binding water, which positively affects the Syneresis properties. The average whey syneresis content of functional *Dahi* was 76.16 ± 0.51,74.81 ± 0.25,74.15 ± 0.11, 71.77 ± 0.23, and70.44 ± 0.16 prepared with the addition of black carrot pomace powder at 0% (T0), 1% (T1), 1.5% (T2), 2.0% (T3), 2.5% (T4) and 3.0 (T5), respectively.

It was observed that from the table, decreased from T0 to T5. T0, i.e., without the addition of tomato pomace powder (0%), was the highest whey syneresis (55.37%) and TP4 with the addition of black carrot pomace powder (2%), was the lowest whey Syneresis (46.37%).

4.5.2 DPPH :

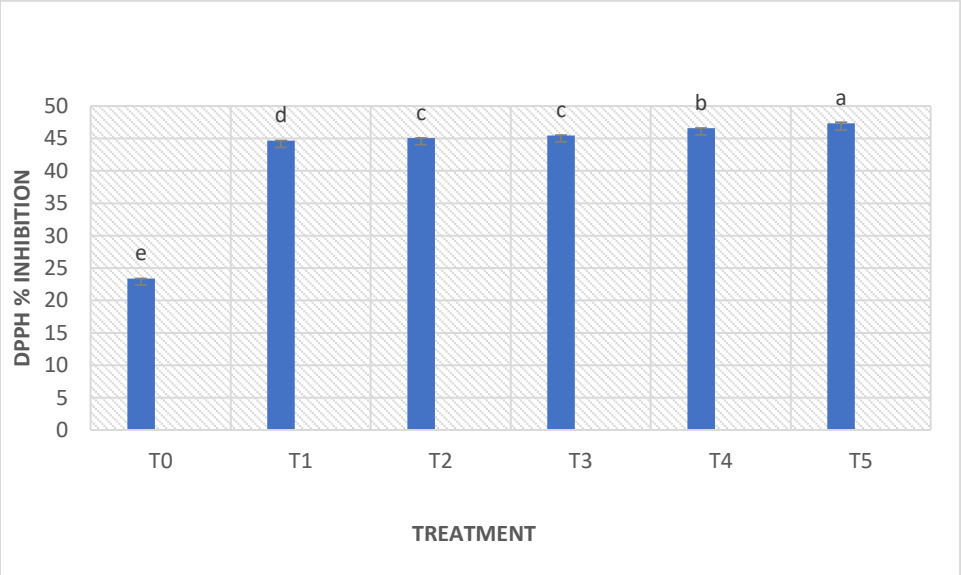


Fig 4.9

4.5.3 Effect of different levels of tomato pomace powder on DPPH radical scavenging activity percentage of functional *Dahi*:

Table 4.3 revealed that the DPPH radical scavenging activity of functional *Dahi* at different concentrations was increased, due to the increased rate of addition of black carrot powder is added. The radical scavenging activity of black carrot incorporated at the different concentrations on DPPH radical scavenging activity is depicted in table 4.3 and. DPPH % of inhibition varied from 23.38 ± 0.02, 44.65 ± 0.04, 45.04 ±0.01, 46.58 ± 0.03 and 47.31 ±0.16 of inhibition in powder incorporated *Dahi*.

It was observed that from the table, increased from T0 to T5. Noticed that from the table 4.4 T0 i.e control (0 %) was the lowest DPPH radical scavenging activity (23.38 ± 0.02) and T5 with the addition of tomato pomace powder (2%) was the highest DPPH radical scavenging activity (47.31 ±0.16 %).

Similarly, the result find out **(Parnell-clunies *et al.*, 2020)** developed fortified yogurt by using tomato pomace powder. Reported that DPPH radical scavenging activity of fortified yogurt increased from

15.82 to 17.56 **(Ankitha*et al.*, 2018)** developed Curcumin Fortified Whey Beverage. Reported that DPPH activity increased from 8.084 to 33.216 at different concentrations.

Crude Fibre :

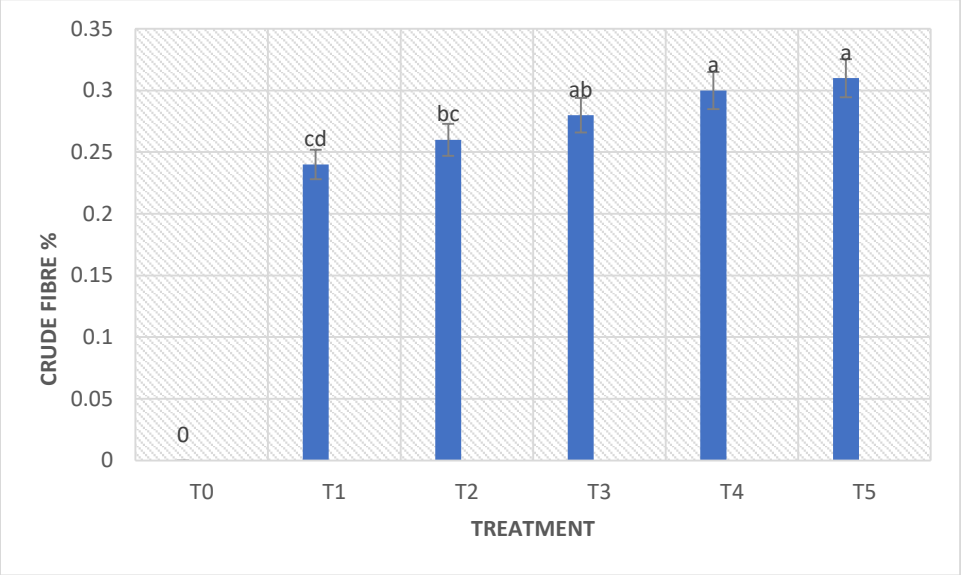


Fig . 5.1

4.6.2 Effect of different levels of black carrot pomace powder on crude Fibre percentage of functional *Dahi*:

The table 4.4 shows that Fibre content significantly increase with rate of addition of black carrot pomace powder , from treatment T0 to T1, T2, T3, T4, T5, addition rate (0%, 1%, 1.5 %2%, 2.5 % and 3%,) are 0,

It was observed from the following table that Fibre content in *Dahi* is directly proportional to the rate of addition of black carrot powder. This study was supported by (Shahidi, 2004), the incorporation of soluble Fibres into the aqueous phase of foods incorporated at a different level at a different rate of addition generally enhances their total solid and Fibre content.

The crude Fibre of different experimental Shrikhand was estimated by the official method described in Analysis of the Association of Official Analytical Chemists (AOAC, 1990), It can be seen from different levels of addition of Fibre with increasing the rate of addition of Fibre, an increasing Fibre content in samples Shrikhand that was observed. Samples fortified with inulin powder had higher Fibre contents because of the presence of higher Fibre content in inulin powder i.e. 9%.

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4.7 Texture profile of probiotic *Dahi* incorporated with Black carrot powder

The textural properties of functional *Dahi* by incorporation of Black carrot powder added measured in terms of fracturability, Cohesiveness, Springiness, Gumminess, Chewiness, and Resiliencies using the Brookfield CT3 texture analyzer. Texture attributes are used as parameters to monitor and control product acceptability.

Table 4.3 Comparative values for textural properties of probiotic *Dahi* incorporated with black carrot powder.

Textural properties	Optimized	Control
Hardness(g)	65.0	64.44
Springiness	0.840	0.864
Chewiness	0.648	-1.509
Cohesiveness	0.415	0.372
Resiliencies	0.026	0.019
Gumminess	0.753	-1.787

4.7 Comparison of Hardness

The data in table 4.3 indicate the of hardness *Dahi* samples. The hardness of functional *Dahi* (65.0 g) and for the control sample (64.44 g) was found to be significantly different.

From the table, it can be evaluated that the hardness of commercial *Dahi* was higher compared to the developed sample. The commercial samples wereanalyzed3 days after the date of manufacture and the samples were kept in refrigerated condition for the time being. **Desai *et al.*,(1991) stated**

that hardness depends upon the minerals content and the Moisture content. An increase in total solid content leads to an increase in hardness also decrease in Moisture content leads to an increase in hardness.

4.7.1 Comparison of Cohesiveness

The data in table 4.7 indicate the Cohesiveness of *Dahi* sample value of the control sample was (0.372) and for the optimized sample was (0.415).

The cohesiveness value of the control sample was (0.372) and for the optimized sample was (0.415) was found to be significantly higher compared to other samples cohesiveness of the controlled sample was lower compared to control and commercial samples and this can be due to powder incorporation. **Dongare *et al.*(2019)** found that the cohesiveness of functional *Dahi* made from buffalo milk was 0.483.

4.7.2 Comparison of Springiness

The data in table 4.7 indicate the springiness of functional *Dahi* incorporated with black carrot powder of Control sample (0.864 mm) was significantly greater than FCMP (0.840 mm).

Control sample (0.92mm) and (0.86 mm). **Kumari and Singh (1992)** found that cow milk *Dahi* had less springiness than buffalo milk *Dahi*.

4.7.3 Comparison of Gumminess

The data in table 4.3indicate the gumminess of *Dahi* samples. There was a significant difference in the Gumminess.**PrakAshrao (2013)** found that gumminess increases with an increase in Fibre content. As the market functional *Dahi* for samples were stored under refrigerated condition for 3 days, which might be the reason for the increase in gumminess.

4.5.6 Comparison of Chewiness

The data in the table 4.3indicate the chewiness of functional *Dahi* samples. The chewiness of the optimized sample (0.648g) was higher significantly higher compared to the controlled sample (-1.059g). It was also found that an increase in chewiness can be due to a decrease in Fat content **(Kumar *et al.*, 2011)**.

4.6 Effect of storage period on the shelf life of optimized *Dahi* (T2) and control sample(T0)

4.6.1 Effect of pH on optimized *Dahi* (T2) and control sample (T0) during storage period at room temperature and refrigeration temperature:

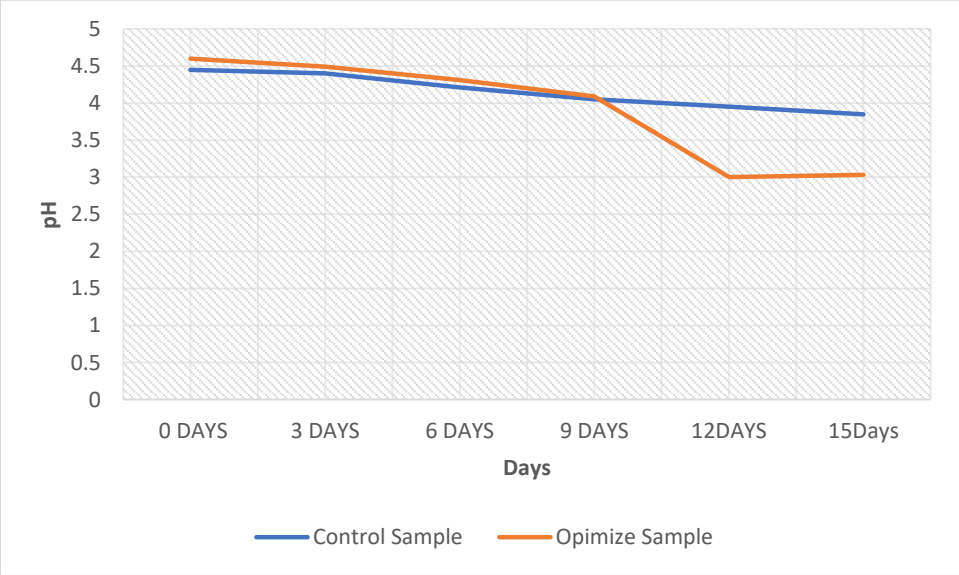

Fig. 5.2

Table 4.4 represent pH decrease significantly from 4.50±0.05 to 4.22±0.01 for control sample T0 (0%) and 4.60±0.05 to 4.32±0.01 (pH) for functional optimized *Dahi* T2 (2%) at room temperature within 2 days after that deteriorated and pH decreased 4.45±0.21 to 3.85±0.14 for the control sample (T0) and 4.60± 0.22 to 4.03±0.19 for optimizing sample(T2) during storage at refrigeration temperature for 15 days respectively

This study was supported by Panda et al., (2006) study of pH of pomegranate peel extracted *Dahi* decreased in all samples with an increase in shelf life and storage period from 4.97 ± 0.07 to 4.87 ±0.09 for the control sample and 4.98±0.04 to 4.72 ±0.09 during storage 0 to 15 days respectively.

The pH was up to 15 days of preservation after that the pH decreased gradually. The initial pH of the control sample was 4.45 at room temperature which was reduced to 2.85 at 15 days of storage and the pH of the optimized sample reduced from 4.60 to 2.65 at room temperature. the pH of probiotic *Dahi* decreases step by step with time during storage. The fresh pH values of the of optimize samples were 4.60 which gradually decreased to 4.31, 3.85, 3.21, and 2.65 respectively for 15 days period at room temperature and the pH value of the optimized sample were 4.60 which gradually decreased to 4.46, 4.31, 4.18 and 4.03 respectively for 15 days. The work is supported by the shelf-life study of Hussain and Shakir (2010), who worked that the pH of apple and apricot jam samples decreased trends during storage time.

4.6.2 Effect of acidity on optimized *Dahi* (T2) and control sample (T2) during storage period at room temperature and refrigeration temperature

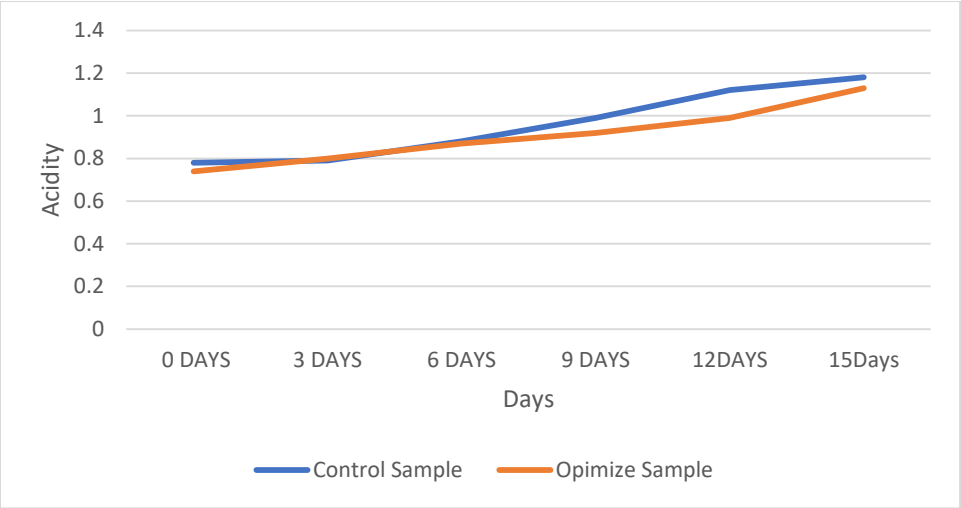

Fig 5.3

Table 4.5 represent acidity decrease significantly 0.77±0.02 to 1.62 ± 0.01 for control T0, (0%) and 0.74±0.02 to 1.01±0.01 for functional optimized lassi T2 (2%) and acidity increases from 0.78 ±0.23

to 1.18 ± 0.33 ± 0.15 for control sample and 0.73 ± 0.18 to 1.13 ± 0.22 for optimize sample during storage at refrigeration temperature for 15 days.

Panda et al., (2006) reported pH and titrable acidity are inversely related to a decrease in pH during storage there was a corresponding increase in titrable acidity. A similar result is found in symbiotic Misti *Dahi*.

4.6.3 Effect of DPPH in functional *Dahi* along with control sample and optimize sample at refrigeration temperature:

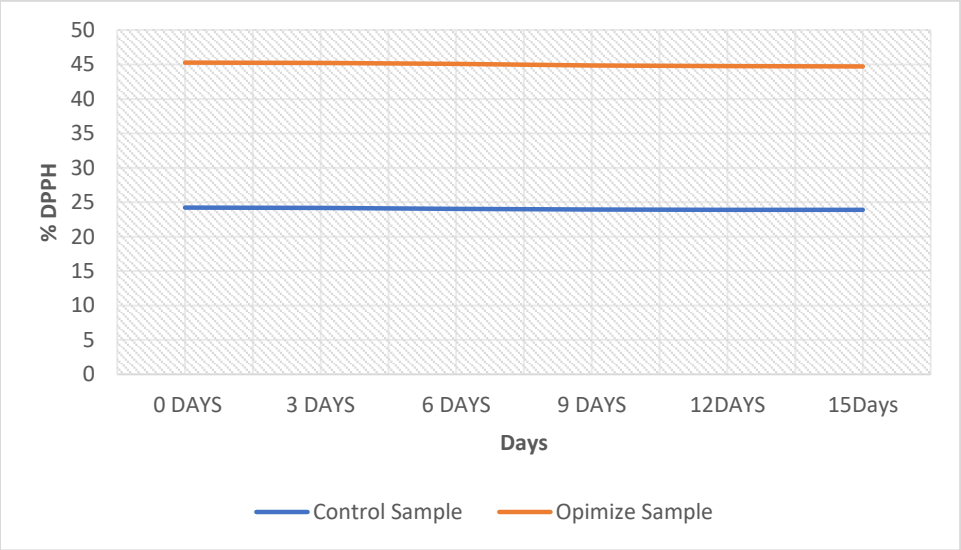

Fig. 5.4

table 4.4 shows that DPPH of control sample reduced from 24.22 ± 0.32 to 23.88 ± 0.32 in control sample T0 (0%) and 45.28 ± 0.28 to 44.73 ± 0.33 in optimize sample at T2 (2%)

This was supported by **Zamora et al. (2004)**Storage temperature and light is a major hindrances when it comes to the stability of phenolic compounds as well as their corresponding antioxidant activity reported a significant decrease in the total antioxidant activity of 26 varieties of teas powder in lassi

within three months of storage at room temperature the result of the present study are also consistent with the finding **Zoric et al**. (20015) who reported a major decline in the antioxidant activity of Marasca sour cherry powder incorporated *Dahi* at 4^0C.

4.6.4 Effect of whey syneresis in functional *Dahi* along with control sample and optimize sample at refrigeration temperature:

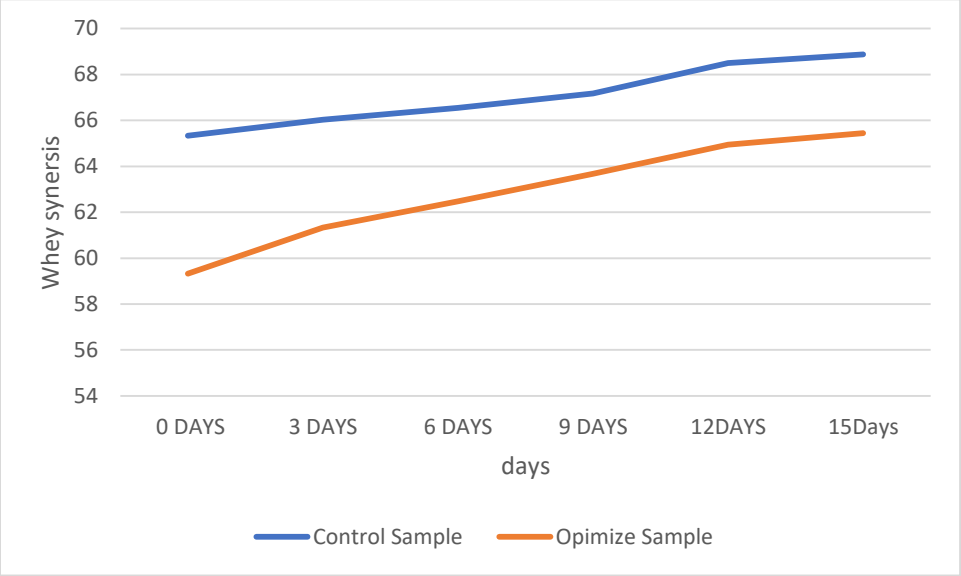

Fig. 5.5

The whey syneresis of the control sample varies from fresh condition 68.13± 0.14 to 65.33± 23 in the control sample and the whey syneresis of the optimizing sample varies from fresh condition 61.86 ± 0.23 to 59.33± 0.23 within 15 days.

The pomegranate peel extracts *Dahi* at the storage significantly increase whey syneresis ($p<0.05$)in all the samples. The control sample had the lowest total solid as compared to extract added *Dahi*with increase I % of extract *Dahi* corresponding increase in total solid content *Dahi* sample was observed. Total solid was significantly increased ($p<0$)during the storage period which could be due to Moisture migration to the surrounding atmosphere Penna et al.,(2006)and Hassan et al., (2010).

Table No. 4.5 Microbial parameter:

Parameters	Treatment	0 days	3 day	6 day	9 day	12 day	15 day
pH	T0	4.45±0.05	4.51±0.05	4.48±0.05	4.41±0.15	4.29 ±0.02	3.85±0.01
	T1	4.60±0.05	4.44±0.05	4.39±0.05	4.35±0.15	4.31±0.02	4.03 ±0.01
Acidity %	T0	0.78±0.02	0.72±0.02	0.76±0.03	0.84±0.02	0.96±0.02	1.18±0.01
	T1	0.73±0.02	0.78±0.02	0.83±0.03	0.88±0.02	0.93±0,02	1.13±0.01
Coliform (cfu/ml)	T0	Nil	Nil	Nil	Nil	Nil	Nil
	T1	Nil	Nil	Nil	Nil	Nil	Nil
Yeast & mould (cfu/ml)	T0	Nil	Nil	10	18	50	55
	T1	Nil	Nil	12	25	60	65

4.6.5 Effect of yeast and mold count in functional *Dahi* along with control sample and optimize sample at refrigeration temperature :

Table 4.5 depicted that there is not found yeast and mold optimized sample until 6 days while in the controlled sample has found yeast and mold was 0 till the 3rd day at 6th days of analysis yeast and mold count was 10 cfu/ml. Yeast and mold count 50 cfu/ml of the control sample and while optimized sample had found 55 cfu/ml and at 15th days yeast and mold count of the control sample was 60 cfu /ml and optimize sample was 65 cfu/ml in 15 days. According to the microbial analysis of Yeast and mold, the count was also observed to be less than the standard value of 100/g. From microbiological analysis, it was observed that the coliform count in *Dahi* was nil throughout the storage period. Considering all these following parameters during the storage period, the shelf life of the control sample can remain good quality for up to 8 days at refrigeration temperature (4 ± 2).while the optimized sample can remain good quality for up to 11 days at refrigeration temperature (4 ± 2). The optimized sample is not of good quality for up to 15 days.

(Pal, 2018) developed functional *Dahi* by incorporating oat Fibre *Dahi* Reported that shelf life of *Dahi* was evaluated for various parameters such as Physico-chemical characteristics, sensory attributes, and microbial quality. Functional *Dahi* was of good quality up to 11 days at 5℃.

Table no. 4.7 Probiotic count during storage:

Duration	Total count (cfu/mg) -control sample			Total probiotic count (cfu/mg) - optimie sample		
	T1	T2	T3	F1	F2	F3
Fresh	9.05×10^9	9.12×10^9	9.17×10^9	5.59×10^9	5.46×10^9	5.38×10^9
2nd	8.05×10^9	8.01×10^9	8.10×10^9	3.86×10^9	3.77×10^9	3.81×10^9
4th	7.45×10^9	7.36×10^9	7.28×10^9	2.48×10^9	2.35×10^9	2.29×10^9
8th	9.25×10^8	9.05×10^8	9.31×10^8	9.47×10^8	9.55×10^8	9.38×10^8
10th	3.52×10^8	3.35×10^8	3.29×10^8	4.32×10^8	4.29×10^8	4.18×10^8
12th	5.81×10^7	5.83×10^7	5.69×10^7	6.39×10^7	6.24×10^7	6.40×10^7
14th	2.45×10^7	3.14×10^7	4.13×10^7	4.69×10^7	4.13×10^7	4.38×10^7
15th	2.10×10^7	2.01×10^7	2.32×10^7	2.32×10^7	3.01×10^7	2.65×10^7

T1, T2, and T3 are a treatment for the control sample, and F1, F2, and F3 are a treatment for the optimized sample.

From the table seen in fresh condition average, lactobacillus count was 5.47 x 10^9 in the control sample and 9.11 x 10^9 in the optimized sample while after 15 days it reduced on average to 2.14 x 10^7 in the control sample and 2.66 x 10^7 optimize sample. The probiotic count of the control sample in fresh condition was 9.05 x 10^9 it was reduced to 8.05 x 10^9, 3.52 x 10^8, 5.81 x $10^{7,}$ and 2.10 x 10^7 on the 10^{th}, $12^{th,}$ and 15^{th} days respectively while on the case of optimizing sample probiotic count is reduced to 5.59 x $10^{9,}$ 4.29 x $10^{8,}$ 6.24 x 10^7 and 2.32 x 10^7 in 10^{th}, 12^{th}, 15^{th} days respectively.

This study was supported by (Dave and Shah, 2005)The viable counts of lactococci and lactobacilli in *Dahi* at different time intervals. The bacterial counts of *Dahi* during storage increased on day 2 of storage and counts of lactococci in fresh samples and after 2 days of storage were 1.17 × 10^7 and 4.62 × 10^9 cfu/mL, respectively, and the counts of lactobacilli were 3.51 × 10^7 and 2.45 × 10^9 cfu/mL, respectively. However, from 2 days onwards, i.e. on the fourth, sixth, and eighth day of

storage, the counts for both lactobacilli and lactococci decreased: the counts of lactococci on the fourth, sixth and eighth day were 2.91 × 10^8, 1 × 10^8 and 1.66 × 10^7 cfu/mL, whereas, the counts of lactobacilli were 1.71 × 10^8, 6.95 × 10^7 and 1.4 × 10^7 cfu/mL, respectively. The results indicated that the storage conditions and time were favoring the growth of lactic acid bacteria initially, but long storage did not favor the growth of lactic acid bacteria in *Dahi*.

Chapter V

SUMMARY AND CONCLUSION

Current consumer trends and evolving needs indicate a significant opportunity for the development of new value-added goods with a variety of health-promoting and disease-preventive features. Natural remedies that are both safe and effective are being developed by the food and dairy industries. The incorporation of numerous plant elements with good medicinal potential, such as antioxidants, phenols, flavonoids, and glycosides into dairy products is gaining appeal in the global market.

The present study aims at the "Development of functional *Dahi* by using black carrot as a source of prebiotic" that was conducted in the Department of Dairy Science & Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India. The project was performed with three major objectives. The first objective was to develop the probiotic *Dahi* using black carrot powder. The second objective was to study the Physico-chemical & antioxidant properties of the product. The third objective was to study the textural and sensory properties of BCP-added probiotic *Dahi*. The project was undertaken with three main objectives. Five trials were examined for sensory attributes (color and appearance, Flavor, Mouthfeel, Body and Texture, and overall acceptability), textural (fracturability, cohesiveness, chewiness, and resilience) for the selection of variants with the optimum level of ingredients.

5.1 Formulation of Functional *Dahi* by Using Black Carrot Pulp Powder:

In the formulation of probiotic *Dahi* by incorporation of black carrot as a source of probiotic first, standardize goat milk at 4% Fat and 8.5 % SNF. Pasteurize this at 85^0 C for 5 min cool it at 60^0C and add black carrot powder 0, 1, 1.5, 2, 2.5, 3.0% and mix properly.

Maintained the temperature up to 37°C inoculate culture 0.2 % and incubate it for 8 hrs and keep it for setting. After setting curd stored it at 4^0 C

5.2 Physico-Chemical, Textural, and Antioxidant activity of Formulated Product

Sensory properties are examined with the help of a 9-point Hedonic rating scale (1 = dislike extremely, 9 = like extremely) was used. The score for the Body and Texture varied from 7.97 to 8.8, for color and appearance it varied from 6.08 to 8.81; for the flavor, it varied from 6.77 to 8.69 and for overall acceptability, it varied from 6.24 to 8.62. Trial T1 showed the best results for all the sensory parameters, as it had the highest mean value of the other variants.

The textural properties of control *Dahi* have fracturability of 8.026 (g) and the optimized product has 7.92 (g), the cohesiveness of control was 0.370 and for optimized it was 0.418 and gumminess for control sample was -1.797 whereas for optimized it was 0.750.

Antioxidant activity of optimized product was 61.50 % and control *Dahi* was 13.66 %. The antioxidant activity was based on the level of BPF powder used. Total phenolic content was found to be 87.23 mg GAE/ g.

According to sensory and textural properties, T1 was found to be having the highest sensory score as well as acceptability, the textural properties were better than the control sample of yogurt, and it also showed the high antioxidant activity. Thus, trial T1 can be taken as the optimized product based on sensory and textural properties. The optimized product (Trial T1) had a composition of black carrot powder 1g, milk 100ml, and both the starter culture @1% each.

The optimized probiohad the proximate composition of protein 3%, Fat 3.4%, Moisture 73.4%, Ash 0.891%, pH 4.24, titrable acidity 0.76 and DPPH inhibition 61.50%.

From the present study, it can be concluded that the developed Antioxidant-rich BCP powder added to probiotic *Dahi* had high sensory and textural properties along with good functional properties. It can be also concluded that Black carrot powder can be added as a natural colorant into many products such as in health drinks, bakeries, and confectionery to increase its antioxidant potential and make it beneficial for health.

From this study, it can be concluded that B can be added as a potential colorant as well as a source of antioxidants in dairy and food products. It is also concluded that due to the high coloring potential, black carrot can be limited to a certain amount in food products, and rather it can be used in combination with other non-colorant antioxidant-rich sources.

5.3 Shelf-life Study of Developed Product

In fresh conditions average lactobacillus count was 5.47×10^9 in the control sample and 9.11×10^9 in the optimizing sample while after 15 days it reduced on average to 2.14×10^7 in the control sample and 2.66×10^7 optimize sample. The probiotic count of the control sample in

fresh condition was 9.05 x 10^9 it was reduced to 8.05 x 10^9, 3.52 x 10^8, 5.81 x $10^{7,}$ and 2.10 x 10^7 on the 10^{th}, $12^{th,}$ and 15^{th} days respectively while on the case of optimizing sample probiotic count is reduced to 5.59 x $10^{9,}$ 4.29 x $10^{8,}$ 6.24 x 10^7 and 2.32 x 10^7 in 10^{th}, 12^{th}, 15^{th} days respectively.

There is not found yeast and mold optimized sample until 6 days while in the controlled sample has found yeast and mold was 0 till the 3^{rd} day at 6^{th} days of analysis yeast and mold count was 10 cfu/ml. Yeast and mold count 50 cfu/ml of the control sample and while optimized sample had found 55 cfu/ml and at 15^{th} days yeast and mold count of the control sample was 60 cfu /ml and optimize sample was 65 cfu/ml in 15 days. According to the microbial analysis of Yeast and mold, the count was also observed to be less than the standard value of 100/g.

APPENDICES

SENSORY SCORECARD FOR

DEVELOPMENT OF FUNCTIONAL *DAHI* BY INCORPORATION OF BLACK CARROT POWDER AS A SOURCE OF PREBIOTIC

Date--------/---------/----------

PRODUCT----------

NAME OF THE PANELIST----------

DESIGNATION----------

Respected Sir/Madam

Kindly evaluate formulated samples of Shrikhand on the 9-Point Hedonic Scale of the following attributes:

Sample Code	Flavor	Color & Appearance	Body and Texture	sweetness	Overall acceptability

1: Dislike extremely
2: Dislike very much
3: Dislike moderately
4: Dislike slightly
5: Neither like nor dislike
6: Like slightly
7: Like moderately
8: Like very much
9: Like extremely

Remarks----------

(Signature of the judge)

STATISTICAL ANALYSIS

1. ANOVA for Color and Appearance

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	11.14167	5	2.228333	14.972	1.04E-06	2.620654148
Within Groups	3.572	24	0.148833			
Total	14.71367	29				

2. ANOVA for Body and Texture

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	21.12267	5	4.224533	113.1571	7.06E-16	2.620654
Within Groups	0.896	24	0.037333			
Total	22.01867	29				

3. ANOVA for Flavor

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4.678667	5	0.935733333	35.76051	2.3E-10	2.620654
Within Groups	0.628	24	0.026166667			
Total	5.306667	29				

4. ANOVA for Mouthfeel

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	52.12567	5	10.42513	584.5869	3.05258E-24	2.620654
Within Groups	0.428	24	0.017833			
Total	52.55367	29				

5. ANOVA for Overall Acceptability

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	27.311	5	5.4622	240.9794	1.08641E-19	2.620654
Within Groups	0.544	24	0.022667			
Total	27.855	29				

6. ANOVA for Moisture

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	17.46278	5	3.492556	48.35846	1.54E-07	3.105875
Within Groups	0.866667	12	0.072222			
Total	18.32944	17				

7. ANOVA for Fat

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.1548	5	0.03096	132.6857	4.51E-10	3.105875
Within Groups	0.0028	12	0.000233			
Total	0.1576	17				

8. ANOVA for Protein

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.168263	5	0.033652676	0.009462	0.999969	2.772853
Within Groups	64.01949	18	3.556638105			
Total	64.18775	23				

ANOVA for pH

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	13.43576	5	2.687152	292.7889831	4.19E-12	3.105875
Within Groups	0.110133	12	0.009178			
Total	13.54589	17				

ANOVA for Acidity

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.073779	5	0.014756	151.7746	2.04E-10	3.105875239
Within Groups	0.001167	12	9.72E-05			
Total	0.074946	17				

ANOVA for Ash

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.157561	5	0.031512	182.9742	6.79E-11	3.105875239
Within Groups	0.002067	12	0.000172			
Total	0.159628	17				

ANOVA for Crude fibre

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.158946	5	0.031789	17339.52	1.03E-22	3.105875
Within Groups	0.000022	12	1.83E-06			
Total	0.158968	17				

ANOVA for DPPH

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1272.449	5	254.4899	24936.41	1.16E-23	3.105875
Within Groups	0.122467	12	0.010206			
Total	1272.572	17				

9. ANOVA for TPC

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.386183	5	0.477237	425.2604	4.55E-13	3.105875
Within Groups	0.013467	12	0.001122			

Total	2.39965	17				

10. ANOVA for Whey syneresis

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	64.76772	5	12.95354	120.8791	7.78E-10	3.105875
Within Groups	1.285933	12	0.107161			
Total	66.05365	17				

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