Technology of Blueberry Incorporated Goat Milk based High Protein Dessert

THESIS SUBMITTED TO
ICAR- NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL
(DEEMED UNIVERSITY)
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE

MASTER OF TECHNOLOGY
IN
(Food Technology)

BY

LAKSHMIPRIYA P R
B.TECH. (FOOD TECHNOLOGY)

DIVISION OF DAIRY TECHNOLOGY

ICAR- NATIONAL DAIRY RESEARCH INSTITUTE

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This is to certify that the thesis entitled **"TECHNOLOGY OF BLUEBERRY INCORPORATED GOAT MILK BASED HIGH PROTEIN DESSERT"** submitted by **LAKSHMIPRIYA P R** towards the partial fulfilment of the requirement for the award of the degree of **MASTER OF TECHNOLOGY IN FOOD TECHNOLOGY** of the **ICAR-NATIONAL DAIRY RESEARCH INSTITUTE (DEEMED UNIVERSITY)**, Karnal (Haryana), India, is a bonafide research work carried out by her under my supervision and guidance and no part of the thesis has been submitted for any other degree or diploma.

(Dr. Heena Sharma)
MAJOR ADVISOR

Dedicated to my Respected guide, beloved parents and twin sister

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ABSTRACT

Goat milk is preferred over bovine milk due to its unique nutritional composition and there has been renewed interest in therapeutic and nutritional characteristics of goat milk because of higher amount of short and medium chain fatty acids (better digestibility) and lower α-s_1 casein (less allergenic). Further, goat milk can be efficiently utilized by converting into value-added products incorporated with natural bioactive such as blueberry. Blueberries have great potential for inclusion in dairy dessert owing to their high antioxidant capacity and several health benefits. However, fermentation of goat milk has always been a challenge for the processors due to lesser firm curd and weaker gel. Certain physical interventions, involving an increase in the total solids or protein content, can be attempted for the preparation of fermented goat milk products. In this study, goat milk based high protein dessert was developed using ultrafiltration technique at different concentration factors and optimizing its formulation using blueberry, sugar and inulin. Ultrafiltered goat skim milk 3X was used for preparation of blueberry dessert as it showed higher protein (%) (8.91 ± 0.15) and total solids (%) (15.58 ± 0.22) than 1X and 2X. Protein curd mass (PCM) obtained after fermentation was further optimized for the incorporation of blueberry crush (BC) (PCM: BC::70:30, 80:20, 90:10), sugar (5%,10%,15%) and inulin (2%, 3%, 4%). Based on sensory analysis and rheological characteristics, PCM: BC with 80:20 ratio, 10% sugar and 4% inulin was optimized and selected for further characterization. The optimized blueberry dessert (OBD) had pH, acidity (% lactic acid), fat (%), protein (%), ash (%) and total solids (%) with the value corresponding to 4.23, 1.37, 1.01, 13.55, 0.93 and 48.79, respectively. Color values of high protein blueberry dessert were significantly ($p < 0.05$) affected with the addition of blueberry crush. The *L**and *b** values of the optimized product significantly decreased ($p<0.05$) with the blueberry addition while, *a** values significantly ($p<0.05$) increased. The antioxidant activity (DPPH, FRAP) and total phenolic content of OBD were significantly higher ($p<0.05$) than control. Control and optimized samples were further stored in polypropylene packaging material and subjected to in-package thermization. Acidity increased significantly ($p<0.05$) with storage for both control and OBD non-thermized and thermized samples. Rheological attributes showed that storage modulus was higher than loss modulus indicating viscoelastic behavior. α –Lactalbumin, β – Lactoglobulin, α, β, Kappa caseins, αs_1-casein were the major protein fractions identified in non-thermized and thermized samples. Storage studies depicted the spoilage of non-thermized control and OBD samples on 7^{th} day and 14^{th} day of storage, respectively while, thermized control and OBD were found to be acceptable upto 28 days under refrigerated condition.

सारांश

ब्लूबेरी शामिल बकरी के दूध *पर* आधारित उच्च प्रोटीन मिठाई की तकनीक

छोटी और मध्यम श्रृंखला फैटी एसिड (बेहतर पाचनशक्ति) और कम α-s_1 कैसिइन (कम एलर्जीनिक) की मात्रा के कारण बकरी के दूध की चिकित्सीय और पोषण संबंधी विशेषताओं में रुचि बढ़ी है। इसके अलावा, बकरी के दूध को ब्लूबेरी जैसे प्राकृतिक बायोएक्टिव के साथ शामिल मूल्य वर्धित उत्पादों में परिवर्तित करके कुशलतापूर्वक उपयोग किया जा सकता है। ब्लूबेरी में अपनी उच्च एंटीऑक्सीडेंट क्षमता और कई स्वास्थ्य लाभों के कारण डेयरी मिठाई में शामिल करने की काफी संभावनाएं हैं। हालांकि, कम सख्त दही और कमजोर जेल के कारण बकरी के दूध का किण्वन हमेशा खाद्य प्रसंस्कारक के लिए एक चुनौती रहा है। किण्वित बकरी के दूध उत्पादों की तैयारी के लिए कुल ठोस या प्रोटीन सामग्री में वृद्धि को शामिल करते हुए कुछ शारीरिक हस्तक्षेपों का प्रयास किया जा सकता है। इस अध्ययन में, विभिन्न सांद्रता कारकों पर अल्ट्राफिल्ट्रेशन तकनीक का उपयोग करके और ब्लूबेरी, चीनी और इनुलिन का उपयोग करके इसके निर्माण को अनुकूलित करके बकरी के दूध पर आधारित उच्च प्रोटीन मिठाई विकसित की गई थी। अल्ट्राफिल्टर्ड बकरी स्किम मिल्क 3X का उपयोग ब्लूबेरी मिठाई की तैयारी के लिए किया गया था क्योंकि इसमें 1X और 2X की तुलना में उच्च प्रोटीन (%) (8.91 ± 0.15) और कुल ठोस (%) (15.58 ± 0.22) दिखाया गया था। किण्वन के बाद प्राप्त प्रोटीन दही द्रव्यमान (पीसीएम) को ब्लूबेरी क्रश (बीसी) (पीसीएम: बीसी :: 70:30, 80:20, 90:10), चीनी (5%, 10%, 15%) के समावेश के लिए और अधिक अनुकूलित किया गया था।) और इनुलिन (2%, 3%, 4%)। संवेदी विश्लेषण और रियोलॉजिकल विशेषताओं के आधार पर, पीसीएम: बीसी 80:20 अनुपात के साथ, 10% चीनी और 4% इनुलिन को अनुकूलित किया गया और आगे के लक्षण वर्णन के लिए चुना गया। अनुकूलित ब्लूबेरी डेज़र्ट (OBD) में pH, अम्लता (% लैक्टिक एसिड), वसा (%), प्रोटीन (%), राख (%) और कुल ठोस (%) का मान क्रमशः 4.23, 1.37, 1.01, 13.55 0.93 और 48.79 के अनुरूप था। उच्च प्रोटीन ब्लूबेरी मिठाई के रंग मूल्य महत्वपूर्ण रूप से (पी <0.05) ब्लूबेरी क्रश के योग से प्रभावित थे। अनुकूलित उत्पाद के एल* और बी* मूल्यों में उल्लेखनीय रूप से कमी आई (पी<0.05) ब्लूबेरी जोड़ के साथ, जबकि, ए* मूल्यों में उल्लेखनीय रूप से वृद्धि हुई (पी<0.05)। ओबीडी की एंटीऑक्सीडेंट गतिविधि (डीपीपीएच, एफआरएपी) और कुल फेनोलिक सामग्री नियंत्रण से काफी अधिक (पी <0.05) थी। नियंत्रण और अनुकूलित नमूनों को आगे पॉलीप्रोपाइलीन पैकेजिंग सामग्री में संग्रहीत किया गया और इन-पैकेज थर्माइजेशन के अधीन किया गया। नियंत्रण और ओबीडी गैर-थर्माइज्ड और थर्माइज्ड नमूनों दोनों के भंडारण के साथ अम्लता में काफी वृद्धि हुई (पी <0.05)। रियोलॉजिकल विशेषताओं ने दिखाया कि भंडारण मापांक विस्कोलेस्टिक व्यवहार को इंगित करने वाले हानि मापांक से अधिक था। α -लैक्टलबुमिन, β - लैक्टोग्लोबुलिन, α, β, कप्पा कैसिइन, αs_1-कैसीन गैर-थर्माइज्ड और थर्माइज्ड नमूनों में पहचाने जाने वाले प्रमुख प्रोटीन अंश थे। भंडारण अध्ययनों में भंडारण के 7 वें और 14 वें दिन गैर-थर्माइज्ड नियंत्रण और ओबीडी नमूनों के खराब होने को दर्शाया गया है, जबकि प्रशीतित स्थिति में थर्माइज्ड नियंत्रण और ओबीडी 28 दिनों तक स्वीकार्य पाए गए हैं।

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LIST OF ABBREVIATIONS

%	Percent
˚C	Degree Celsius
µg	Microgram
µm	Micrometer
µmol	Micromole
ACE	Angiotensin-converting enzyme
ACN	Anthocyanins
AOAC	Association of Analytical Chemists
BC	Blueberry crush
Ca	Calcium
CAGR	Compounded Annual Growth Rate
Cfu	Colony forming unit
CLA	Conjugated linoleic acid
$CuSO_4$	Copper(II) sulphate
EFA	Essential fatty acid
FAO	Food and Agriculture Organization
G	Gram
GABA	γ-aminobutyric acid
GSM	Goat skim milk
H	Hour
H_2SO_4	Sulfuric acid
HCl	Hydrochloric acid
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
K_2SO_4	Potassium sulphate
L	Liter
Ltd	Limited
MCT	Medium-chain triglycerides
mg	Milligram

Mg	Magnesium
mL	Mililitres
MRS	De Man, Rogosa and sharpe agar
MUFA	Monounsaturated fatty acids
N	Normality
NaOH	Sodium hydroxide
NCDC	National Collection of Dairy Cultures
NPN	Non-protein nitrogen
OBD	Optimized blueberry dessert
P	Phosphorous
Pa.s	Pascal second
PCM	Protein curd mass
pH	Negative log of the hydrogen ion concentration
PP	Polypropylene
SD	Standard Deviation
SDS- PAGE	Sodium dodecylsulphate-polyacrylamide gel electrophoresis
SMP	Skim milk powder
TPC	Total plate count
TS	Total solids
UF	Ultrafiltration
UFM	Ultrafiltered concentrated skim milk
Zn	Zinc

CHAPTER-1

Introduction

INTRODUCTION

Goat (*Capra hircus*), a ruminant and hollow-horned mammal, belongs to the genus *Capra* and family *Bovidae*. Demand for the goat milk has been increasing in many developed and developing countries because of growing populations, increasing levels of disposable incomes and the connoisseur interest in goat milk products, especially cheeses and yoghurt. Goat milk consumption has proven to be advantageous to those afflicted with cow milk allergies and other gastrointestinal ailments. Asia has the largest proportion of the world's goat milk production (53%), followed by Africa (25%), Europe (17%), Americas (4%), and Oceania (<1%) (Miller *et al.,* 2019). In India, the heads of the goats are close to 148.88 million showing an increase of 10.1% over the previous census. India occupies first position in goat milk production with production figure of 6.2 million tonnes and it has been estimated to be 8.2 million tonne by 2024 (FAOSTAT, 2019). The global goat milk market size was $ 8.5 billion in 2018 and is expected to reach $ 11.415 million by 2026. Geographically, Asia- pacific region dominates the global milk product markets. Because of high nutritional content and health benefits of goat milk and milk products, these have gained popularity across the globe. With such high demand and production of goat milk, there is immense scope for value-addition of goat milk in order to improve its consumer acceptability and sensory quality.

Goat milk is preferred to cow milk since it offers important nutrients to infants and has significant therapeutic effects (Shahid *et al.,* 2021).As far as the composition is concerned, goat milk has almost similar lactose as cow milk, however there are variations in protein composition and their structure. Further, as compared to cow milk, goat milk has comparatively lower fat content but it is richer in medium chain fatty acids (Eknaes *et al*., 2009). Therefore goat milk is an important part of the diet, particularly for infants and people suffering from malnutrition (Turkmen, 2017). It is also considered to be a natural functional food with chemical qualities similar to cow milk and human milk. Goat milk and its products are valuable to the dairy sector and have been used for the treatment of jaundice, dengue fever (Haenlein, 2004; Mahendru *et al*., 2011; Deshwal *et al*., 2020). Due to its unique characteristics, such as higher digestibility, hypoallergenicity and probiotic delivery, there is a growing recognition of goat milk's relevance. However, the goaty flavour is another issue that has arisen as a result of the high level of free octanoic acids in goat milk, which may limit its adoption by few people (Alqahtani *et al.,* 2021).

The most popular milk-based products in the world are fermented milks due to their high nutritional and therapeutic values. It has been estimated that nearly 8 per cent of the milk produced in India is used in the preparation of fermented milks (Aneja *et al.,* 2002). Over the

years, fermented milks became an indispensable supplement to the stable food consumed every day. Fermented milk products play an important role in the human daily diet. Global fermented milk product market was USD 63.1 billion in 2018 and is expected to register 5.3% CAGR during the period 2019-2024. Some of the popular fermented milk products are shrikhand, *dahi*, *lassi*, yoghurt, kumiss, kefir, acidophilus milk and bulgarian butter milk (Kosikowski, 1997). Value added products are being prepared or developed by goat milk producers all over the world by adopting certain technological interventions. However, in addition to its unpleasant goaty flavour, the manufacture of fermented goat milk products has technological challenges, particularly delicate coagula, lower quantities of α_{s1}-casein, greater micellar dispersion, and higher concentrations of colloidal calcium and all these have been linked to decreased firmness in goat milk products. As a result, goat milk yoghurts coagulum is soft and less viscous, with a high amount of syneresis and a weaker gel. Various ways to improve goat milk yoghurt texture, such as membrane processing, enzymatic cross-linking of milk proteins, high-pressure homogenization and the use of in situ exopolysaccharides generating cultures, have been tested to address these technological challenges. Traditional methods of improving goat milk yoghurt texture, either by increasing overall solid content or adding various stabilisers, are still the most widely utilised. Cyclodextrin and fruit juices have, on the other hand, been successfully used to conceal the goaty flavour in goat yoghurt.

Further, dairy products are considered as the ideal host of functional ingredients based on topmost preference by the consumers. Dairy desserts are more or less like gel or semisolid soft textured light flavoured products made from whole or skimmed milk, sugar, thickeners such as starch and hydrocolloids and addition of other functional ingredients. Dairy desserts make an important contribution to the diet and these type of products are high in calcium and vitamin D, as well as phosphorus, potassium, magnesium, riboflavin, niacin, essential fatty acids and proteins (Ozcan *et al.*, 2019). Consumers are provided with an option to choose from a vast variety of dairy desserts each with its own appearance, texture and flavor (Jahromi and Niakousari, 2018). Therefore, incorporation of various functional ingredients to goat milk and milk products would enhance sensory and functional value of the product. They are commonly consumed by various groups of people because of their nutritional value. The industries dealing with preparation of dairy desserts are anticipated to flourish in the coming years owing to novel dietary trends that include healthy and low fat foods. Researchers have utilized various herbs (Giloy, aloevera, arjuna, sage, basil etc.), fruits (berries, papaya, mango, watermelon) bioactive compounds (curcumin) etc. for incorporation in dairy products for their value addition, improved functionality and overall acceptability (Oraon *et al.*, 2017).

Food and Agriculture Organization (FAO) has designated blueberry as one of five human healthy foods rich in polyphenols, anthocyanins and other nutritive active components (Cheng *et al*., 2020). The worldwide production of blueberry is 552,505 tonnes and the major producers are America and Canada (80%). With increasing demand of blueberries, imports to India has also been increasing with figure of about 100 tonnes in 2020 (The Hindu, 2020). Blueberry (*Vaccinium sp.*) is a fruit with great potential for inclusion in functional products due to its high antioxidant capacity (Hou *et al.*, 2019).Due to its strong antioxidant activity against free radicals and reactive species, the blueberry is recognised as a "longevity fruit" and is considered as one of the richest sources of antioxidants, among all fruits and vegetables (Prior *et al*., 1998). This activity is most likely the principal mechanism by which its intake lowers the chances of developing various diseases, including chronic non-communicable diseases triggered by oxidative processes (Reque *et al.*, 2014). Blueberries have been shown to have effective preventive and inhibitory effects on oxidative damage caused by diseases such as cardiovascular diseases and cancer (Zhang *et al*., 2021).

Although goat milk yoghurt has various health-related bioactivities, it has lower antioxidant capacity. The addition of phenolic-rich components to goat milk yoghurt could increase the products value owing to antioxidant benefits (Dimitrellou *et al*., 2020; Aires *et al*., 2017 ; Verruck *et al*., 2019).

The consumption of blueberry has also been associated with several health benefits. However, fermentation of goat milk always has always been a challenge for the processors owing to its unique protein profile. Fermented goat milk often yield less firm curd and weaker gel. Methods applied to address this issue includes the physical interventions which involves an increase in the total solids or protein content. Moreover, goat milk based protein-rich products incorporated with natural bioactive possess the challenges such as poor fermentation characteristics, astringency, precipitation and alteration in functional properties. Furthermore, addition of polyphenol- rich bioactive compounds such as blueberries extract/pulp/crush may pose certain other technological challenges including protein- polyphenol interactions, color, flavour stability and others. Therefore, keeping all these technological challenges in mind, the present study is envisaged with the following objectives:

Objectives:

1. Optimization of processing conditions and formulation for blueberry incorporated goat milk based high protein dessert

2. Determination of shelf life of goat milk based high protein dessert

CHAPTER-2

Review of Literature

REVIEW OF LITERATURE

This chapter provides in depth scientific information generated in the past related to various aspects of goat milk and its significance, focusing mainly on incorporation of blueberry as a functional ingredient, quality interventions and fermentation in goat milk, utilization of inulin as fat replacer etc. Various previously scientific papers published related to this field have also been presented here for their relevance and interest.

2.1 Goat Milk

The value of goat milk has increased globally due to its high medicinal virtues for human health (Pal, 2014). Goat milk contains 3.4% protein, 3.8% fat, 4.1% lactose, and 0.8% ash. Goat milk contains higher amount of Ca, Mg and P than cow and human milk (Park *et al.,* 2007). Three fatty acids viz., caproic, caprylic and capric have great medicinal values for patients suffering from a variety of ailments. Further, 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 (Yangilar, 2013). Goat milk is recognized as 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 owing to higher content of medium chain fatty acids. Further, it is considered as a naturally homogenized milk, in which its fat globule size (0.73 to 8.58 µm) is smaller than cow milk (0.2 to 20 µm). Usually goat milk contains lower or traces of α_{s1} casein and higher β lactoglobulin content than cow milk, the difference being in the genetic polymorphism of proteins which makes it as an ideal choice for those suffering from cow milk allergy. β- Casein is the major component of the casein fraction in goat milk as compared to the cow milk. However, lower amounts of α_{s1} casein present in goat milk (5.6%) results in softer gel products, a higher water holding capacity and a lower viscosity. Moreover, the flavour of goat milk is more intense than cow milk, which can restrict the acceptance of its derivatives by consumers (Gomes *et al.,* 2013).

Goat milk and milk products are preferred increasingly for their health and nutritional benefits, including greater digestibility and lipid metabolism as compared to cow milk. It also possess health promoting compounds, such as bioactive peptides, conjugated linoleic acids and oligosaccharides. The lower heat stability, higher buffering capacity due to higher phosphate content and unique protein composition are also main attributes of goat milk. Goaty flavour due

to higher content of MCFA generate processing challenges for conversion of goat milk into variety of dairy products.

2.1.1 Goat milk proteins

Goat milk has six principle proteins: β-lactoglobulin, α-lactalbumin, k-casein, β-casein, α_{s1}casein, α_{s2}-casein. β- Casein is shown to be the major component of the casein fraction in goat milk, whereas α_{s1}-casein is the major casein protein in cow milk (Haenlein, 2004). Goat milk has markedly different levels of α_{s1} and α_{s2}-casein from those in cow milk (Kumar *et al.*, 2012).While casein are the major proteins in milk, whey or serum protein fractions consist mainly three components, namely bovine serum albumin, β-lactoglobulin, and α-lactalbumin. Non protein nitrogen (NPN) content in goat (0.4%) and human milk (0.1%) are much higher than in cow milk (0.2%). However, goat milk has comparable levels of lactoferrin (20-200 µg/mL), transferrin and prolactin to those of cow milk. Goat milk contain lyzozyme on an average 25 µg (Park *et al.,* 2007). Xanthine oxidase activity of goat milk is less than 10% of that cow milk. Xanthine oxidase has been implicated in the spontaneous development of undesirable oxidized flavours in market milk and other dairy products. It has also been observed that, peptides formed from goat milk casein by proteases tasted much less bitter than those from cow milk casein. Average amino acid composition of goat and cow milk shows higher levels of 6 of the 10 essential amino acids: threonine, isoleucine, lysine, cysteine, tyrosine, valine in goat milk (Haenlein, 2004).

Table 2.1 Average protein composition of goat milk, cow milk, and human milk

Components	Goat milk	Cow milk	Human milk
Protein (%)	3.4	3.2	1.2
Total casein (g/100mL)	2.11	2.70	0.4
α_{s1} (% of total casein)	5.6	38.0	-
α_{s2} (% of total casein)	19.2	12.0	-
β(% of total casein)	54.8	36.0	60-70
κ(% of total casein)	20.4	14.0	7.0
Immunoglobulins			
IgA (µg/mL)	30-80	140	1000
IgM (µg/mL)	10-40	50	100
IgG (µg/mL)	100-400	590	40

Minor proteins			
Lactoferrin (µg/mL)	20-200	100	2000
Lysozyme (µg/mL)	25	0.13	100-390

Jenness (1980); Renner (1980); Park and Haenlein (2004)

2.1.2 Goat milk lipids

Goat milk is naturally homogenised, with smaller fat globules than cow milk. Goat milk fat globules have an average diameter of 3.49 µm (Kumar *et al.*, 2012). While, fat globule size of cow milk is 4.55 µm. The free lipids and bound lipids content of goat milk total fat is 97-99 % and1-3 % respectively (Park *et al.,* 2007). Butyric acid (C4:0), caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), and linoleic acid (C18:2) are all higher in goat milk, but stearic acid (C18:0) and oleic acid (18:1) are lower (Haenlein, 2004). Goat milk have more short and medium chain fatty acids, representing 15- 18 % of short chain fatty acids as compared to 5 – 9 % of cow milk. These fatty acids which gives characteristic goaty odour to goat milk are responsible for the easy absorption and digestion of the milk fat, in which the enzyme lipase act more specifically on short chain fatty acids than long chain (jenness, 1980; Luke and Keith,1992; Park,1994). The free fatty acids in goat milk have another important difference in the amount of essential fatty acids (EFAs) and conjugated linoleic acids (CLAs). Goat milk have more essential fatty acids like arachidonic and linoleic acids and conjugated linoleic acids, that are positional and geometric isomer of linoleic acid (Luke and Keith, 1992;Sonu *et al.*, 2020). The susceptibility of goat milk to spontaneous lipolysis is higher than that of cow milk.

2.1.3 Goat milk Carbohydrates

The major carbohydrate present in goat milk is lactose. Goat milk contains average lactose content of 4.45%, which is about 0.2-0.5% less as compared to cow milk which is 4.66% (Park *et al.,* 2007).

2.1.4 Goat milk minerals

Goat milk contains higher minerals such as calcium, phosphorous, potassium, magnesium and chlorine and lower sodium and sulphur contents than cow and human milk (Yangilar, 2013). Fe content is lower in goat and cow milk, however higher iodine contents was found in goat and

cow milk than human milk, which would be important for human nutrition, since iodine and thyroid hormones are involved in the metabolic rate of physiological body functions (Kumar *et al.,* 2012). There is significant difference among the calcium contents of goat milk (134 mg/100 g Ca) and cow milk (119 mg/100 g Ca). Selenium is an essential trace mineral and is a component of glutathione peroxidase, which detoxifies the peroxidase. Selenium deficiency is correlated with liver necrosis, abnormal cardiac function, and even in cancer. Goat and human milk contain higher concentrations of selenium than cow milk (Park *et al.,* 2007).

Table 2.2 Minerals and vitamin content of goat milk

Minerals	Goat milk (mg/100g)	Vitamins	Goat milk (per 100g)
Calcium	134	**Vitamin A (IU)**	185
Phosphorous	121	**Vitamin D (IU)**	2.3
Magnesium	16	**Thiamine (mg)**	0.068
Potassium	181	**Riboflavin (mg)**	0.14
Sodium	41	**Niacin (mg)**	0.27
Chlorine	150	**Pantothenic acid (mg)**	0.31
Sulphur	28	**Vitamin B6 (mg)**	0.05
Iron	0.07	**Folic acid (µg)**	1.0
Copper	0.05	**Biotin (µg)**	1.5
Manganese	0.032	**Vitamin B12 (µg)**	0.065
Zinc	0.56	**Vitamin C (mg)**	1.29
Iodine	0.022		
Selenium	0.00133		

(Park *et al.,* 2007)

2.1.5 Goat milk vitamins

Vitamins are organic compounds contained in milk. There is major difference in vitamin A content of goat and cow milk. Goat milk supplies adequate amounts of vitamin A and niacin, and excess of thiamine, riboflavin and pantothenate for a human infants. Vitamin A deficiency is quite common among youngsters and is responsible for 1–2 million fatalities and half a million incidents of blindness each year, so the vitamin A concentration of goat and cow milks may be the most relevant difference among the other vitamins. Goats convert all β-carotene in

their milk to vitamin A, thus goat milk is whiter and contains more vitamin A than cow milk (Park *et al.*, 2007; Kalyankar *et al.,* 2016).

Given the lack of vitamin B_{12} and in particular, folic acid feeding newborns solely goat milk might result in "goat milk anaemia - megaloblastic anaemia," as folic acid is required for the production of haemoglobin (which causes anaemia when deficient) (Park *et al.,* 2009). Whereas goat and cow milk are deficient in pyridoxine (B_6), vitamin C and vitamin D.

2.1.6 Physico-chemical properties of goat milk

The specific gravity of cow milk and goat milk is almost similar and generally found in the range of 1.023 to 1.030. Titratable acidity (expressed as percentage of lactic acid) is also similar to that of cow milk and generally observed from 0.11 to 0.18. Viscosity at 27˚C is marginally lower than that of cow milk. The refractive index of goat milk is also almost close to cow milk. The electric conductivity of goat milk is found in the range of 0.0101 to 0.0188 ohm^1cm^{-1}, whereas pH value of goat milk found in the range of 6.5 to 6.9 as against 6.6 to 6.8 in case of cow milk (Kumar *et al.,* 2012).

Goat milk is white in colour compared with cow milk, which is yellowish because of the presence of carotene. Goat milk contains higher levels of capric, caproic, caprylic and lauric acids than cow milk, which is correlated to goaty flavour in goat milk. Goat milk is alkaline in nature, due to the higher protein content and a different arrangement of phosphates. Lack of the agglutinating protein that causes the clustering of fat globules and the rapid separation of cream (Jandal, 1996) are the reasons for natural homogenization of goat milk.

2.1.7 Health benefits of goat milk

Goat is one of the major contributor for the development of especially dairy products in developing countries. Goat milk has better alkalinity, digestibility and buffering capacity. It has certain therapeutic values in human nutrition and medicine (Coni *et al.,*1999). Due to the absence or lower amount of α_{S1} casein, goat milk is an alternative to people those who are sensitive to cow milk (Jandal, 1996). Consumption of the goat milk improves the intestinal absorption of copper, owing to higher content of cysteine in goat milk which is about 83 mg/ 100g as compared to 23 mg/100g of cow milk (Barrionuevo *et al*., 2002). Medium chain triglycerides, capric and caprylic acids are used for the medical treatments for various clinical disorders, because of their unique metabolic ability in providing direct energy not like as deposited in adipose tissues and also the action of lowering serum cholesterol level and inhibiting the deposition of cholesterol. Various disorders includes epilepsy, malabsorption syndromes, cystic fibrosis, gall stones, coronary by- pass, infant malnutrition, premature infant

feeding, hyperlipo- proteinemia etc. Presence of higher amount of polyunsaturated fatty acids, monounsaturated fatty acids and medium chain triglycerides provides beneficial effects to human health and also reduce the cardiovascular conditions (Alferez *et al*., 2001).

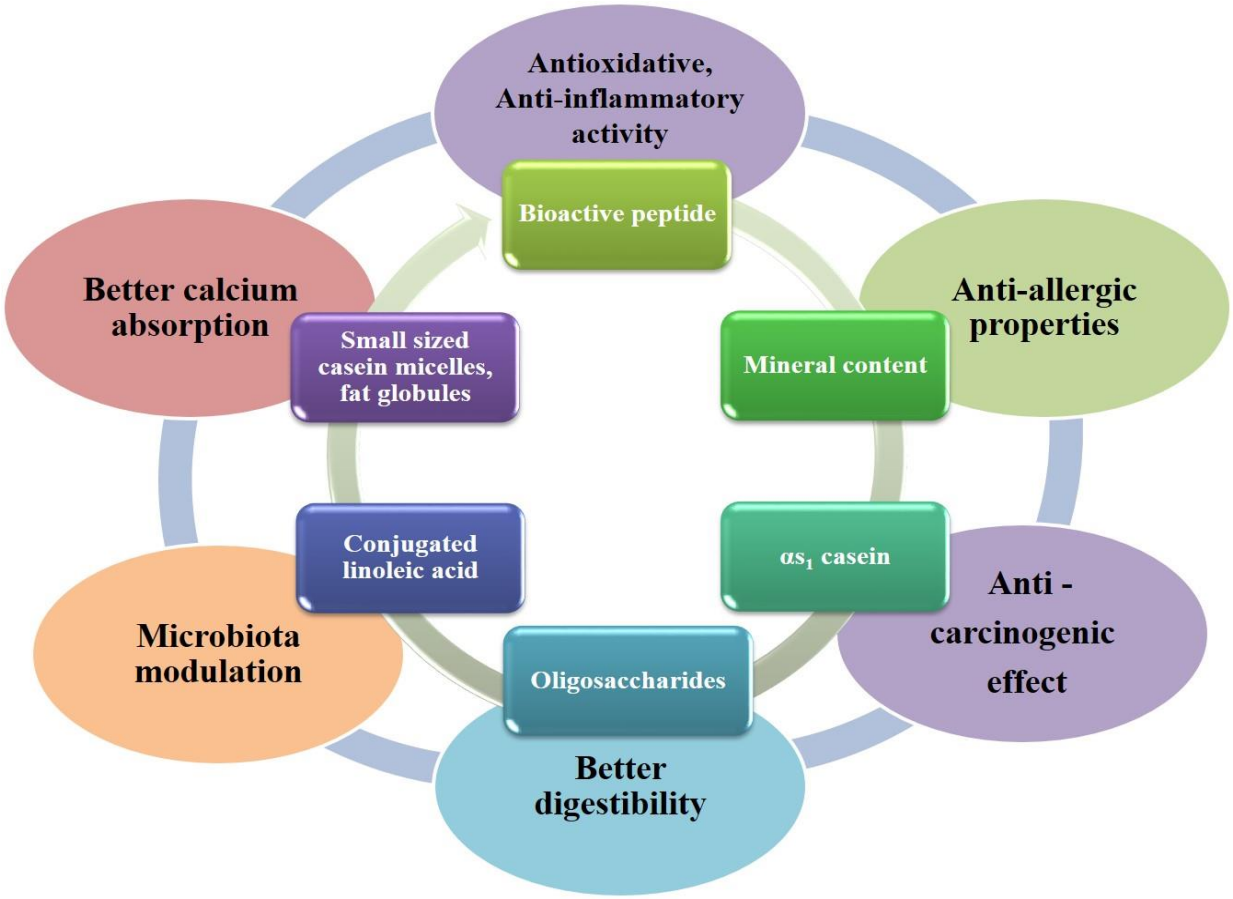


Figure 2.1 Health benefits of goat milk

However, better digestibility of goat milk is also attributed to lower curd tension than cow milk. As the fat globules size are smaller in goat milk, it makes a better dispersion and a greater surface area of the fat for better digestion by lipases (Jandal, 1996). Goat milk also possess health-promoting compounds, such as bioactive peptides, which provide several protective health benefits such as anti-diabetic, prevention of cancer, anti-inflammatory, antihypertensive, anti-atherogenic and antioxidant properties. Conjugated linoleic acids has significant role in stimulation of immune system (Park, 2010). Medium chain triglycerides are energy substrate which are readily available. Goat milk have higher amount of MCTs than cow milk so that it has major role in providing energy, as these are more rapidly metabolized to provide energy (Papamandjaris *et al*., 1998)

2.1.8 Fermented goat milk

Since ancient times, fermentation is known to be the best method for the preservation using growth of particular microorganisms in food. Because of certain minerals, such as calcium, zinc and magnesium, being a part of several enzyme complexes involved in lactose fermentation, they may impact and stimulate the formation of lactic acid bacteria in goat milk. Furthermore, microbes grow more quickly in the presence of higher quantities of specific amino acids, the higher whey protein content could be relevant in this regard. The physiological effects are frequently attributed to the products microflora. Due to its capacity to increase the shelf-life of milk, improve the texture and flavour of the resulting products, and enhance health benefits, fermentation is a commonly utilised food processing technique in dairy production (McKevith and Shortt, 2003). The metabolites involved in milk fermentation help to confer chemical, biochemical, and nutritional properties (Garcia *et al.,* 2014). Lactic acid bacteria utilise lactose in goat milk into lactic acid during the fermentation process, which is responsible for the acidity in goat milk yoghurt with pH of 4.6. When the acidity rises to a certain extent, the goat milk could be coagulated. Meanwhile, acidity is also an important factor affecting the shelf life of yogurt. For the evaluation of nutritional quality of dairy products, milk protein is an important component. (Li *et al.*, 2020).There is a reduction in pH and the development of three dimensional structure of yoghurt gel owing to bacterial action. Colloidal calcium phosphate is dissolved as the net negative charge decreases, which increases the aggregation and attraction of proteins. In the initial stage, casein micelles establish covalent links with denatured whey proteins, which, along with a drop in pH, results in the production of a chain of hydrophobic and electrostatic bonds, eventually forming the semi-solid structure of yoghurt. Syneresis is also recognized as important quality index of yoghurts, which is a major visible defect during the storage. Syneresis occurs when the gel structure weakens and the yoghurt's gel capacity to entrap water phase is compromised. As a result, there is an increased expulsion of whey, due to the reduction in net negative charge of the casein micelles. Lactic acid bacteria have a proteolytic system that includes extracellular cell wall-bound proteinases that start the breakdown of milk proteins into oligopeptides, peptide transporters that take the peptides into the cell and various intracellular peptidases that break down the peptides into shorter peptides and amino acids (Liu *et al.,* 2010). This can cause peptides with bioactive characteristics to be released from fermented dairy products (Maqueda *et al.,* 2012). Gobbetti *et al*. (2002) reported that the kind of dairy product, the method used and most importantly, the bacterial strain all influence proteolytic activity. In some circumstances, a mixture of chosen yeasts and lactic acid bacteria is employed to produce peptides with well-known health advantages Thus, lactic acid

bacteria screening led to the selection of a mixed starter including *Streptococcus thermophilus* and several *Lactobacillus* strains (*casei, helveticus, plantarum*) to produce goat milk with γ-aminobutyric acid (GABA) and ACE-inhibitory peptides (Minervini *et al*., 2009). ACE inhibitory peptides have been found in caprine milk fermentation products such as kefir (Quirós *et al*., 2005). Fermentation of ultrafiltered goat milk with lactic acid bacteria, including *Lactobacillus plantarum* C4, a strain with documented probiotic potential in terms of *in vitro* intestinal microbiota regulation, has recently been reported (Meca *et al*., 2015). Two types of fermentation in industrial scale are ethanol or lactic acid fermentation. Fermentation is known to enhance the benefits of dairy products, and fermented goat milk alternatives have been demonstrated to offer numerous health benefits, including antiallergenic, anticarcinogenic and probiotic qualities. It has also been demonstrated that the nutritional value of goat milk increases during fermentation and that it loses its distinctive goaty flavour which is unacceptable to many consumers (Slacanac *et al.,* 2004). Fermentation is also a vehicle for better bioavailability of micronutrients in milk. *in vitro* bioaccessibility of Ca, Mg, P and Zn from the fermented goat milk samples was determined by Bergilos- Meca *et al.* (2013) and it was found that bioavailability of Zn and Mg was not only higher than goat milk but also than fermented cow milk.

2.1.9 Fermented goat milk products

2.1.9.1 Yoghurt and fermented milk products

Yogurt is currently gaining popularity around the world as people are more aware of the health advantages of fermented dairy products. It is a fermented dairy product that can be made by employing certain mixture of starter culture using milk, cream or skim milk (Pal, 2014). Fermented goat milk products have been shown to be an excellent diet for persons who are allergic to cow milk. Yogurt made from goat milk is reported to be higher in fatty acids, protein and minerals (Costa *et al*., 2014). Folic acid is essentially non-existent in goat milk, which is one disadvantage. This difficulty could be remedied in a fermented product by adding folate producing bacteria during fermentation, Sanna *et al*. (2005) reported that fermented goat milk with a mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, resulted in a yoghurt with a considerable amount of folate and good sensory qualities. Damunupola *et al.* (2014) studied about the evaluation of quality characteristics of goat milk yogurt incorporated with beetroot juice, it was observed that the goaty flavour of the yoghurt was masked by the addition of beetroot juice, and it also increased the moisture content, while

lowered the total solids. Further, incorporation of 4% of beetroot juice was found to be acceptable by sensory evaluation.

Quality characteristics and antioxidant activities of goat milk yogurt with added jujube pulp studied by Feng *et al.* (2018). Jujube pulp, which is rich in polysaccharides and polyphenols have aromatic flavour, high antioxidant capacity, incorporated into goat milk yogurt showed that it conceals the goaty flavour. Physicochemical, microbiological, textural, sensory and antioxidant properties showed positive effect with the addition of jujube pulp during storage. Teshome *et al.* (2017) studied about the incorporation of mango and papaya juice for the development of fruit flavoured yoghurt. Results revealed that physicochemical, microbiological and sensory properties were significantly ($p< 0.05$) affected by the addition of fruit juices. Higher acidity and decreased fat, protein, total solids of fruit flavoured yoghurt were reported as compared to plain yoghurt. Furthermore, 15% mango flavoured yoghurt achieved highest score as compared to papaya and plain yoghurt.

Chakka is a concentrated product made from *dahi* after whey has been drained (fermented milk product). *Shrikhand*, a famous and traditional delicacy in Western India, is made by mixing *Chakka* with sugar and other seasonings (Prajapati and Nair, 2003). Chhana is a traditional Indian milk product that has been heated and acid coagulated (Park and Guo, 2006, Pandya and Ghodke, 2007). It is utilised as a basic material for the production of sweets, especially in Indian eastern states, however Chhana made from goat milk is slightly acidic. Kefir, another fermented milk product contains natural probiotics, and has lesser acidic flavour, a yeasty scent, and foams when stirred (Otles and Cagindi, 2003; Adriana and Socaciu, 2008). In kefir fermentation, replacing goat milk with black rice extract had no effect on the viability of the microbes but did lower the pH. By replacing goat milk with black rice extract and/or adding inulin, the antioxidant activity of kefir could be increased. Moreover, a dose of at least 2.0 mL of kefir made from goat milk and black rice extract had similar effects on diabetics as glibenclamide as an anti-diabetic medication (Pal *et al*., 2014).

2.1.9.2 Icecream and frozen desserts

Ice cream is a popular value-added dairy product created by freezing a pasteurised mixture and agitating it to incorporate air and achieve a consistent consistency (Pal, 2014). Silva *et al*. (2016) created goat milk ice cream with varying concentrations of carob powder. They discovered that goat milk ice cream with carob powder added at a rate of 12% was the most acceptable in terms of all sensory qualities. Ranadheera *et al*. (2013) developed a technology for producing acceptable quality chocolate flavoured probiotic ice cream from goat milk using

a probiotic bacterial culture that included *Lactobacillus acidophilus* LA-5, *Bifidobacterium animalis* subsp. *lactis* BB-12, and the novel probiotic *Propionibacterium jensenii* 702. The freezing technique used to make the ice cream resulted in decrease in viable cell counts; nevertheless, when held at -20 °C for 52 weeks, the viable numbers of all probiotics remained at 10^7 to 10^8 cfu g^{-1}.The chemical, physical, and organoleptic properties of ice cream made from cow, goat and sheep milk were evaluated for their suitability for ice cream production by (Konar and Akin, 1997; Pandya and Ghodke, 2007). The most popular ice cream was made using goat milk, followed by cow milk. According to Correia *et al.* (2008), goat milk ice cream has a softer texture and distinct melting properties. Cajá (*Spondiasmombim L.*), a distinctive fruit of the Brazilian Cerrado, was used to flavour goat milk frozen yoghurt, with varied amounts of cajá pulp (0 % control, 20 %, 30% and 40%). Sensory acceptance testing revealed that product with 20% and 30% cajá pulp were the mostly accepted (Oliveira *et al*., 2016).

2.1.9.3 Goat milk cheese

Mesopotamia was the birthplace of goat cheese. The milk was most likely turned into soft cheese, and eventually hard, ripened goat cheeses were formed in the Mediterranean basin (Park, 2001). Goat cheese can be made using both raw and pasteurised milk. The production of goat cheese from raw milk is illegal in several countries due to food safety concerns (e.g. brucellosis). The type of milk used has a big impact on the final product (Loewenstein *et al*., 1980). Goat cheese is made in many different varieties all around the world. Many different elements influence the maturation or ripening of goat milk and other species milk cheeses (Park, 1990). The majority of goat cheeses are created by delayed coagulation, leaving the curd with whey until dipped into moulds, and drying the cheeses before ripening. Proteolysis and lipolysis are two important biochemical processes involved in the multidimensional phenomena of cheese ageing, which involves a variety of chemical, physical, and microbiological changes in a controlled environment. Goat milk has shorter renneting time than cow milk and the weak consistency of gel is good for human digestion but reduces the yield of its cheese (Pacinovski *et al*., 2015). The short-chain fatty acids (C_6–C_{10}) give goat milk cheese its pungent flavour. Because, ewes milk has relatively low lipase activity, a similar flavour may develop in ewes milk cheese if moulds, etc., grow (as in Roquefort cheese) or if some goat milk is added. The short texture of goat milk cheese is due to the low level of α- $_{S1}$ casein in goat milk (ranging from 0 to 20% of total casein). Goat milk curd has a stronger syneresis than cow milk curd (Pal *et al*., 2017). Mehaia (2002) used ultrafiltration (UF) and traditional procedures to make fresh soft white cheese (Domiati-type) from goat milk. In comparison to cheeses prepared using the

standard procedure, ultrafiltered cheeses exhibited higher pH, moisture content and ash, but decreased protein and fat content.

2.1.9.4 Goat milk whey products

Goat milk whey has more α- lactalbumin (Pandya and Ghodke, 2007), however it is frequently wasted or given to animals as a nutritional supplement, and there is little information on it. Whey goat milk flavoured beverage, tablets (chewable), whey protein concentrate, and athletic supplements are among the various items derived from whey goat milk.

2.2 Ultrafiltration in goat milk

Ultrafiltration (UF) is a pressure driven process using a semi permeable membrane to separate macromolecules or colloids from liquids. It is a separation technique for the concentration and separation of substance that contains molecules from 500 to 300000 Da. By ultrafiltration, the macromolecule concentration in the retentate increases such as casein, whey protein etc. as their size is larger than the membrane pores while, the lactose and minerals, the small molecules in milk in soluble phase, are removed by membrane process into the permeate solution. UF of milk can change the chemical and physicochemical properties of milk and in turn can affect the properties of milk products. There has been increasing popularity of UF as fractionation technology owing to its utilization in the production of highly value-added dairy ingredients. UF has also been successfully utilized for the quality improvement of goat milk based fermented milks and other dairy products.

Milk UF is a good concentration approach that does not impair the nutritional value of the milk, and the resulting fermented milks were reported to have better textural and sensorial characteristics and improved nutritional values (e.g., proteins, Ca and Fe) (Rinaldoni *et al*., 2009; Domagała, 2012). Comparing with raw milk and skim milk, ultrafiltered concentrated skim milk (UFM), shows high fat and dry extract values. Ultrafiltration is a process that modifies the mineral distribution, increases the Ca and Mg solubility. Increased casein concentration of 85%, ash, density, acidity, whey proteins and the low fat content make the milk nutritionally superior (Montoro *et al*., 2015).

Figure 2.2 Ultrafiltration membrane Unit

Mehaia and El- Khadragy (1998) observed that there is decreased rennet coagulation time of goat milk with an increase in volume concentration ratio during ultrafiltration. The role of ultrafiltration in increasing the quality of bovine milk yoghurt has been reported by a number of researchers. In terms of sensory scores, textural features, and tendency of whey separation, good quality *dahi* can be produced by ultrafiltration (Meena *et al*., 2015). Earlier, Ultrafiltration was used to increase the quality of cow milk plain yoghurt (Narayana and Gupta, 2013). The authors determined that there were no significant variations in flavour, appearance, or textural qualities between yoghurt manufactured from straight ultrafiltered (UF) retentate and milk standardised with UF retentate. As a result, using either UF retentate or UF retentate supplemented milk to improve yoghurt quality may be recommended. Ultrafiltration has been used in a variety of applications recommended for improving the quality of yoghurt. Biliaderis *et al*. (1992) showed the rheological, sensory and chemical properties of skim milk and ultrafiltered skim milk retentate yoghurts. Milk was concentrated to 12%, 14%, and 16% total solids, respectively. The onset gelation time reduced as the solids concentration increased, and UF samples had lower onset times. In addition, using the UF process to concentrate skim milk

to 12 percent solids resulted in yoghurt with ideal taste ingredient profiles, texture and other sensory qualities.

Various processing techniques that may improve the qualitative features of goat milk yoghurt were reviewed by Nahar *et al.* (2007) and Domagala (2008, 2009). Vacuum concentration, addition of milk powder to increase total solids content, ultrafiltration, addition of cross-linking enzymes were some of the methods used. Another methods include adding folate-producing bacteria to the starter culture (Pal *et al*., 2005).

Table 2.3 Research studies on goat milk interventions

Methods	**Research studies**
Milk solids enrichment	When it comes to yoghurt manufacture, goat milk yoghurt has a lower coagulum than cow milk yoghurt. Additionally, goat milk products are less appealing to consumers. As a result, adding bovine skim milk powder to goat milk is a popular method to boost overall solids and consumer acceptance. Bruzantin *et al*. (2016) evaluated the effect of adding bovine skim milk powder to goat milk, and the results showed that goat milk yoghurt containing added bovine SMP received outstanding sensory scores and the lowest score for bitter flavour. In comparison to the control, textural characteristics and overall acceptance were much greater.
Ultrafiltration	The manufacturing of yoghurt and yoghurt beverages has long relied on membrane processing. Separation and fractionation of proteins, fat and colloidal salts from lactose, soluble minerals, non-protein nitrogen (NPN), water-soluble vitamins, and water are the main principles of ultrafiltration in milk (Hinrichs, 2001; Fox *et al*., 2004). Because vitamin B12 and folic acid are protein bound, they are retained during UF of milk (Ford *et al*., 1969).

	The original taste of food products and the nutritional value of heat-sensitive components are preserved when concentrated using membrane filtration rather than thermal evaporation. Ultrafiltration was used to concentrate goat skim milk to volume concentration ratios (VCR) of 2, 3, 4 and 5. All fat, casein, whey protein nitrogen, 19 % non-protein nitrogen, 78.1% TS, 78.6% ash and 3.5 % lactose are retained after ultrafiltration of goat skim milk (Mehaia and El- Khadragy, 1998).

2.3 Blueberry

Blueberries are perennial flowering plants and are the excellent source of health restoring phytochemicals belongs to the section *cyanacoccus* and *vaccinium* species native to North America and other European countries where it is more cultivated and commercialized. Popular berries are strawberries, blueberries, blackberries, raspberries, cranberries, red currants, black currants, chokeberries, wolfberries, huckleberries and lingonberries etc. Among these, blueberry possess highest antioxidants and is regarded as a quintessential functional food ingredient. Blueberry (*Vaccinium sp.*) belongs to the family *Ericaceae*. This plant is native to the USA and Southern Canada, growing wild in hilly and woodland regions. Several types of blueberries are commonly available, depending on the growing season and harvesting time. The three prominent varieties grown are highbush (*V. corymbosum*, *V. ashei*), lowbush (*V. angustifolium*) and evergreen (*V. darrowii*). The berries are known to be nutrient storehouse with plentiful fibres, tannins, anthocyanins, proanthocyanidins, vitamin C, ellagic acid, omega3 fatty acids, carotenoids, minerals etc. (Patel, 2014). A variety of anthocyanins occur in blueberry, the chief types being monoarabinosides, monoglucosides and monogalactosides of cyanidin, petunidin, peonidin, delphinidin and malvidin. Although many blueberry species are native to North America, they are now grown in practically every country, including Australia, New Zealand and Europe.

Blueberries are a healthy fruit since they are high in carbohydrates, vitamins, and minerals (Liu *et al*., 2015). Blueberries are also high in dietary fibre, which accounts for 3–3.5% of the fruit's weight (Michalska and Lysiak, 2015). United States Department of Agriculture (USDA) human nutrition centre recommends its inclusion in diet (U.S. Department of Agriculture, Agricultural Research Service, 2013). As they are rich in bioactive compounds, blueberries have several

beneficial health and therapeutic properties. Blueberry is known as 'Longevity fruit'because of its high antioxidant activity against reactive species and the free radicles. Among the fruits blueberries have highest antioxidant property and have the capacity to cure several chronic and non- communicable diseases.

Patel (2014) studied about the blueberry as functional food and dietary supplement and considered it to be the natural way to ensure holistic health. It was suggested that the bioactive components of blueberry and precise mechanisms mediate the disease remediation. Berries, especially blueberry, are touted as rich sources of anthocyanins, which are antioxidants reducing risk of heart disease and cancer. Studies have found that blueberries may help diminish body fat, which is considered to be beneficial since having too much body fat has been linked to conditions like heart disease and diabetes.

Table 2.4 Phytochemical profile of blueberry

Nutrients	Per 100 g
Energy	57 Kcal
Protein	0.74 g
Fat	0.33 g
Carbohydrate	14.49 g
Dietary fibre	2.40 g
Ash	0.24 g

Patel (2014)

Table 2.5 Minerals content of blueberry

Nutrients	mg Per 100 g
Calcium	6.00
Copper	0.28
Iron	0.06
Magnesium	6.00
Manganese	0.34
Phosphorous	12.00
Potassium	77.00
Selenium	0.10
Sodium	1.00
Zinc	0.16

Patel (2014)

Table 2.6 Vitamin content of blueberry

Nutrients	mg Per 100 g
Ascorbic acid	9.70
Thiamine	0.04

Riboflavin	0.04
Niacin	0.42
Pantothenic acid	0.12
Pyridoxine	0.05
Folic acid	6.00 µg
Retinol	54.00 IU
Tocopherol	0.57 mg ATE

Patel (2014)

2.3.1 Major bioactive components in blueberry

Blueberries are rich source of bioactive components and major bioactives includes anthocyanin, ellagic acid and proanthocyanidin. Furthermore, blueberries are high in numerous phytochemicals such as ascorbic acid and phenolics. The bioactive qualities of the phytochemical elements are linked to many of the reported favourable health effects associated with blueberry eating. Ascorbic acid, flavonols (including kaempferol, quercetin and myricetin), hydroxycinnamic acids (including caffeic acids, ferulic acids and coumaric acids), hydroxybenzoic acids (including gallic acids and procatchuic acids), pterostilbene, resveratrol, and ACNs are the most common bioactive components found in blueberries. The possible health advantages of blueberries have gotten a lot of attention in recent years because of bioactive components (Chen *et al*., 2010). Blueberry anthocyanins have been shown in numerous studies to improve human health and reduce risk factors for cardiovascular disease. Blueberry anthocyanins are powerful antioxidants that also reduce inflammation, especially in the stomach and protect against cardiovascular disease by lowering systemic micro inflammation (Cassidy *et al*., 2018; Grosso *et al*., 2017; Tran and Tran, 2021). Furthermore, these substances lower the chance of developing diabetes and obesity. And also, these compounds provide indirect neuroprotection and may be useful adjuvants in the treatment of cancer (Proestos, 2018). Blueberries are high in ascorbic acid, a water-soluble molecule that has a variety of roles in living systems, including improving immunity and lowering inflammation (Liu *et al*., 2010). Vitamin C (ascorbic acid) is an antioxidant. It can be found in a variety of blueberry species and cultivars. 100 g of blueberries give 10 mg of ascorbic acid on average. This is one-third of the daily dietary recommendations intake (Capra, 2006; Prior *et al*., 1998). Different species have been shown to have high levels of ascorbic acid. Phenolic compounds are a diverse collection of chemical compounds that contain one or more aromatic rings with a conjugated aromatic system as well as one or more hydroxyl groups. Water-soluble (phenolic acids, flavonoids and quinones) and water-insoluble compounds (Condensed tannins). Phenolic compounds can be found in free or conjugated forms with sugars, acids and

other biomolecules (Skrovankova *et al*., 2015). Phenolic compounds in blueberries consists of Stilbenoids, tannins, hydrolyzable tannins (gallotannins and ellagitannins) and condensed tannins (proanthocyanidins) and flavonoids, including flavan-3-ols, ACNs and their polymeric condensation products. Tannins have been proposed to have therapeutic potential in the treatment of diabetes in two ways: i) they may lower glucose levels by delaying intestinal glucose absorption and exerting an insulin-like effect on insulin-sensitive tissues and (ii) they may delay the onset of insulin-dependent T2D by regulating the antioxidant environment of pancreatic b-cells (Serrano *et al*., 2009). Proanthocyanidins, also known as condensed tannins, are the most widely distributed products of plant secondary metabolism in nature (Gu *et al*., 2003). Other berries, such as blackberries, black raspberries, red raspberries, and strawberries, contain mostly ellagitannins, whereas blueberries contain predominantly proanthocyanidins (Seeram, 2008). As a result, the specific chemical structures of tannins may be linked to the unique biological features of blueberries. Individual classes of tannins may be responsible for the various biological effects of blueberries on neuronal function in different parts of the brain and behaviour in ageing animals. Flavonoids are a heterogeneous collection of benzo-c-pyron derivatives found in abundance in fruits and vegetables, as well as in food and beverages (Shi *et al*., 2017). Many of flavonoids physiological benefits have been attributed to their antioxidant and free radical scavenging properties, which have been shown to have beneficial health effects on chronic diseases such as cancer and neurodegenerative disorders (Lau *et al.*, 2007; Neto, 2007; Nile and Park, 2014). Blueberries have also been shown to have high levels of flavonoid molecules, making them one of the foods with the most antioxidant activity (Barberis *et al*., 2015; Borges *et al*., 2010; Moyer *et al*., 2002). One of the best studied flavonoids, quercetin, possesses antioxidative and anti-carcinogenic properties that protect against oxidative stress (Heo and Lee, 2004). Faria *et al*. (2010) have shown that anthocyanins (ACNs), pigments that contribute to the intense colours in blueberries, have a variety of bioactive properties, including anti-inflammatory, antioxidant and anticancer properties. Peonidins, pelargonidins, malvidins, delphinidins, cyanidins and petunidins are the most frequent anthocyanidin aglycones (Li *et al.*, 2011).

2.3.2 Health benefits of blueberry

Various health benefits include antioxidant, anti-inflammation, neuro-protection, antimetastatic, cardio-protective, antimicrobial, renoprotective, opthalmoprotective, anti-diabetic, hepato-protective, gastro-protective, anti-osteoporotic, anti-aging have been reported. It was reported that blueberries owing to their high content of ellagic acid, foil endogenous oxidative DNA damage leading to diminished cancer risks (Aiyer *et al.*, 2008). It was reported

that a 3% blueberry diet fed for 8 weeks was capable of protecting the kidneys from oxidative damage and reduced markers of renal oxidative stress were observed (Shaughnessy *et al.*, 2009).

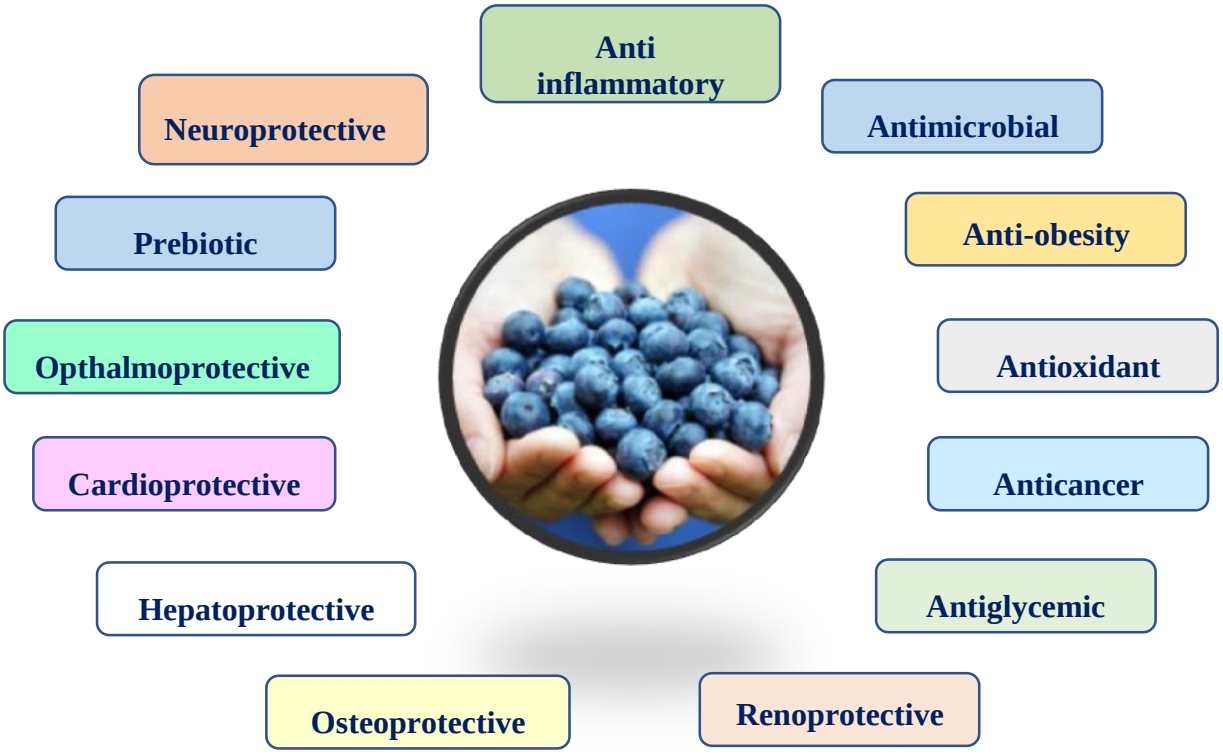
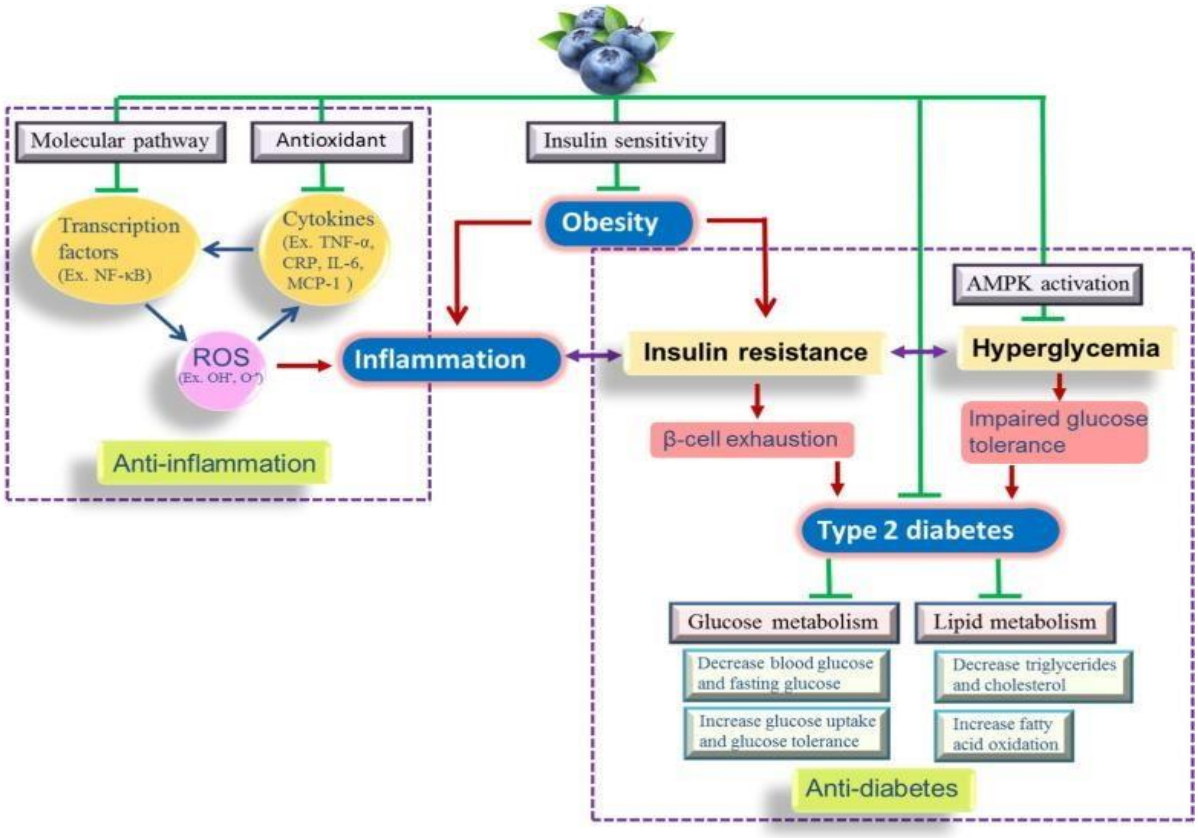


Figure 2.3 Health benefits of blueberry

Blueberry is having high antioxidant activity due to the presence of proanthocyanidin and anthocyanidin, which are responsible for enhancing the redox status of the body (Huang *et al.*, 2012).

Anthocyanins found in blueberries are powerful antioxidants that can efficiently combat reactive oxygen species in the body. Both in vitro and in vivo research has been done on the ability. In vitro research revealed that extracts from the fruit of several blueberry cultivars have varying antioxidant activity. Polaris anthocyanin extracts exhibited the highest antioxidant capacity of the ten blueberry types evaluated. Polaris dramatically increased the cellular activity of superoxide dismutase (SOD), catalase (CAT) and decreased malondialdehyde (MDA) in HepG2 cells in an *in vitro* research (Li *et al.*, 2018).



Source : (Shi *et al.*, 2017)

Figure 2.4 Effect of blueberry on obesity and related comorbidities

2.3.3 Blueberry added yoghurt

Boycheva *et al.* (2011) studied about the quality characteristics of yoghurt from goat milk supplemented with fruit juice (Aronia and blueberry juice). Dynamics of acidification, number of lactic acid bacteria, fatty acid compositions were investigated. They found that milk coagulated at lower acidity and in shorter time with higher lactic acid bacterial counts in yoghurt. There was an increased content of unsaturated fatty acids and monounsaturated fatty acids and also, yoghurt incorporated with blueberry juice showed least syneresis. Dimitrellou *et al.* (2020) studied quality characteristics of yoghurt added with grapes and berries and analyzed the physicochemical parameters including pH, acidity, reducing sugar, color, syneresis, total phenol count and antioxidant activity. Result showed that with increased antioxidant and phenolic content, juices have potential to be used in yoghurt production.

Table 2.7 Studies on blueberry added yoghurt

Title	Key findings	Reference
Effect of the Addition of Blueberries on Selected Physicochemical and Sensory Properties of Yoghurts	The physicochemical and sensory features of blueberries and sugar added to yoghurts were studied on the 1st, 10th and 20th days after being stored in the refrigerator. pH, total solids, protein, fat, ash, viscosity, syneresis, Hunter's *L**, *a** and *b** values, flavour, body, texture, appearance and colour were all measured in the samples. The physicochemical and sensory qualities of yoghurts were significantly affected by blueberry and sugar mass fractions, as well as storage time. When blueberries were incorporated into yoghurt, the syneresis increased while the pH, fat, protein, ash, viscosity and whiteness decreased. Only the flavour of yoghurts was improved by adding sugar in terms of flavour, body, texture, colour and appearance, panellists preferred samples with 25% blueberries and 4% sugar.	Cinbas and Yazici (2008)
Color stability of fruit yoghurt during storage	The stability of anthocyanins in yoghurts containing strawberry, sour cherry and blueberry fruit preparations was tested over an 8-week period in a refrigerated environment. The rate of anthocyanin degradation and colour changes were examined between stirred yoghurts and fruit-on-the-bottom yoghurts (fruit preparation was on the bottom of the package). The concentration of anthocyanin in fruit yoghurts reduced significantly after storage, especially in the first two weeks. There were differences in the rate of colour degradation between yoghurts made from various fruit species. The pigments in stirred yoghurt with strawberry, sour cherry and blueberry preparations had half-lives of 5.5, 6.7 and 19.0 weeks, respectively. During the preservation of blueberry yoghurt for several days, there was a considerable change in the pigment profile was observed.	Scibisz *et al.* (2019)

2.4. Inulin

Inulin is a polysaccharide which consists of fructose molecules in linear chain with glucose moiety at the terminal end. It is a group of naturally occurring polysaccharides produced by many types of plants. It belongs to a class of dietary fibres known as fructans. It is FDA approved as a dietary fibre. The other names are alatin, helenin and meniantin (Tiwari *et al.,* 2015). The unique aspect of the structure of inulin is its β-(2→1) bonds. These linkages prevent inulin from being digested like a typical carbohydrate and are responsible for its reduced caloric value and dietary fibre effects (Niness, 1999). Karimi *et al*. (2015) define inulin and FOS as functional dietary ingredients since they alter physiological and biochemical processes, resulting in improved health and a lower risk of various diseases. Inulin is employed in the development of innovative foods for technological reasons in addition to its nutritional benefits. It's used to replace fat or sugar, as a low-calorie bulking agent and as a texturizer (Tungland and Meyer, 2002). Its capacity to bind water molecules and form a particle gel network has been attributed to its fat mimic capabilities (Franck, 2002). Staffolo *et al*. (2004) observed no variations in viscosity or acceptability when yoghurt fortified with 1.3 percent inulin was compared to a control yoghurt. The addition of inulin to reduced fat ice cream resulted in an increase in viscosity values (Jonsson *et al.,* 2004), a decrease in freezing point (SchallerPovolny and Smith, 2001), and an improvement in sensory properties (Jonsson *et al.,* 2004). ElNagar *et al*. (2002) discovered that the sensory texture profiles of low fat yoghurt -ice cream with inulin were identical to the high fat reference sample but not the low fat control sample. Inulin and oligofructose have been thus, used in many countries to replace fat or sugar and reduce the calories of foods such as ice cream, dairy products, confections and baked goods.

2.4.1. Inulin as fat replacer in dairy desserts

Inulin has wide applications in various types of foods because of their large number of health promoting functions. Dairy products represent one of the most highly studied food matrices for supplementation of inulin. Incorporation of inulin in a variety of foods specially dairy products is mostly due to two reasons. One reason can be attributed towards the various physiological functions which confer to the consumer (*i.e.,* dietary fibre, prebiotic etc.). Other reason is the different technological properties of inulin and its functionality in the food matrix (*i.e.,* mimic texture modifier etc.). Staffolo *et al.* (2003) studied influence of dietary fibre addition on sensory and rheological properties of yoghurt. Dietary fibres like Apple fibre, wheat, bamboo or inulin is added. Rheological characterization done by dynamic, shear, compression-

extrusion assays. Results showed that, with storage at 4ºC for 21 days there is no syneresis with dietary fibre addition. The water activity, pH and color parameters were stable with storage time. Physicochemical and sensory analysis of inulin enriched desserts were done by Tomas *et al.* (2009) and they found that the addition of different types of inulin like short chain, native chain and long chain in whole and skim milk, skim milk long chain inulin shows similar characteristics and flow behaviour as that of whole milk without inulin. Skim milk showed same creaminess, consistency intensity. This inulin addition has effect on pseudoplasticity and consistency. Addition of inulin in shrikhand upto 3 % reduces the titratable acidity, pH, moisture, fat, protein and increases the viscosity, fibre, ash, carbohydrate and total solid content. Microbiological parameters like total plate count and yeast and mould count increased. Shrikhand scored high score for flavour, body, texture, colour, sweetness and overall acceptability with 3% inulin addition.

CHAPTER-3

Materials and Methods

MATERIALS AND METHODS

A detailed technical program was devised indicating the plan of experiments. This chapter deals with the details of raw materials, experimental details, methodologies adopted to determine the physico-chemical, functional, organoleptic, rheological, microbiological analysis of products. Details of statistical procedures adopted during the study, is also discussed under appropriate sub-headings.

3.1 MATERIALS

3.1.1 Raw material (Goat milk)

The goat milk was procured from Cattle yard of National Dairy Research Institute, Karnal. The goat milk was warmed to 40 - 45°C for the separation of cream for ultrafiltration. Cream separation of fresh goat milk was done by using cream separator (Kamdhenu Engineering Pvt. Ltd., Ambala, India). Frozen blueberries were procured from Anusaya fresh India Pvt.Ltd., Navi Mumbai.

3.1.2 Yoghurt culture

Yoghurt culture NCDC- 263, used for the fermentation of goat milk was procured from the National Collection of Dairy Cultures (NCDC) of the institute. The ampoule of the culture was cleaned thoroughly by wiping with cotton soaked in absolute alcohol and the tip was broken by tapping. Sterile skim milk (0.4mL) was added to the ampoule using a sterile pipette. The suspension was transferred to another sterilized test tube containing 10 ml of sterilized skim milk. The contents were mixed with constant shaking followed by incubation of these test tubes at 37°C for 12-14 h. After completion of the incubation period, test tubes containing culture were stored under refrigeration till further use.

For mother culture preparation, skim milk was sterilized in an autoclave at 15 psi for 15 min. Ten milliliter of sterilized skim milk poured in, thoroughly cleaned and sterilized glass tubes. Glass tubes (20 mL volume) were inoculated with seed culture and incubated at 37°C in the incubator. For bulk culture preparation, 100 mL of skim milk in conical flask was autoclaved at 15 psi for 15 min, cooled to room temperature and inoculated with the help of 10 mL graduated sterilized pipette. Flask was incubated at 42°C for further use. Culture was added at the rate of 2% for preparing yoghurt samples.

3.1.3 Packaging Materials

Sterile containers of polypropylene material of 30 mL was procured from Himedia Laboratories Ltd. and 250 mL was procured from local market.

3.1.4 Media for Microbiological analysis

The microbiological media *i.e.,* Plate Count Agar (PCA), Violet Red Bile Agar (VRBA) and Potato Dextrose Agar (PDA), Lactobacillus Agar (MRS) and M17 agar for different microbiological analysis were procured from Himedia Laboratories Ltd., Mumbai.

3.2 EQUIPMENTS

3.2.1 Ultrafiltration module

A lab scale ultrafiltration unit (Merck Millipore Life Sciences Pvt. Ltd. California, USA) fitted with plate and frame module having polyethersulfone membrane of molecular weight cut- off 10 kDa and surface area of 0.1 m^2 was used for ultrafiltration of goat skim milk. Transmembrane pressure was kept at 1.0 Kg/cm^2 during ultrafiltration. After the operation, the plant was rinsed with hot water (40°C) for 10 minutes, cleaned with 0.1 N NaOH solution (45°C) for 20 minutes and then final rinsing was performed by using hot water for 10 minutes. Cleaning with water was continued till it regained its original flux. The membrane cassette was taken out of the plant and stored in 0.1 N NaOH solution under refrigeration conditions.

Figure 3.1 Equipment of Ultrafiltration Unit

3.2.2 High shear Disperser

A T18 basic ultra-turrax unit (Ika-Werke, Staufen, Germany) was used to treat the samples at high shear. The shear rate of 6000 rpm for 10 minutes was used.

3.2.3 Rheometer

Rheometer (Model- MCR 52, Anton Paar, Germany) was used to measure the rheological properties of samples. The samples were evaluated for the amplitude sweep, Flow curve and frequency sweep test. Data was analysed using the supplier's RHEOPLUS/32 Service V3.61 software.

3.2.4 Colorimeter

Color Flex (Hunter Associates Laboratory, Inc., Reston, VA, USA) colorimeter equipped with dual beam xenon flash lamp and Universal software was used for measurement of color of the product in terms of the *L**, *a** and *b** color coordinates.

3.2.5 pH meter

The pH of the samples was measured using pH meter (Model: Cyberscan pH2100, EUTECH Instruments, Thermo Fisher Scientific, Mumbai, India). Prior to the test, the pH meter was calibrated using the standard buffers of pH 4.0 and 10.00 and standardized using pH buffer of 7.0 at 20 °C.

3.2.6 Refrigerated centrifuge

Refrigerated Centrifuge (Model No. 2-16PK, Sigma Laborzentrifugen GmbH, Germany) with rotor number 12071 (suitable for 250 mL centrifuge tube; rpm: 8000, max.) was used for the centrifugation of sample solutions at prescribed temperatures and speed of rotation during the separation of whey and protein curd mass.

3.2.7 Muffle furnace

Muffle furnace (Modern Industrial Corporation, Bombay, India) was used for dry digestion.

3.2.8 Double distillation unit

Double distillation unit was used for double distilled water (Bhanu Scientific Instruments, Mumbai, India).

3.3 ANALYTICAL METHODS

All the chemicals used in the present study were of analytical grade (AR) and procured from reputed suppliers. The reagents required for analysis were freshly prepared from chemicals by adopting standard procedures/protocols. The procedures were standardized and reagents were stored under desired conditions wherever required.

3.3.1 Physical/ Physico-chemical analysis

3.3.1.1 pH and Acidity

The pH of samples was determined with the help of pH meter ((PHAN LABINDIA Model, Labtek Engg. Pvt. Ltd. India) fitted with a combination electrode. About 50 mL of sample was taken for determining pH. The electrode assembly was calibrated with suitable standard buffer of pH 4.0, 7.0 and 9.2 respectively.

Titratable acidity of samples was measured as % lactic acid by standard method described in IS:1479 (part I)- 1960. Five gram dessert sample was taken in a beaker and distilled water was added to make the volume upto 50 mL. The samples were mixed uniformly by shaking for 5 minutes. Ten milliliter of aliquot sample was taken in 100 ml beaker and titrated against 0.1 N NaOH till faint pink color is developed, which should persist for at least 30 seconds. Results were expressed as % lactic acid.

$$\text{Titratable acidity (\% lactic acid)} = \frac{\text{Titre value} \times N_{NaOH} \times \text{Volume made up} \times 90 \times 100}{\text{Volume taken for estimation} \times \text{wt of sample} \times 1000}$$

3.3.1.2 Color measurement

Color values (*L**, *a**, *b**) of samples were measured using Hunter Lab Colorimeter (MiniScan XE Plus, Hunter Associates Laboratory, Reston, Virginia, USA). Before the test, the instrument was calibrated with standard black and white tiles as specified by the manufacturer. The *L** value represents the lightness of the product from 100 for perfect white to 0 for black. Redness/greenness and yellowness/ blueness are the chromaticity dimensions (a^* and *b**), respectively.

Where *L**, *a** and *b** are measured values and L-99.24. a- 1.6, b-1.35 are the color values of the standard white tile.

3.3.1.3 Total solids

Total solids content of samples were measured by standard method described in IS: SP part XI (1981) with some modifications**.**

Procedure:

Clean and dry empty shallow flat-bottom dish of aluminum, nickel, stainless steel, porcelain or silica was weighed accurately. Five grams of prepared sample was weighed in the dish. 1-2 drops of phenolphthalein solution was added to the sample in the dish and neutralized with sodium hydroxide (0.1N) solution to a faint pink color. The volume of 0.1N sodium hydroxide required to neutralize the sample was noted. The dish was placed on a boiling waterbath till the water was removed from the sample. The under-surface of the dish was wiped and placed in the oven maintained at 100±2°C for 3 hours. Dish was removed from the oven and placed in a desiccator for cooling. It was heated and re-weighed at hourly intervals till successive weighing does not vary by more than 0.5 mg. Half of the weight of 0.1 N sodium hydroxide used for neutralization was deducted from the residue after drying. Total solids contents of samples were calculated as follows:

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

$$\text{Total solids} = \frac{100 \times (W2 - a)}{W1}$$

Where,

N = Normality of NaOH

T.V. = Titre value

W2 = Weight of residue left after drying (g)

W1 = Weight of the sample taken (g)

a = Half the volume of 0.1 N NaOH used for titration

3.3.1.4 Protein Estimation

Crude protein content in the sample was determined by macro Kjeldahl method (AOAC, 2000). Two grams of sample was weighed and transferred to Kjeldahl digestion tube.It was followed by addition of digestion mixture (15g of K_2SO_4 and 1mL of 5% solution of $CuSO_4$) and 25 mL of concentrated H_2SO_4. The contents in the tube were digested to a transparent clear fluid by heating in digestion chamber.

The digested content was transferred to a 500 mL distillation flask and diluted with 300 mL of distilled water. The contents were distilled with 75mL of 50% sodium hydroxide and the liberated ammonia was collected in 50 mL saturated boric acid containing 2-3 drops mixed indicator (one part of 0.2% alcoholic methyl red and five parts of 0.2% alcoholic bromocresol green solution). Approximately 150 mL of the distillate was collected in a 250 mL conical flask.

The contents of the flask were titrated against 0.1N HCl. Blank was determined using distilled water in place of sample. The total nitrogen and percent protein were calculated as follows:

$$\% \text{ Nitrogen} = \frac{14.007 \times (V - B) \times N \times 100}{1000 \times W}$$

Where,

V = amount of HCl required for sample (mL)

B = amount of HCl required for blank (mL)

N = Normality of HCl used

W = weight of sample (g)

% Total Protein = % Total nitrogen x conversion factor*

*conversion factor = 6.38

3.3.1.5 Fat

The fat content of samples was determined by Rose-Gottlieb method described in IS:1479 (Part II) – 1961.

Procedure:

About ten grams of the prepared sample was weighed accurately into the Mojonnier fat extraction tube. 1 mL of concentrated ammonia solution (Sp.gr. -0.88) was added in the tube

and mixed well in the lower bulb. After mixing, 10 mL of alcohol (95% v/v) was added in the tube and mixed by allowing the liquid to flow back and forth between the two bulbs. 25 mL of Diethyl ether (Sp.gr. – 0.720) was added; tube was tightly closed with the stopper and shaken vigorously for one minute. This step was repeated using 25 mL of petroleum ether (boiling point: 40-60°C) in place of diethyl ether. The tube was allowed to stand on the flat bottom of the lower bulb until the ethereal layer was clear and completely separated from the aqueous layer, usually for not less than 30 min, or centrifuged until clear. The supernatant was carefully decanted as much as possible into a suitable flask by gradually bringing the cylindrical bulb of the tube into a horizontal position.

The extraction and decantation operation was repeated twice by using 15 mL each of diethyl ether and petroleum ether. The solvents were carefully distilled from the flask and the residual fat was dried in an oven maintained at 100±1°C for one hour and cooled to room temperature in a desiccator. It was repeatedly dried, cooled and weighed until successive weighing do not show a difference of more than 1 mg. The fat content of sample was calculated as follows:

$$Fat\ \% = \frac{W1 - W}{W2}\ X\ 100$$

Where,

W = weight of empty conical flask

W_1 = weight of sample with conical flask after drying (g)

W_2 = weight of sample taken for the test

3.3.1.6 Total ash

The ash content of product was determined by gravimetric method described in IS:1479 (Part II) – 1961.

Procedure:

Two grams of sample was weighed and transferred to pre-weighed silica crucible. The weighed sample was charred till the smoke ceased from it. The crucible was then transferred to muffle furnace maintained at 550±5ºC and incinerated until light grey ash was obtained. The crucible were then cooled in desiccator and weighed.

$$\text{Ash \%} = \frac{\text{Weight of crucible with sample after ashing - Weight of empty crucible}}{\text{Weight of sample}} \text{ X } 100$$

3.3.1.7 Lactose

The lactose content of optimized product was determined by Lane-Eynon method as described in IS: SP: 18, Part XI (1981). As per the method, a well mixed sample 10 mL was accurately taken in a 100 ml volumetric flask followed by the addition of 30- 40 ml warm water at 40-45°C. The contents were mixed thoroughly and 1.5 ml of acetic acid (10%) was added. The contentswere mixed well and the final volume was made up with distilled water up to the mark. Flask was kept undisturbed for 30 minutes and the contents were filtered through whatman filter paper Grade 42. The first few ml of the filtrate was discarded and the rest was collected in a clean dry 100 ml Erlenmeyer flask fitted with stopper. Using two different pipettes, 5 ml of each of Fehling A and Fehling B solution were taken in 250 mL conical flask and test solutionwas added under boiling condition. It was boiled for about 15 seconds and rapidly further quantities of the solution were added until only faintest perceptible blue color remains. Then 2to 5 drops of 1% methylene blue was added and titration was completed by adding the test solution drop wise. Factor was calculated from the table given in manual corresponding to titre and correction was applied.

$$\text{Lactose percentage} = 5 \times \frac{V1}{V2}$$

Where,

V1= volume in mL, of standard lactose solution used

V2= volume in mL, of sample filtrate used

3.3.2 Dynamic Rheological Properties

The rheological measurements were performed on dynamic rheometer (MCR 52, Antonpaar, Ostifildern, Germany) using cone and plate configuration (CP-75) of 75 mm diameter at20°C.The samples were loaded on rheometer base plate and allowed to rest for 10 minutes to preventthe influence of structure modification during sample handling and loading. For dynamic viscoelastic determination, rheological measurements were performed by frequency sweep testwithin the LVE range of the product at constant shear rate of 1 Pa. The mechanical spectra wasobtained recording storage modulus (G') and loss modulus (G'') as a function of

frequency (0.01 to 100 Hz). Each test was carried out in triplicate.

3.3.2.1 Apparent viscosity

The apparent viscosity of the retentates, protein curd mass and blueberry dessert were determined at 20°C at variable shear rates ranging from 0 to 100s^{-1}.

3.3.3 Microbiological analysis

3.3.3.1 Total plate count

Plate Count Agar was used to enumerate the total viable count of samples as per the standard method described by AOAC pour plate method, (2002). For estimation, dessert sample was collected in pre-sterilized test tube and serially diluted to 10^{-8} dilution in sterile saline blanks. 1 mL was taken from 10^{-4} and 10^{-5} dilution and pour plated on plate count agar. To each plate, 12-15 mL of plate count agar, previously melted and cooled to 42 to 44˚C, was added. The contents were mixed thoroughly by rotating the dish in clockwise and anti-clockwise direction. The mixture was allowed to solidify. The plates were then inverted and incubated at 37±0.5˚C for 48 h and colonies were counted as cfu/g.

Log cfu/g = log (average no. of colonies x dilution factor)

3.3.3.2 Lactic acid bacteria count

Lactic acid bacterial population was estimated in dessert samples using lactic agar supplied by MS. HiMedia Laboratories Pvt. Ltd. For estimation, dessert sample was collected in pre-sterilized test tube and serially diluted to 10^{-8} dilution in sterile saline blanks. 1 mL was taken from 10^{-4} and 10^{-8} dilution and pour plated on lactic agar. The plates were then incubated at 37°C for 24 hr. After the period of incubation, the plates were observed for growth of bacteria and numbers of colonies were counted. The results were expressed as log colony forming unit (cfu) per gram of sample as follows:

Log cfu/g = log (average no. of colonies x dilution factor)

3.3.3.3 Coliform count

Total coliform count was determined as per standard procedure described in IS: 5401-2002: Detection and estimation of coliform bacteria of foodstuffs, using violet red bile agar (Sisco Research Laboratories Pvt. Ltd., Mumbai). The plates were incubated at 37°Cfor 24 h followed by enumeration.

Log cfu/g = log (average no. of colonies x dilution factor)

3.3.3.4 Yeast and mold count

Yeast and mold count was determined as per the standard procedure described in IS: 5403-1999: Method for yeast and mold count of foodstuffs, using potato dextrose agar medium (Sisco Research Laboratories Pvt. Ltd., Mumbai). The plates were then incubated at 25°C for 3-5 days followed by enumeration**.**

Log cfu/g = log (average no. of colonies x dilution factor)

3.3.3.5 Streptococcus enumeration

Streptococcus bacterial population was estimated in dessert samples using M17 agar supplied by MS. HiMedia Laboratories Pvt. Ltd as procedure described. For estimation, dessert sample was collected in pre-sterilized test tube and serially diluted to 10^{-8} dilution in sterile saline blanks. 1 mL was taken from 10^{-4} and 10^{-8} dilution and pour plated on M17 agar. The plates were then incubated at 37°C for 24 hr.

After the period of incubation, the plates were observed for growth of bacteria and numbers of colonies were counted. The results were expressed as log colony forming unit (cfu) per gram of sample as follows:

Log cfu/g = log (average no. of colonies x dilution factor)

3.3.4 Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using hand casted vertical electrophoresis system. The electrophoresis was conducted according to the method of Laemmli (1970) with slight modifications.

a) Stock Acrylamide/Bisacrylamide (30%) solution: Approximately 29.2 g of acrylamide and 0.8 g of bisacrylamide were dissolved in milli-Q water and total volume was made to 100 mL. The solution was stored at 4°C.

b) 1.5 M Tris-HCl (pH 8.8, Running gel buffer): Approximately 18.15 g of tris was dissolved in 50 mL of milli-Q water. The pH was adjusted to 8.8 with 1.0 M HCl and total volume was made up to 100 mL with milli-Q water. The buffer was stored at 4°C.

c) 0.5 M Tris-HCl (pH 6.8, Stacking gel buffer): 6.05 g of tris was dissolved in 50 mL of milli-Q water and pH was adjusted to 6.8 with 1.0 M HCI. The total volume was made up to 100 mL, and the buffer was stored at 4°C.

d) 10% SDS: 10 g of Sodium Dodecyl Sulphate was dissolved in 50 mL of milli-Q water and volume was made to 100 mL. The solution was then stored at room temperature

e) 5X Electrode buffer (25mM Tris, 192 mM glycine, 0.1% SDS, pH 8.3): 15 g of Tris, 72 g Glycine and 5 g of SDS were dissolved in milli-Q water and pH was adjusted to 8.3. The total volume was made up to 1000 mL with milli-Q water. The solution was then stored at room temperature.

f) Sample buffer: The following were mixed to prepare sample buffer: 12.5 mL of SDS stacking gel buffer, 2.5 ml of 0.4% bromophenol blue, 10 mL of glycerol, 20 mL of 10% SDS and 50 mL of milli-Q water. 5 mL of β-Mercaptoethanol was added before using the sample buffer.

g) 10% Ammonium persulphate (APS): 100 mg of APS was dissolved in 1 mL of milli-Q water APS was freshly prepared each time.

h) Stacking gel (4%, 10 mL): Water-3.35 mL, 30% acrylamide/bisacrylamide mixture-4.0 mL, 0.5 M Tris- HCl (pH 6.8)-2.5 mL, 10% APS – 0.050 mL and TEMED- 0.015 mL.

i) Separating gel (12%, 15 mL): Water-5.0 mL, 30% acrylamide/bisacrylamide mixture-6.0 mL, 1.5 M Tris-HCl (pH 8.8)-3.8 mL, 10% SDS-0.150 mL, 10% APS-0.150 mL and TEMED-0.009 mL.

j) Coomassie brilliant blue staining solution: 0.25% Coomassie brilliant blue (CBB) R-250 in methanol/glacial acetic acid/water (2.5:1:6.5) (v/v).

k) De-staining solution: was prepared by adding the methanol/glacial acetic acid/water in the ratio 1:1:8 (v/v).

Preparation of sample

4 ml of sample was mixed with 15 mL of sample buffer (M Tris-HCl, pH 6.8, 2% (w/v) SDS, 7% (v/v) glycerol, 5% β-Mercaptoethanol (v/v)) for 30 minutes at 40°C. The obtained suspension was then centrifuge at 2600 g for 20 minutes. The clear solution beneath the upper layer was carefully taken and diluted with sample buffer in 2:1. The solutions were frozen. Prior to electrophoresis, samples were heated at 90°C for 5 minutes and cooled down to the room temperature.

Preparation of gel

A pair of dry glass rectangular plates was taken and a spacer was placed between both side de plates. The gel casting assembly was secured by clamping with the clamps. The assembly was allowed to stand on the side clamps. The separating gel after addition of A was immediately deposited between the assembled glass plates of the gel equipment such the level remains slightly below the comb. Water about 200 mL was carefully overlaid pipetting down the side of the glass plates. The gel was allowed to polymerize at room temperature for at least 30-40 min. The stacking gel was prepared and was carefully deposit on top of the polymerized separating gel, after removing water from the top of the separating gel, until the cavity was full. Comb was then inserted into the stacking gel solution, avoid trapping of air bubbles underneath the comb. The stacking gel was allowed to polymerize at least 30 min at room temperature. Comb was removed and sample wells were overlaid with enough water to fill completely. clamps were then removed and the plates were fixed into the electrophoretic unit.

Electrophoresis Run

Diluted and chilled electrode buffer was poured appropriately into the electrophoretic unit. The overlaid was removed from the polymerized stacking gel and the upper reservoir filled with electrode buffer. 20 µL of the sample and 8 µL of the marker were loaded into the well using a micropipette. The electrophoretic unit was connected with the power supply and the electrophoresis was carried out at a voltage of 60 V for 30 minutes until the dye front reaches the bottom of the stacking gel layer and after that 110 V for almost 1.5 hour.

Staining and Destaining:

At the end of electrophoresis, the gel was removed from the electrophoretic unit and kept for staining in the staining solution for 2 h. After staining, the gels were transferred to destaining solution at room temperature till protein bands are completely visible. The protein bands from the sample were compared with that from the control and marker. The destained gel was photographed.

3.3.5 Antioxidant properties

3.3.5.1 DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay

Antioxidant capacity based on DPPH (2,2 diphenyl-1-picryl hydrazyl) radical for the dessert samples were analysed following the method given by Brand Williams *et al.* (1995).

For DPPH activity, 100 µL of blueberry dessert extracted sample was mixed with 2.9 mL of DPPH reagent and incubated at room temperature for 30 min in the dark. The absorbance of DPPH activity was estimated at 517 nm. The standard curve for DPPH activity was plotted between 10 and 100 µmol/g range and the standard curve equation was $y = 0.086x - 8.874$ ($R^2 = 0.995$).

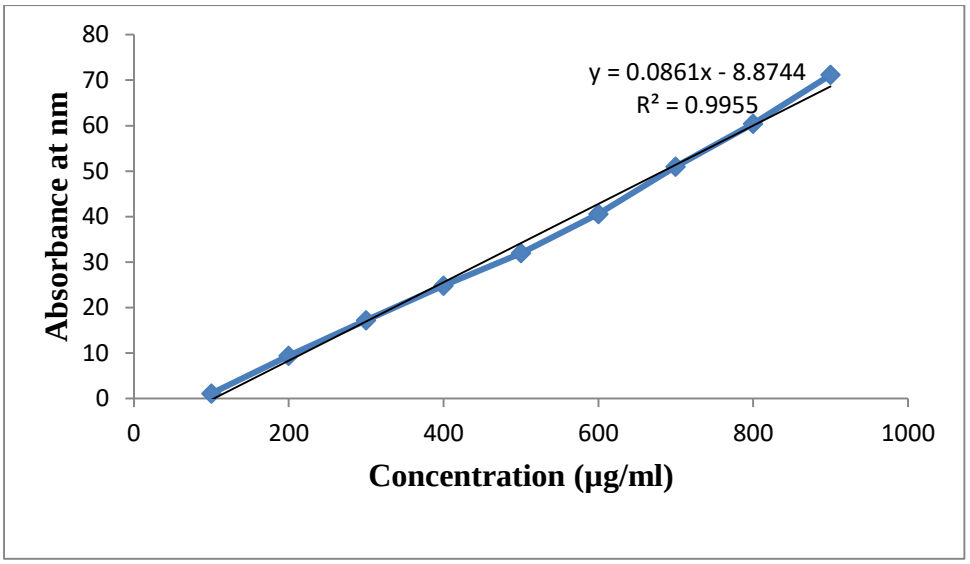


Figure 3.2 Standard curve for DPPH

3.3.5.2 Total Phenolics

Antioxidant capacity based on Total phenolics for the dessert samples were analysed following the method given by Quitao-Teixeira *et al.* (2009).

Total phenolics were estimated using the Folin-Ciocalteau method. Briefly, 500 µL of the blueberry dessert extracted sample and 2.5 mL of Folin-Ciocalteau reagent (diluted in 1:10 ratio) was taken in a test tube. After 5 min, 2 mL of sodium carbonate solution (7.5 g in 100 mL distilled water) was added and incubated at room temperature for 90 min in the dark. The absorbance of the samples was measured at 765 nm using a spectrophotometer (UV1800, Shimadzu, Japan). The standard curve was plotted for a range of 10–100 µg/g and results were expressed as µg gallic acid equivalent/g using a standard curve equation; y = 0.010x + 0.001 (R^2 = 0.995).

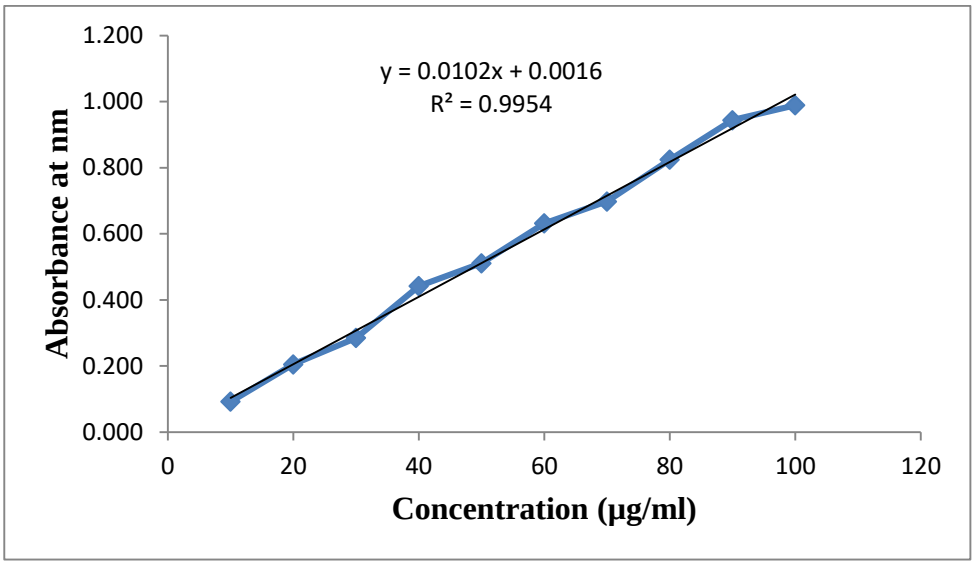


Figure 3.3 Standard curve for total phenolics

3.3.5.3 Total Flavonoid content

Antioxidant capacity based on Total flavonoid content for the dessert samples were analysed following the method given by Quitao-Teixeira *et al.* (2009).

Total flavonoid content was estimated using aluminum chloride solution (2 g/100 mL in ethanol). Two mL each of blueberry dessert extracted sample and aluminum chloride solution was taken in a test-tube followed by incubation at room temperature for 1 h and absorbance measurement at 420 nm. Quercetin was used as standard and the standard curve was plotted for a range of 1–10 µg/g. The results were expressed as µg Quercetin/g using a standard curve equation; $y = 0.281x - 0.085$ ($R^2 = 0.996$).

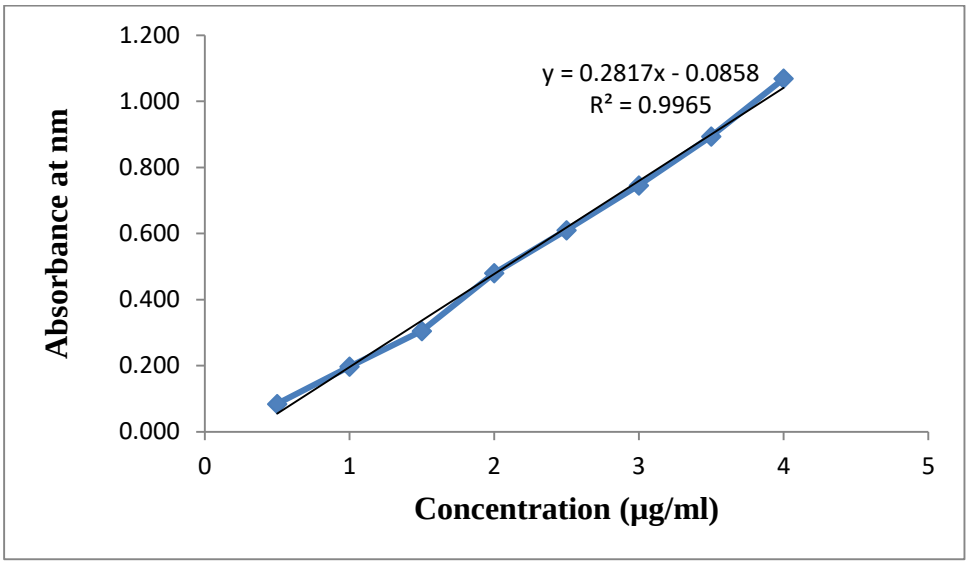


Figure 3.4 Standard curve for total flavonoids

3.3.5.4. Ferric Reducing Antioxidant Power Assay

Antioxidant capacity based on ferric reducing antioxidant power assay for the dessert samples were analysed following the method given by Benzie *et al.* (1996).

While FRAP activity was estimated by mixing 500 µL of blueberry dessert extracted sample with 3.5 mL of FRAP reagent followed by incubation at 37 ◦C for an hour maintained using a BOD incubator (Sanco Make Lab Equipments, India). The absorbance of FRAP assay was estimated at 593 nm. while the standard curve for FRAP assay was plotted for 10–100 µmol/g range and the standard curve equation was y = 0.005x + 0.013 (R^2 = 0.994). Trolox was used as a standard for both the analysis and the results were expressed as µmol Trolox Equivalent (TE)/g.

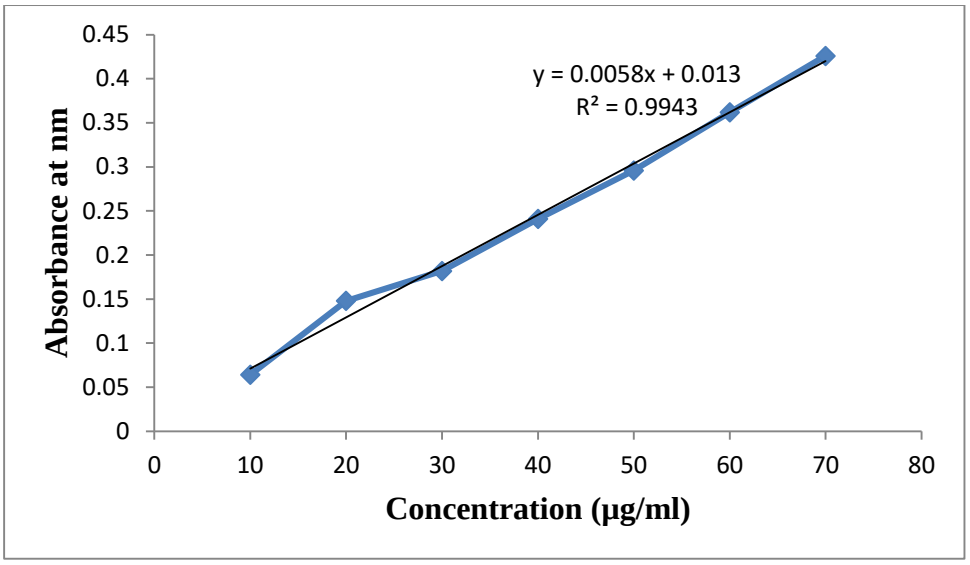


Figure 3.5 Standard curve for Ferric Reducing Antioxidant Power Assay

3.3.6 Sensory evaluation of dessert

Blueberry dessert samples were filled in PP sterile containers and stored at 4ºC in a refrigerator. The samples were then given to 6 trained panelists including the faculty of National dairy research institute, Karnal. Samples were analyzed for different sensory attributes of flavor, body and texture, color and appearance and overall acceptability using semi- structured linear intense scale.

3.3.7 Statistical Analysis:

The data obtained from the various experiments during development of blueberry incorporated goat milk based high protein dessert was subjected to analysis of variance (i.e., one way ANOVA and two way ANOVA) by employing SPSS software 23.0. Each experiment was performed thrice in triplicates

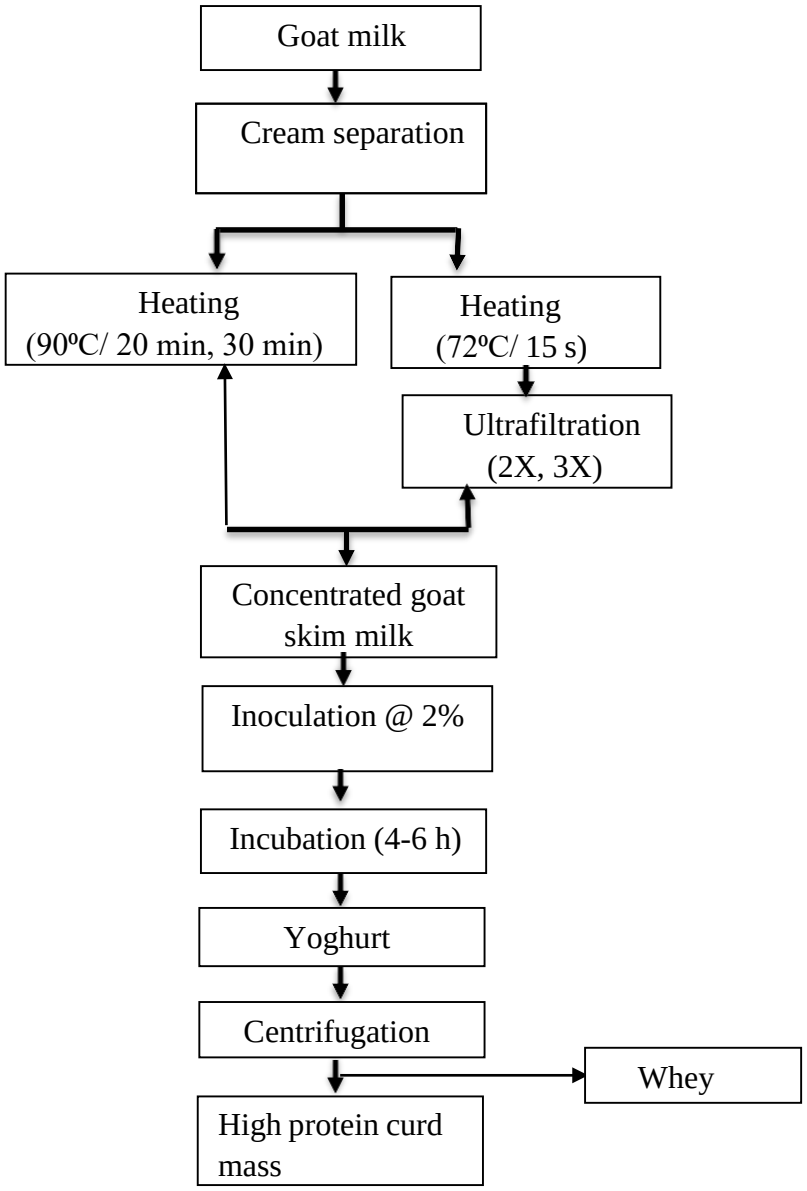
3.4 Plan of work:

Objectives:

1. Optimization of processing conditions and formulation for blueberry incorporated goat milk based high protein dessert
2. Determination of shelf life of goat milk based high protein dessert

3.4.1 Optimization of processing conditions and formulation for blueberry incorporated goat milk based high protein dessert

3.4.1.1 Process optimization of blueberry incorporated goat milk based high protein dessert



Flow chart 3.1 Preparation of high protein curd mass

The Goat milk will be collected from cattle yard of NDRI. Milk will be heated to 40-45ºC for cream separation. Then, collected skim will be concentrated by either heating at 90ºC/ 30 min,20 min or heating at 72ºC/15 s and then ultrafiltration (2X, 3X). The concentrated skim milk collected from these steps will be inoculated with 2% NCDC 263 culture and incubated for 4-6 h till the pH becomes 4.6. The prepared yoghurt will then be centrifuged to obtain high proteincurd mass and the obtained whey will be collected for the analysis as mentioned in the flow chart 3.1.

Parameters to be studied:

Table 3.1 Analysis of whey

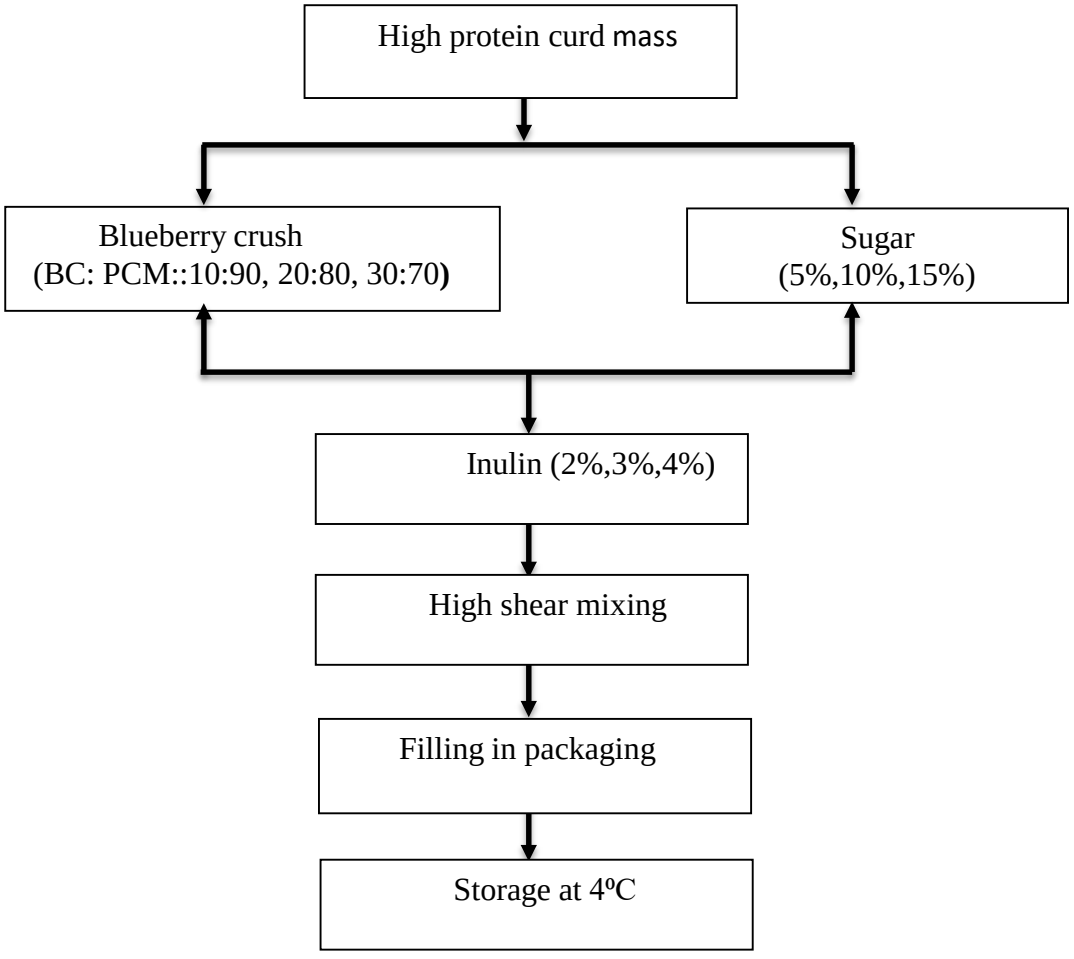
Parameters	**Method**
Total solids	By direct forced air oven drying (AOAC, 2016)
Lactose	Lane Eynon method (IS: IP: 18, Part XI 1981)
Protein	Kjeldahl method (AOAC, 2016)

Table 3.2 Analysis of protein curd mass

Parameters	**Method**
pH	pH meter
Acidity	Titrimetric method (AOAC, 2016)
Rheological analysis	Rheometer (MCR 52, Anton paar)

3.4.1.2 Formulation optimization of blueberry incorporated goat milk based high protein dessert

Protein curd mass (PCM) optimized from the first activity will be used for the formulation optimization of blueberry crush and sugar as presented in flowchart 2. In first step, the level of blueberry crush in the range of ratios (BC: PCM=10:90, 20:80, 30:70**)** and sugar in the range of 5-20 % will be optimize. After optimization of the blueberry crush and sugar, the second step include optimization of inulin levels which will be incorporated at the levels of (1 – 4%). The optimized product will then be subjected to high shear mixing and the products will be filled in packaging materials (polypropylene cups) and further stored at 4°C.



Flowchart 3.2 Preparation of blueberry dessert by optimizing the level of blueberrycrush, sugar and inulin

3.4.1.3 Physicochemical characteristics of high protein dessert

The resultant blueberry incorporated goat milk based high protein dessert will then be subjected to following physicochemical analysis:

Parameters to be studied:

Table 3.3 Physicochemical analysis of optimized blueberry dessert

Parameters	Method
pH	pH meter
Acidity	Titrimetric method (AOAC, 2016)
Fat	Majonnier method IS:1479 (part II, 1961)
Protein	Kjeldahl method (AOAC, 2016)
Total solids	By direct forced air oven drying (AOAC, 2016)
Ash	Gravimetric method (AOAC, 2016)
Color	Hunter Lab colorimeter
Protein pattern	SDS PAGE (Liu *et al*., 2018)

Table 3.4 Rheological parameters of optimized blueberry dessert

Parameters	Method
Rheological analysis	Rheometer (MCR, 52, Anton paar)

Table 3.5 Antioxidant properties of optimized blueberry dessert

parameters	Method
DPPH	Brand-Williams *et al.* (1997)
Total phenolics	Quitao-Teixeira *et al.* (2009)
Total flavonoid	Quitao-Teixeira *et al.* (2009)
FRAP	Benzie *et al.* (1996)

Table 3.6 Microbiological characteristics of optimized blueberry dessert

Parameters	Method
Total viable count	pour plate method AOAC, (2004)
Coliform count	IS:5401(2002)
Yeast and mold	IS:5403(1999)
Lactic acid bacteria	pour plate method AOAC, (2004)
Streptococcus count	pour plate method AOAC, (2004)

Table 3.7 Sensory evaluation of optimized blueberry dessert

Parameter	Method
Sensory evaluation	Intensity score card

3.4.2 Determination of shelf life of goat milk based high protein dessert

3.4.2.1 Determination of shelf life of dessert at refrigeration temperature

For determination of shelf life of goat milk based high protein dessert, the optimized product would be subjected to two different processing and packaging conditions. The samples will be analyzed at 7 days interval for 1 month at 4±1ºC.

- In- package thermization (60ºC for 10 minutes)
- Packaging in Polypropylene cups (PP cups)

Parameters to be studied:

Table 3.8 Physicochemical characteristics of optimized blueberry dessert during storage

Parameter	Method
pH	pH meter

Acidity	Titrimetric method (AOAC 2016)
Color	Hunter lab colorimeter

Table 3.9 Microbiological characteristics of optimized blueberry dessert during storage

Parameter	**Method**
Total viable count	Pour plate method AOAC, (2004)
Coliform count	IS: 5401 (2002)
Yeast and mold	IS:5403 (1999)
Lactic acid bacteria	Pour plate method AOAC, (2004)
Streptococcus count	Pour plate method AOAC, (2004)

Table 3.10 Rheological parameters of optimized blueberry dessert during storage

Parameter	**Method**
Viscosity	Anton paar rheometer
Frequency sweep	Anton paar rheometer

Table 3.11 Antioxidant properties of optimized blueberry dessert during storage

Parameter	**Method**
DPPH	Brand-Williams *et al.* (1997)
Total phenolics	Quitao-Teixeira *et al.* (2009)
Total flavonoid	Quitao-Teixeira *et al.* (2009)
FRAP	Benzie *et al.* (1996)

Table 3.12 Sensory evaluation of optimized blueberry dessert during storage

Parameter	**Method**
Sensory evaluation	Intensity score card

CHAPTER-4

Results and Discussion

This chapter deals with the aspects related to process optimization, physico-chemical, textural, storage aspects, microbiological and antioxidant properties of blueberry incorporated goat milk based high protein dessert. In the first objective of the study, goat skim milk concentration levels were optimized to improve the textural and rheological attributes of the goat milk based fermented base. In the second part of the study, blueberry, sugar and inulin levels were standardized for preparation of blueberry incorporated goat milk based high protein dessert and optimized product was analyzed for various quality parameters. The optimized product was analyzed for various storage characteristics such as physicochemical, rheological, microbial, sensory and antioxidant properties. The obtained results during this investigation are presented and discussed in this chapter here under.

Objectives:

1. Optimization of processing conditions and formulation for blueberry incorporated goat milk based high protein dessert
2. Determination of shelf life of goat milk based high protein dessert

4.1 OPTIMIZATION OF PROCESSING CONDITIONS AND FORMULATION FOR BLUEBERRY INCORPORATED GOAT MILK BASED HIGH PROTEIN DESSERT

4.1.1 Process optimization of blueberry incorporated goat milk based high protein dessert

4.1.1.1 Effect of Heating (90^0 C/20 min and 30 min) on physicochemical and rheological properties of goat skim milk

Raw goat milk was passed through cream separator (at 40ºC) for obtaining goat skim milk (GSM). The collected GSM was subjected to heat treatment (90ºC) for a duration of 20 and 30 minutes**.** Heat treated GSM was analyzed for changes in terms of pH, acidity, total solids (TS), lactose and protein. The results obtained are presented in Table 4.1**.** With increase in duration of heat treatment (at 90ºC) *i.e.,* from 20 to 30 minutes, there was a significant ($p<0.05$) decrease in pH and subsequent increase in acidity while, significant increase ($p<0.05$) in total solids content of GSM was observed. High pH of the milk upon heat treatment might be attributed to the lower whey protein attached to the casein micelles (Jose *et al.,* 2015). Slight increase in protein content of the GSM samples was also recorded with increase in duration of heat treatment however, the increase did not differ significantly ($p>0.05$). Our results also corroborated with the findings of Desouky *et al.* (2017), who also observed similar compositional changes upon subjecting the goat milk to heat treatment.

Table 4.1 Effect of heat treatment on proximate composition and viscosity of goat skim milk

Parameters	H 20	H 30
pH	6.24 ± 0.01[b]	6.12 ±0.00[a]
Acidity (% LA)	0.19 ± 0.00[b]	0.22 ±0.00[a]
Total solids (%)	11.32 ± 0.19[b]	13.58 ±0.28[a]
Fat (%)	0.20 ± 0.05[a]	0.19 ± 0.02[a]
Lactose (%)	5.00 ± 0.06[a]	4.7 ±0.04[b]
Protein (%)	5.37 ± 0.22[a]	5.69 ±0.27[a]
Viscosity at 50 s^{-1} shear rate (Pa.s)	0.05 ± 0.01[a]	0.15± 0.09[b]

[ab]Values (Mean ± SD; n=9) with different superscripts in a row are significantly different at the level of $p<0.05$
H20: Goat skim milk heated at 90°C for 20 min; H30: Goat skim milk heated at 90°C for 30 min.

Apparent viscosity of heat treated GSM samples were measured at shear rate from 0 to 100 s^{-1} using rheometer. The results obtained were plotted as apparent viscosity and shear rate in figure 4.1. Apparent viscosity of the both heat treated GSM samples (*i.e.*, 90º C/20 min and 90º C/30 min) decreased with increase in shear rate indicating shear dependent behavior of liquid samples. For the low-fat products and/or skim milk, that generally comprises higher protein content, heat treatment plays significant and crucial role in determining the viscosity (Mc Carthy *et al.*, 2022). Our results revealed that the GSM heated at 90º C/30 min exhibited three times higher apparent viscosity (at 50 s^{-1} shear rate) as compared to GSM heated at 90º C/20 min (Table 4.1). This could be attributed to relatively higher protein denaturation of GSM heated at 90º C/30 min than the other sample, which increases the water binding capacity and thus, reflected as higher apparent viscosity **(**Somer *et al.*, 2012). Highest viscosity was observed for unstandardized skim milk (@9% TS) as compared to standardized skim milk (permeate) owing to increased volume fraction of protein (Murphy *et al.*, 2018).

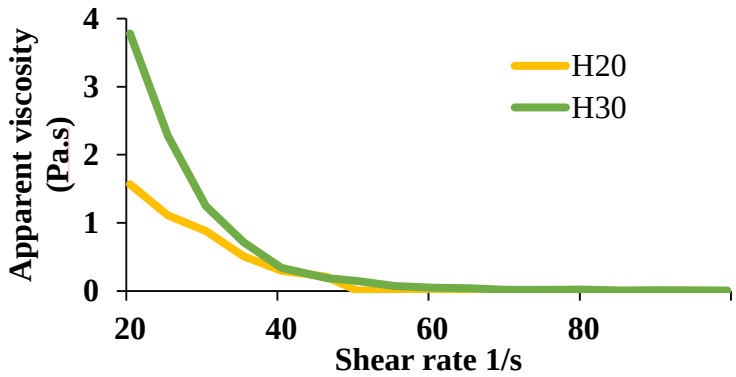


Figure 4.1. Effect of duration of heat treatment on apparent viscosity (at 50 s^{-1} shear rate)

(H20: Goat skim milk heated at 90°C for 20 min; H30: Goat skim milk heated at 90°C for 30 min)

4.1.1.2 Effect of ultrafiltration on physicochemical and rheological properties of goat skim milk retentate

Ultrafiltration (UF) is a membrane process by which proteins and fats are concentrated and majority of lactose and some soluble minerals are removed from a solution. The GSM was subjected to UF at three different volume concentration ratios (VCR) to get 1X, 2X and 3X retentate concentrations. Further, the obtained retentates were analyzed for physico-chemical composition and the results are tabulated in the Table 4.2. All the parameters (pH, acidity, TS and protein) except, lactose and pH significantly increased ($p<0.05$) with the increase in the GSM concentration. Meanwhile, pH and lactose values (% LA) significantly decreased ($p<0.05$) from 6.52 to 6.22 and 5.03 to 4.27 in 1X and 3X concentrations, respectively. The decrease in lactose content could be attributed to the removal of lactose through membrane process which subsequently goes into permeate part of UF (Deshwal *et al.*, 2020). Similar findings were reported by several authors (Park *et al.*, 2007; Domagala and Kupiec, 2003; Mehaia and El- Khadragy, 1997; Espinoza and Calvo, 1998; Meena *et al.*, 2016).

Apparent viscosity of UF GSM samples (1X, 2X and 3X) were measured at shear rate from 0 to 100 s^{-1} using rheometer. The results obtained were plotted as apparent viscosity versus shear rate as shown in figure 4.2. All the samples exhibited shear thinning behavior *i.e.*, apparent viscosity of the samples decreased with increase in shear rate. Further, results revealed that the maximum apparent viscosity (0.22 Pa.s) was observed for 3X GSM samples followed by 2X (0.14 Pa.s) and 1X (0.03 Pa.s) samples (Table 4.2). Higher protein concentration and thus, subsequent casein particles lead to denser protein matrix. This results in enhanced firmness and water holding capacity of the gel (Sodini *et al.*, 2004).

Table 4.2 Effect of different ultrafiltration concentrations on proximate composition of goat skim milk

Ultrafiltered milk	1X	2X	3X
pH	6.52 ± 0.00^{c}	6.29 ± 0.00^{b}	6.22 ± 0.00^{a}
Acidity (% LA)	0.14 ± 0.00^{a}	0.15 ± 0.00^{b}	0.16 ± 0.00^{c}
Total solids (%)	8.86 ± 0.05^{a}	11.64 ± 0.12^{b}	15.58 ± 0.22^{c}
Lactose (%)	5.03 ± 0.05^{c}	4.72 ± 0.06^{b}	4.27 ± 0.07^{a}
Protein (%)	3.61 ± 0.02^{a}	6.30 ± 0.14^{b}	8.91 ± 0.15^{c}
Viscosity at 50 s^{-1} shear rate (Pa.s)	0.03 ± 0.01^{a}	0.14 ± 0.05^{b}	0.22 ± 0.32^{c}

[ab]Values (Mean ± SD; n=9) with different superscripts in a row are significantly different at the level of $p<0.05$

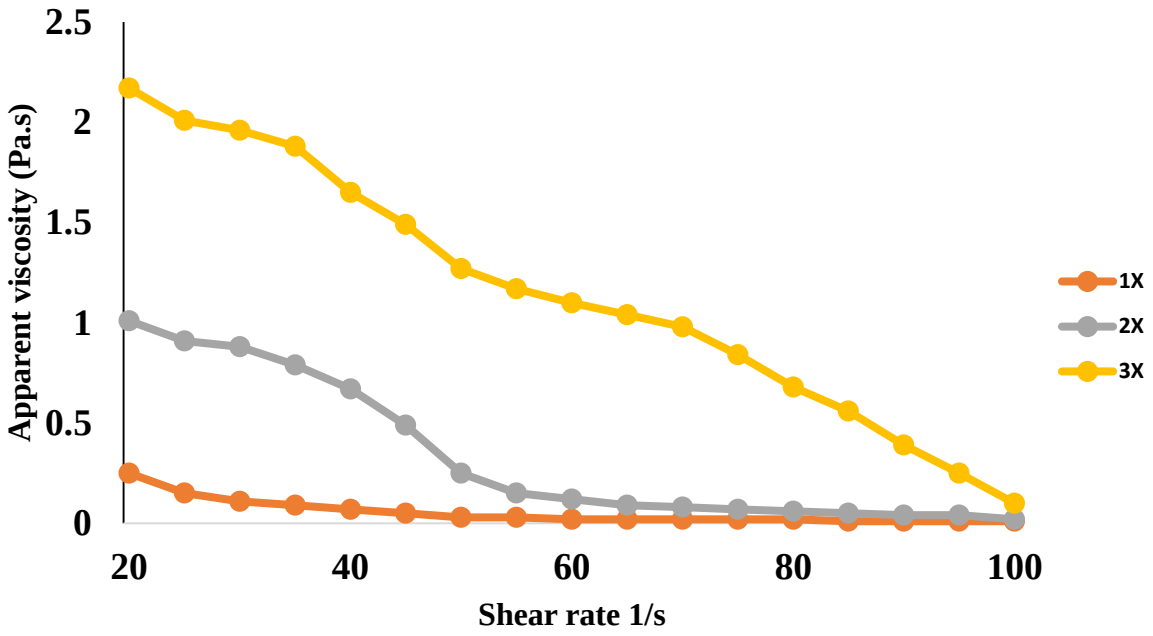


Figure 4.2 Apparent viscosity of GSM retentate as affected by shear rate

4.1.1.3 Physicochemical analysis of whey and protein curd mass obtained by fermentation of goat skim milk retentate

Yoghurt was prepared form different concentrated GSM samples (1X, 2X and 3X) following standard protocol as mentioned in section 3.4.1.1. The obtained yoghurts were centrifuged to obtain high protein curd mass (PCM) and whey. The resultant whey and PCM samples were further analyzed for various quality attributes and results are presented in Table 4.3 and Table 4.4. It can be seen from the Table 4.5 that higher acidity (0.19% LA) and lactose (5.08%) were observed in 3X whey samples. The higher pH (4.53) and lower acidity (0.15% LA) values in 1X whey could be attributed to its lower protein, total solids and mineral content. Lactose content of whey samples increased with increase in concentration of GSM base that was used for PCM preparation. However, the increase was not significantly ($p>0.05$) different among various samples.

Optimum pH and acidity are critical to determine sensorial and aesthetic quality of fermented dairy product. Significantly higher and lower ($p<0.05$) pH values were observed in 1X PCM (4.56) and 3X PCM (4.51), respectively. Acidity and protein content of PCM samples markedly increased ($p<0.05$) with increase in concentration of GSM base. 3X PCM sample exhibited significantly higher ($p<0.05$) protein (20.25%) and acidity (1.53% LA) than 1X PCM samples.

Table 4.3 Physico-chemical composition of whey obtained from centrifugation of yoghurt samples prepared from different concentrations of goat skim milk

Parameters	1X whey	2X whey	3X whey
pH	4.53 ± 0.00^{c}	4.40 ± 0.00^{b}	4.28 ± 0.02^{a}
Acidity (% LA)	0.15 ± 0.00^{a}	0.17 ± 0.00^{b}	0.19 ± 0.01^{c}
Total solids (%)	6.20 ± 0.44^{a}	6.42 ± 0.32^{b}	6.86 ± 0.22^{ab}
Protein (%)	1.30 ± 0.44^{a}	1.74 ± 0.21^{b}	1.73 ± 0.06^{b}
Lactose (%)	4.94 ± 0.24^{a}	5.02 ± 0.36^{a}	5.08 ± 0.24^{a}

[ab]Values (Mean ± SD; n=9) with different superscripts in a row are significantly different at the level of $p<0.05$

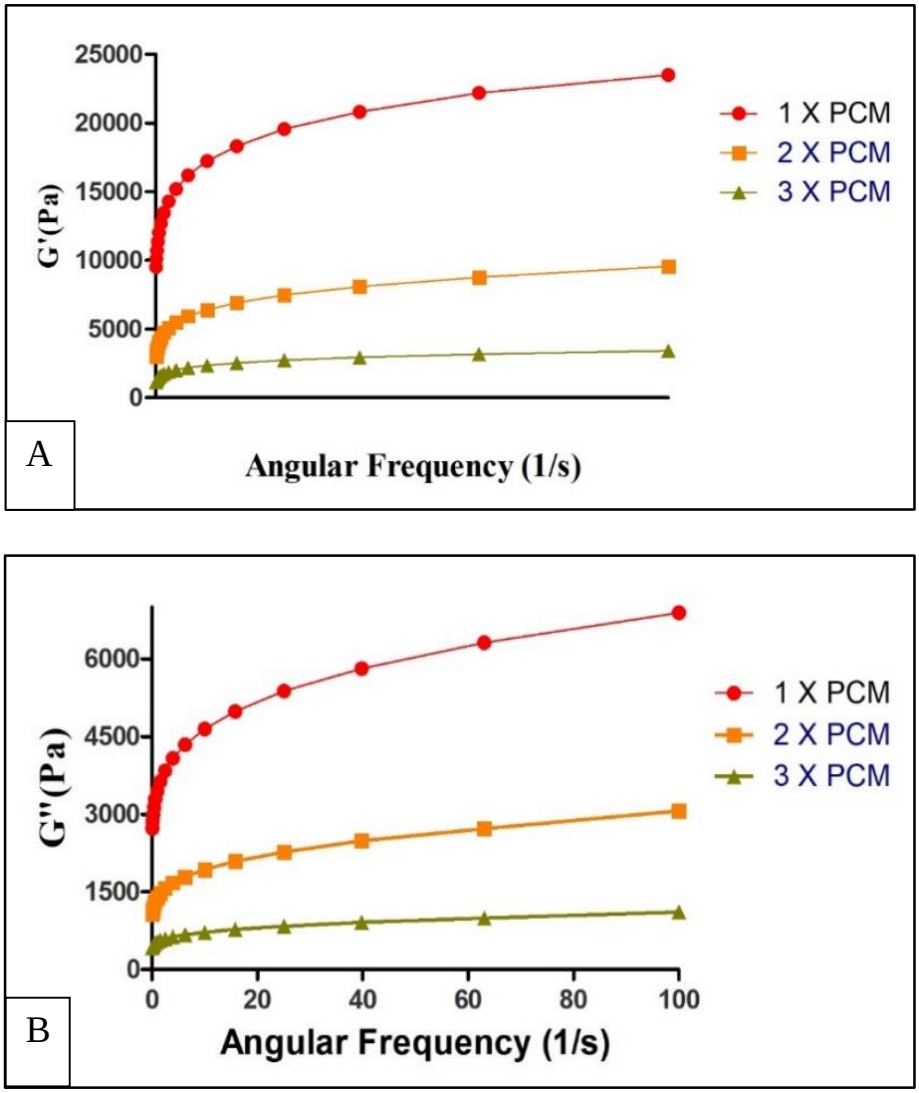


Figure 4.3: Rheological attributes (A) storage modulus (B) Loss modulus of protein curd mass

Apparent viscosity of the PCM samples obtained from flow curve at 50 s^{-1} shear rate are presented in Table 4.4. The maximum (18.9 Pa.s) and minimum (17.3 Pa.s) apparent viscosity values were recorded for samples PCM 3X and PCM 1X, respectively. Frequency sweep test was performed to check the frequency dependency of PCM samples and results are plotted in figure 4.3 (A & B). Storage modulus of PCM samples was found to be higher than loss modulus indicating the viscoelastic behavior of PCM. This similar viscoelastic behaviour for high concentrations protein gels was also studied and confirmed by prasanna *et al.* (2013) and Koksoy and Kilic (2004*).*

Table 4.4 Physico-chemical analysis of protein curd mass (PCM) obtained from fermentation of different concentrations of goat skim milk

Parameters	1X PCM	2X PCM	3X PCM
pH	4.56 ± 0.38^{a}	4.54 ± 0.21^{a}	4.51 ± 0.33^{a}
Acidity (%LA)	1.29 ± 0.50^{a}	1.32 ± 0.20^{b}	1.53 ± 0.22^{ab}
Protein (%)	10.85 ± 0.42^{a}	18.75 ± 0.51^{b}	20.25 ± 0.74^{b}
Viscosity at 50 s^{-1} shear rate (Pa.s)	17.3 ± 2.35^{a}	17.8 ± 1.53^{a}	18.9 ± 1.63^{b}

^{ab}Values (Mean ± SD; n=9) with different superscripts in a row differ significantly ($p<0.05$)

4.1.2 Formulation optimization of blueberry incorporated goat milk based high protein dessert

4.1.2.1 Optimization of blueberry crush for preparation of goat milk based high protein dessert

Protein curd mass (PCM) and blueberry crush were mixed in different ratios (70:30, 80:20, 90:10) for optimization of formulation of high protein dessert and the level of blueberry crush was optimized on the basis of sensory scores. Appearance of any product is first and foremost sensory attribute which influences the acceptability of that particular product by the consumer (Kumar *et al.*, 2017). In our study, higher scores for color values (5.66) were obtained for BD 70: 30 sample followed by BD 80:20 sample. These significant variations might be attributed to the fact that blueberry crush addition imparts color to the product. Thus, it could be interpreted that samples having higher crush levels were recorded with higher appearance scores indicating the significant impact of addition of blueberry crush to the aesthetic appearance of the product. Similar trend was also observed for fruity flavour scores as it was also affected by varying the ratio of blueberry crush and PCM. Among flavor scores, all the attributes were significantly affected by the incorporation of blueberry crush. Fruity and sweet

flavor were significantly enhanced ($p<0.05$) as the level of blueberry crush increased from 10 to 30%. Furthermore, samples containing higher crush levels (*i.e.*, 70:30) had also shown significantly higher ($p<0.05$) acidity and sour taste values than other two samples. Regarding body & texture scores, addition of blueberry crush reduced thickness and lumpiness of high protein dessert significantly ($p<0.05$) which might be due to the dilution effect caused by incorporation of crush which ultimately, resulted in lower total solids content (Chand *et al.,* 2022). Overall acceptability of the product is the culmination of the scores of all other sensory attributes and thus, is significantly influenced by other attributes selected for optimization (Talukder *et al.*, 2013). Overall acceptability scores of BD 70:30, BD 80:20 and BD 90:10 were recorded as 6.83, 8.00 and 6.00, respectively. Therefore, BD 80:20 ratio was selected for further investigations as optimized ratio for preparing good quality high protein blueberry crush incorporated dessert.

Table 4.5. Sensory scores of dessert samples during optimization of blueberry crush and PCM

Attributes		**BD 70:30**	**BD 80:20**	**BD 90:10**
Appearance	**Color**	5.66 ± 0.57^{c}	4.16 ± 0.76^{b}	1.5 ± 0.50^{a}
	Free whey	Nil	Nil	Nil
Flavour	**Creamy**	6.00 ± 1.00^{a}	6.16 ± 1.25^{a}	5.50 ± 0.50^{a}
	Goaty	Nil	Nil	0.40 ± 0.17^{a}
	Fermented	5.67 ± 1.15^{a}	5.16 ± 0.28^{a}	5.00 ± 0.00^{a}
	Cooked	0.03 ± 0.05^{a}	0.10 ± 0.17^{a}	Nil
	Fruity	6.40 ± 0.69^{c}	4.93 ± 0.40^{b}	2.83 ± 0.28^{a}
	Sweet	5.33 ± 1.25^{b}	4.66 ± 1.04^{b}	1.83 ± 0.28^{a}
	Acidic	5.83 ± 0.28^{b}	4.66 ± 0.28^{ab}	3.66 ± 1.15^{a}
	Bitter	0.20 ± 0.26^{a}	0.26 ± 0.25^{a}	0.03 ± 0.05^{a}
	Sour	8.16 ± 0.28^{b}	6.00 ± 1.00^{a}	5.83 ± 0.76^{a}
	Astringent	1.83 ± 1.25^{a}	1.80 ± 1.04^{a}	1.00 ± 0.00^{a}
Body & Texture	**Thickness**	4.30 ± 1.50^{a}	5.66 ± 1.15^{b}	8.00 ± 0.50^{c}
	Lumpiness	0.66 ± 0.76^{a}	1.00 ± 1.32^{a}	1.50 ± 0.86^{a}
	Ropiness	Nil	Nil	Nil
Overall acceptability		6.83 ± 0.28^{a}	8.00 ± 0.50^{b}	6.00 ± 0.05^{a}

[ab]Values (Mean ± SD; n=9) with different superscripts in a row differ significantly ($p<0.05$)

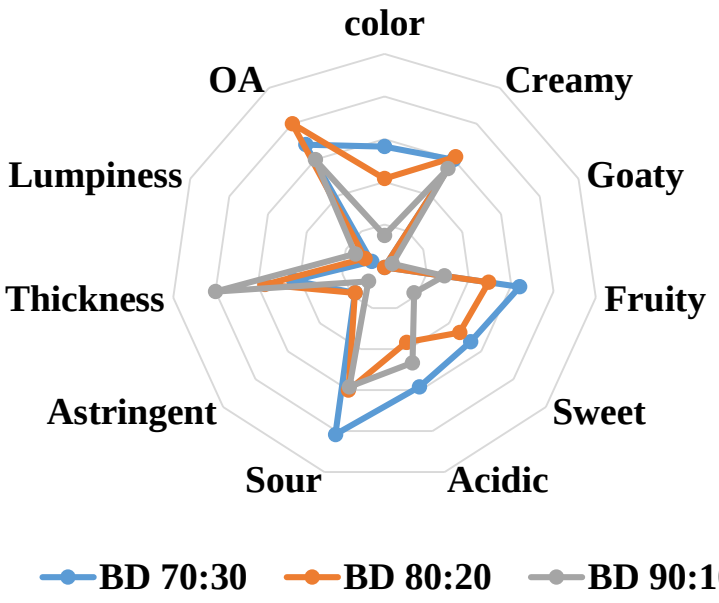


Figure 4.4: Sensory analysis (spider web diagram) for the optimization of blueberry crush and protein curd mass

4.1.2.2 Optimization of sugar for preparation of blueberry crush incorporated high protein dessert

Based on the results of previous activity, high protein curd mass and blueberry crush were mixed in the ratio of 80:20 and further, subjected to the incorporation of sugar at three different levels (5%, 10% & 15%). The sugar incorporated resultant product was then evaluated for sensory attributes for final optimization step. Generally, sensory analysis include different sciences for the better understanding of sensory properties of a product and consumers response to these properties (chambers *et al.*, 2003). It is evident from the Table 4.6 that the appearance of the product was not affected ($p>0.05$) due to sugar addition. While, flavor attributes notably, fruity and sweetness were influenced significantly ($p<0.05$) with increase in the sugar level from 5 to 10% in the mix of high protein curd mass and blueberry crush. Furthermore, body & texture scores also showed similar results wherein, thickness of the product decreased ($p<0.05$) as the level of sugar increased from 5% to 15%. The optimum and desired thickness of the products was observed to be at 10% sugar level. Hygroscopic nature of the sugar ultimately resulting in the increased apparent viscosity might have affected the total solids concentration in the product (Chand *et al.*, 2022) and thereby, yielding significantly ($p<0.05$) lower scores for thickness as the sugar level was increased. Finally, overall acceptability of the product seemed to be influenced by thickness, fruity and sweetness scores of the product. In our study, highest overall acceptability scores were observed for BD 80:20 with 10% sugar level (8.66 ± 0.28) followed by 15% sugar (7.16 ± 0.28). Therefore, based on the sensory evaluation, sugar

level of 10% was selected as optimized level for the preparation of good quality high protein blueberry crush incorporated dessert.

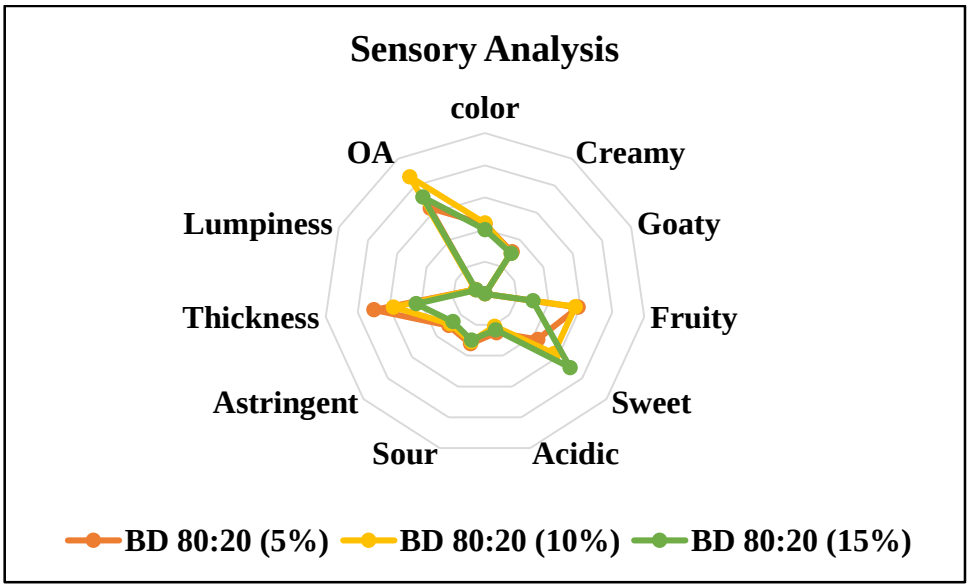


Figure 4.5: Sensory analysis (spider web diagram) for the optimization of sugar in blueberry incorporated high protein curd mass

Table 4.6 Sensory scores of high protein dessert samples during optimization of sugar

Attributes		BD 80:20 (5%)	BD 80:20 (10%)	BD 80:20 (15%)
Appearance	**Color**	4.33 ± 0.57^{a}	4.40 ± 0.20^{a}	4.00 ± 0.73^{a}
	Free whey	Nil	Nil	Nil
Flavour	**Creamy**	3.10 ± 0.56^{a}	3.00 ± 0.72^{a}	3.00 ± 0.76^{a}
	Goaty	Nil	Nil	Nil
	Fermented	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}
	Cooked	Nil	Nil	Nil
	Fruity	5.85 ± 0.57^{b}	5.67 ± 0.62^{b}	3.00 ± 0.48^{a}
	Sweet	4.33 ± 1.57^{a}	5.67 ± 0.98^{a}	7.00 ± 0.00^{b}
	Acidic	2.52 ± 0.63^{a}	2.10 ± 0.84^{a}	2.33 ± 0.77^{a}
	Bitter	0.03 ± 0.05^{a}	0.03 ± 0.05^{a}	0.03 ± 0.05^{a}
	Sour	3.23 ± 0.47^{a}	3.10 ± 0.26^{a}	3.00 ± 0.32^{a}
	Astringent	3.00 ± 0.40^{a}	2.86 ± 0.32^{a}	2.64 ± 0.59^{a}
Body & Texture	**Thickness**	6.98 ± 0.61^{c}	5.76 ± 0.55^{b}	4.33 ± 0.92^{a}
	Lumpiness	0.66 ± 0.21^{a}	0.65 ± 0.32^{a}	0.59 ± 0.22^{a}
	Ropiness	Nil	Nil	Nil
Overall acceptability		6.33 ± 0.57^{a}	8.66 ± 0.28^{c}	7.16 ± 0.28^{b}

4.1.2.3 Optimization of inulin for the preparation of blueberry crush incorporated high protein dessert

As the final optimization step, inulin was used as fat replacer in the product BD 80:20 containing 10% sugar at three different levels (3%, 4% and 5%). Based on rheological properties and sensory scores, the level of inulin was optimized. Apparent viscosity of the resultant products was represented as viscosity versus shear rate (Fig 4.6). Results revealed that, irrespective of the inulin levels, the viscosity of all the products decreased with increasing shear rate. However, the highest viscosity (at shear rate of 50 s^{-1}, Pa.s) was found to be in product incorporated with 4% inulin (16.77 ± 1.26) followed by 3% inulin level (12.17 ± 1.09). It has been reported that increased viscosity of the products occur due to the incorporation of inulin as the latter, being a polysaccharide, enhances the solids-not-fat content of the product (Chand *et al.*, 2021). Similar increase in viscosity of the yoghurt drinks owing to the addition of inulin was reported by Allgeyer *et al.* (2010). Moreover, three-dimensional structure of caseins is reported to get rearranged upon the addition of inulin which subsequently led to the formation of firm gels (El-Kholy *et al.*, 2020).

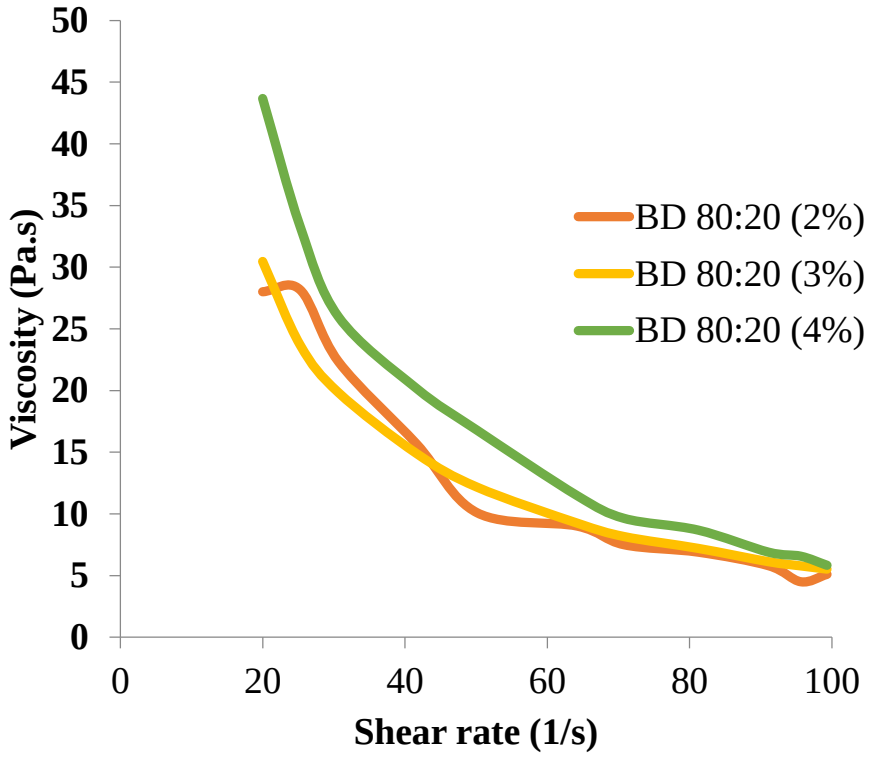


Figure 4.6: Apparent viscosity (Pa.s) of different levels of inulin incorporated blueberry dessert

Further, sensory scores of the product were not significantly influenced ($p>0.05$) by the addition of different levels of inulin except appearance and thickness. Decreased color scores of the products with increased levels of inulin ($p<0.05$) might be due to lighter color perceived by the sensory panelists. Moreover, the fat replacer nature of the inulin leading to more intense homogenous structure and firmness in the products ultimately, resulted in enhanced thickness perception of high protein dessert (Pimentel *et al.,* 2013). These results also corroborated with the rheological properties of the products containing different levels of inulin discussed in this section earlier. Therefore, based on rheological properties and sensory evaluation, inulin level of 4% was selected as optimized level for the preparation of good quality high protein blueberry crush incorporated dessert.

4.1.3 Characterization of physicochemical and rheological properties of blueberry incorporated goat milk based high protein dessert

4.1.3.1. Physicochemical, color and rheological characteristics of optimized blueberry dessert

The physicochemical, color and apparent viscosity of optimized blueberry dessert and control samples results were presented in Table 4.7. The pH, acidity (% LA), fat (%), protein (%), ash (%) and total solids (%) of optimized blueberry dessert was 4.23, 1.37, 1.01, 13.55, 0.93 and 48.79, respectively. Significantly ($p<0.05$) higher pH and TS in the optimized product than control samples could be attributed to added crush in the optimized product. Protein content of the optimized product was significantly ($p<0.05$) lower than control samples as a result of dilution effect. Addition of blueberry crush in optimized product significantly enhanced ($p<0.05$) fat content than control samples. The reason for this could be due to naturally existence of fat in blueberry crush which could have also contributed to optimized product fat.

Color values of high protein blueberry crush incorporated dessert were significantly ($p<0.05$) affected with the addition of fruit crush. The maximum L^* value (87.20) was observed for control sample which might be due to the ability of milk proteins to scatter the light in visible spectrum, that resulting in whitening effect of control sample (Sameer *et al.,* 2020). The L^* and b^* values of the optimized product significantly ($p<0.05$) decreased with the blueberry addition while, a^* values significantly ($p<0.05$) increased. Higher a^* value of dessert sample than control was because of blueberry addition which increased the red color of the samples (Cinbas and Yazici, 2008).

Viscosity is an important parameter for dairy and fruit based desserts. The apparent viscosity of the optimized blueberry crush incorporated goat milk based high protein dessert

was significantly higher ($p<0.05$) (12 Pa.s) than the control dessert (7.03Pa.s). This increase in apparent viscosity of the optimized dessert could be attributed to inulin addition as it has the ability to impart viscosity and improve mouthfeel in low fat-based products (Passephol *et al.,* 2008).

Table 4.7 Physico-chemical and rheological properties of blueberry incorporated goat milk based high protein dessert

Parameters	Control	Optimized product
pH	4.54 ± 0.01^{b}	4.23 ± 0.01^{a}
Acidity (%LA)	1.12 ± 0.10^{a}	$1.37^{b}\pm0.05^{b}$
Fat (%)	0.70 ± 0.08^{a}	1.01 ± 0.14^{b}
Protein (%)	20.82 ± 0.25^{b}	13.55 ± 0.22^{a}
Ash (%)	1.43 ± 0.07^{b}	0.93 ± 0.00^{a}
Total Solids (%)	30.32 ± 0.06^{a}	48.79 ± 0.31^{b}
Color		
L*	87.20 ± 0.07^{b}	70.42 ± 0.05^{a}
a*	-0.72 ± 0.31^{a}	3.55 ± 0.57^{b}
b*	6.17 ± 0.06^{b}	3.37 ± 0.18^{a}
Apparent viscosity (Pa.s)	7.03 ± 0.86^{a}	12.00 ± 2.62^{b}

[b]Values (Mean ± SD; n=9) with different superscripts in a row significantly different $p<0.05$

Figure 4.7: Optimized blueberry incorporated goat milk based high protein dessert

4.1.3.2 Antioxidant and microbiological properties of blueberry incorporated goat milk based high protein dessert

The antioxidant properties of blueberry incorporated goat milk based high protein dessert were analyzed and compared with control dessert. The obtained results are presented in Table 4.8. In general, the antioxidant activity (in terms of DPPH and FRAP) of blueberry incorporated goat milk based high protein dessert was significantly higher ($p<0.05$) than control dessert samples due to addition of blueberry crush. Several authors investigated the antioxidant properties and reported that blueberry pulp is a good source of antioxidants owing to the presence of phenolic acids, tannins and flavonoids etc. Presence of higher amount of anthocyanins and proanthocyanins have already been reported which are held responsible for higher antioxidative properties (Huang *et al.,* 2012). The DPPH and FRAP activity for blueberry incorporated goat milk based high protein dessert were observed to be (169.85 µmol/g) and (447.32 µmol/g) respectively. Phenolic compounds are an excellent source of antioxidants and are reported to have a prominent role in preventing diseases like alzheimer's, cardiovascular diseases etc. Significantly higher ($p<0.05$) total phenolics (143.64 µg GAE/g) and flavonoids (2.96 µg QE/g) were observed for blueberry incorporated goat milk based high protein dessert than control dessert.

Table 4.8 Antioxidant properties of blueberry incorporated goat milk based high protein dessert

Antioxidant activity	Control	Optimized product
DPPH (µmol TE/g)	89.84 ±8.23[a]	169.85 ±10.12[b]
Total phenolic (µg GAE/g)	98.84 ± 16.75[a]	143.64 ± 22.50[b]
FRAP (µmol TE/g)	290.78± 25.32[a]	447.32 ± 36.89[b]
Total flavonoid (µg QE/g)	2.12 ± 0.01[a]	2.96 ± 0.06[b]

[ab]Values (Mean ± SD; n=9) with different superscripts in a row significantly different $p<0.05$

Microbiological characteristics of optimized blueberry dessert and control was represented in the Table 4.9. The total viable count of blueberry incorporated goat milk based high protein dessert were slightly higher than the control dessert, however the difference was statistically insignificant ($p>0.05$). Coliform and yeast and mold count in both optimized and control dessert samples were found to be absent. Significantly higher ($p<0.05$) *Streptococcus* count and lower *Lactobacillus* counts were observed in optimized product than control dessert. It has been reported that presence of live *Lactobacillus and Streptococcus* strains might further enhance the functional properties of dairy based fermented products (Akin *et al.,* 2007).

Table 4.9 Microbiological characteristics of blueberry incorporated goat milk based high protein dessert

Parameter (log cfu/g)	Control	Optimized product
Total viable count	6.23±0.84[a]	6.28±0.28[a]
Coliform count	Nil	Nil
Yeast and mold	Nil	Nil
Lactobacillus count	5.95±0.25[b]	5.84 ±0.30[a]
Streptococcus count	5.77± 0.11[a]	6.30±1.14[b]

[ab]Values (Mean ± SD; n=9) with different superscripts in a row significantly different p<0.05

4.1.3.3 Sensory analysis of optimized blueberry incorporated goat milk based high protein dessert

Table 4.10: Sensory analysis of blueberry incorporated goat milk based high protein dessert

Sensory attributes		Control	OBD
Appearance	**Color**	1.76±0.25[a]	4.50±0.50[b]
Flavour	**Creamy**	3.6±0.61[a]	4.70±0.26[b]
	Goaty	0.83±0.76[b]	Nil
	Fermented	5.23±0.25[b]	5.06±0.11[a]
	Cooked	0.10±0.10[b]	Nil
	Fruity	Nil	4.83±0.28[b]
	Sweet	Nil	5.16±0.28[b]
	Acidic	6.70±0.25[b]	5.90±0.36[a]
	Bitter	Nil	0.03±0.05[b]
	Sour	6.83±0.28[b]	5.90±0.36[a]
	Astringent	Nil	2.90±0.36[b]
Body & Texture	**Thickness**	3.50±0.50[a]	6.16±1.04[b]
	Lumpiness	Nil	1.16±1.04[b]
	Ropiness	Nil	Nil
Overall acceptability		4.90 ± 0.36[a]	6.76± 0.75[b]

[ab]Values (Mean ± SD; n=9) with different superscripts in a row significantly different p<0.05

Table 4.10 represents sensory analysis of optimized product. Based on sensory analysis of optimized product and control, it was observed that, there was significant difference in the color of control and OBD which could be due to the addition of blueberry crush. Goaty flavor

was masked by the addition of blueberry crush. Sweetness and fruity flavor was significantly ($p<0.05$) higher in OBD, as the control sample was not added with blueberry crush and sugar. Thickness of the OBD was found to be higher which may be due to the addition of inulin. The overall acceptability score was 4.90 and 6.76 for control and OBD respectively.

4.2 DETERMINATION OF SHELF LIFE OF GOAT MILK BASED HIGH PROTEIN DESSERT

The optimized blueberry crush incorporated goat milk based high protein dessert was investigated for storage stability by following two different approaches: 1. Non-thermized product stability 2. Thermized product stability. The conditions adopted for thermization are described under section 3.4.2.1. The changes occurred during this investigation are herewith presented below.

4.2.1 Storage stability of non-thermized blueberry incorporated goat milk based high protein dessert

4.2.1.1 Physico-chemical characteristics of non-thermized dessert samples during refrigerated storage

The pH, acidity and color values of the optimized and control dessert samples were evaluated and results are presented in (Table 4.11). pH values of control and OBD were found to be 4.52 and 4.38, respectively. Due to the solubilisation of κ-casein, acidification of milk occurs, which leads to the destruction of the internal structure of casein mycelium (Cusmenco *et al.,* 2021). Cinbas and Yazici (2008) reported that the blueberry pulp added yoghurt showed lower pH than control owing to the lower pH of the blueberry pulp. Significant decrease in pH and increase in acidity values ($p<0.05$) were observed for both control and optimized dessert with progression in storage duration. Sugar addition in the optimized product might have helped in the growth of lactic acid bacteria during storage which could utilise lactose and produce more acid (Cinbas and Yazici, 2008; Amal *et al.,* 2016). Spoilage of dessert samples was observed on 14^{th} and 21^{st} day for control and optimized products, respectively. Further, color is one of the most important attributes of the dairy desserts that also determine sensory acceptability to some extent. The L^* and b^* values of optimized dessert samples were significantly lower ($p<0.05$) than control, while a^* values of optimized product was found to be significantly higher ($p<0.05$) than control. Similar findings were reported by Cinbas and Yazici (2008) such as decrease in the L^* value and b^* value while an increase in a^* value in blueberry added yoghurt owing to its purple red color of crush. All color attributes (L^*, a^* & b^*) of control and optimized desserts differed significantly ($p<0.05$) throughout the storage. In case of optimized product, it was observed that with increase in storage period, L^* and a^*

values were not affected significantly ($p>0.05$) whereas, *b** values increased significantly ($p<0.05$). Slight decrease in *L** value was observed during storage which could be attributed to intra-molecular, inter-molecular, co-pigmentation and self-association reaction resulting into pigment stabilization (Trigueros *et al.,* 2014). Baria *et al.* (2021) studied the color properties of black carrot anthocyanins in yoghurt and they also observed non -significant difference in *L** value, a decrease in *a** value and increase in *b** value indicating the decrease in blue color of the product owing to the pH reduction that led to the change in structure of the anthocyanin pigment.

Table 4.11 Effect of refrigerated storage on physico-chemical characteristics of non-thermized blueberry incorporated goat milk based high protein dessert

Parameters		Day 0	Day 7	Day 14	Day 21
pH	**Control**	4.52 ± 0.01^{bA}	4.39 ± 0.00^{aA}	Spoiled	
	OBD	4.38 ± 0.01^{cB}	4.27 ± 0.00^{bB}	3.92 ± 0.03^{a}	Spoiled
Acidity (% LA)	**Control**	1.23 ± 0.00^{aA}	1.50 ± 0.00^{bA}	Spoiled	
	OBD	1.44 ± 0.00^{aB}	1.71 ± 0.00^{aB}	1.98 ± 0.00^{b}	Spoiled
L*	**Control**	87.20 ± 0.07^{aA}	87.35 ± 0.08^{aA}	Spoiled	
	OBD	70.42 ± 0.05^{aB}	70.87 ± 0.15^{aB}	70.66 ± 0.05^{a}	Spoiled
a*	**Control**	-0.72 ± 0.31^{aA}	-1.33 ± 0.00^{bA}	Spoiled	
	OBD	3.55 ± 0.57^{aB}	3.38 ± 0.06^{aB}	3.31 ± 0.05^{a}	Spoiled
b*	**Control**	6.17 ± 0.06^{aA}	6.39 ± 0.08^{aA}	Spoiled	
	OBD	3.37 ± 0.18^{aB}	4.01 ± 0.06^{bB}	4.36 ± 0.09^{c}	Spoiled

* Means±SD from triplicate determinations; OBD: Optimized blueberry crush incorporated goat milk based high protein dessert.
^{abc}. Means with different superscript in row differ significantly ($p<0.05$).
^{AB}. Means with different superscript in column differ significantly ($p<0.05$).

4.2.1.2 Antioxidant properties of non- thermized dessert samples during refrigerated storage

Antioxidant properties (DPPH and FRAP), total phenolics and total flavonoids were studied for both control and optimized non-thermized dessert samples during storage (Table 4.12) and Figure 4.7 (A,B,C &D respectively). Blueberries are rich in antioxidants and polyphenols especially anthocyanins. DPPH, FRAP, total phenolics and total flavonoids were found to be significantly decreased ($p<0.05$) with storage. On Day 0, significantly higher ($p<0.05$) DPPH (169.85 µmol TE/g) and FRAP values (447.32 µmol TE/g) were recorded for optimized non-thermized dessert samples than control. FRAP values for both control and optimized dessert samples decreased significantly ($p<0.05$) with increase in storage period. Lower DPPH scavenging activity of 109.04 (µmol TE/g) was observed for optimized dessert samples on day 7 of storage, however it did not differ significantly ($p>0.05$) as compared to day 14. Total phenolic and flavonoid content of both dessert samples decreased significantly

($p<0.05$) with increase in storage period. The total flavonoid content (µg QE/g) of optimized dessert decreased significantly ($p<0.05$) from 2.96 to 1.38 from day 0 to 14, respectively. Significantly higher ($p<0.05$) phenolic content (µg GAE/g) of 143.64 was noticed for optimized non-thermized dessert as compared to control (98.84) on day 0 of storage. Dimitrellou *et al.,* 2020 reported significant difference in total phenolic content and DPPH activity of control and fruit added yoghurt and with storage, the total phenolic and DPPH content (antioxidant activity) was decreased due to the interaction of proteins and polyphenols. Compared to control, the optimized product has higher antioxidant, phenolic and flavonoids due to the presence of phytochemical compounds in fruit, thus indicating higher capacity to decrease the oxidative damage by inhibiting free radicals. The decrease in antioxidant activity (flavonoid, phenolics) in samples might have been due to the loss of anthocyanin activity and the interaction of milk and polyphenol reaction (Ibhaze *et al.,* 2022).

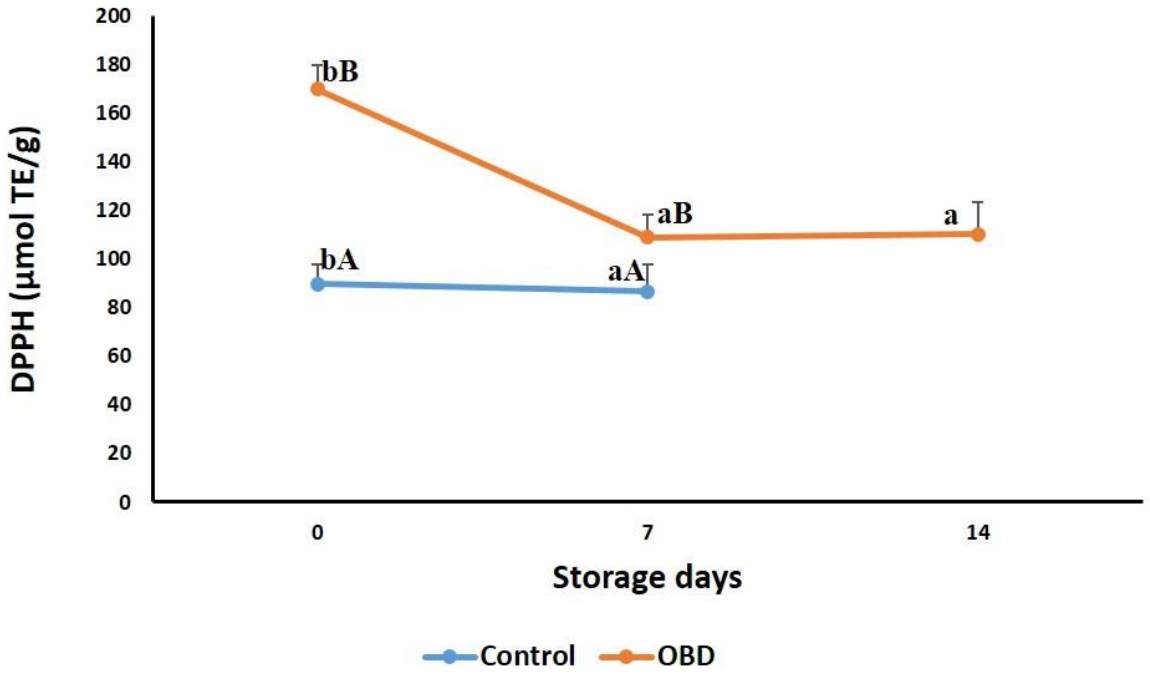
Table 4.12 Effect of refrigerated storage on antioxidant properties of non-thermized blueberry incorporated goat milk based high protein dessert

Samples	Day 0	Day 7	Day 14
DPPH (µmol TE/g)			
Control	89.84 ± 8.23^{bA}	86.6 ± 11.39^{aA}	Spoiled
OBD	169.85 ± 10.12^{bB}	109.04 ± 9.54^{aB}	110.21 ± 13.05^{a}
FRAP (µmol TE/g)			
Control	290.78 ± 25.32^{bA}	265.48 ± 33.02^{aA}	Spoiled
OBD	447.32 ± 36.89^{cB}	417.2 ± 49.23^{bB}	335.36 ± 35.22^{a}
Total Phenolics (µmol GAE/g)			
Control	98.84 ± 16.75^{bA}	95.84 ± 17.89^{aA}	Spoiled
OBD	143.64 ± 22.50^{cB}	97.24 ± 18.65^{bA}	87.84 ± 31.01^{a}
Total flavonoid (µg QE/g)			
Control	2.12 ± 0.01^{bA}	1.40 ± 0.09^{aA}	Spoiled
OBD	2.96 ± 0.06^{bB}	1.44 ± 0.04^{aA}	1.38 ± 0.25^{a}

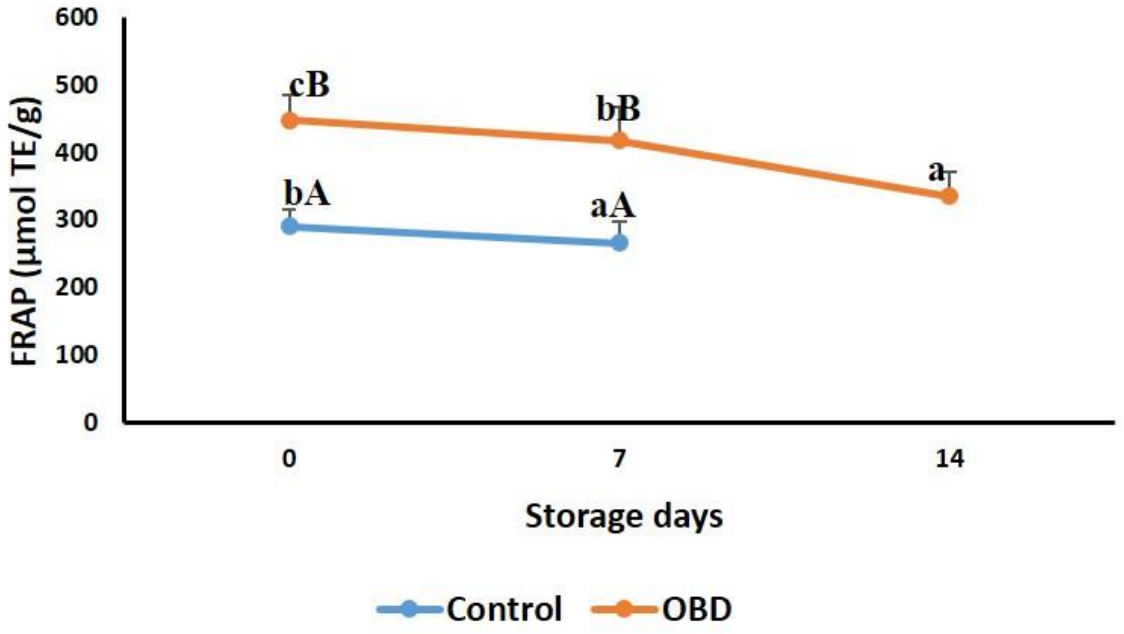
* Means±SD from triplicate determinations. OBD: Optimized blueberry crush incorporated goat milk based high protein dessert.

^{abc}Means with different superscript in row differ significantly ($p<0.05$).

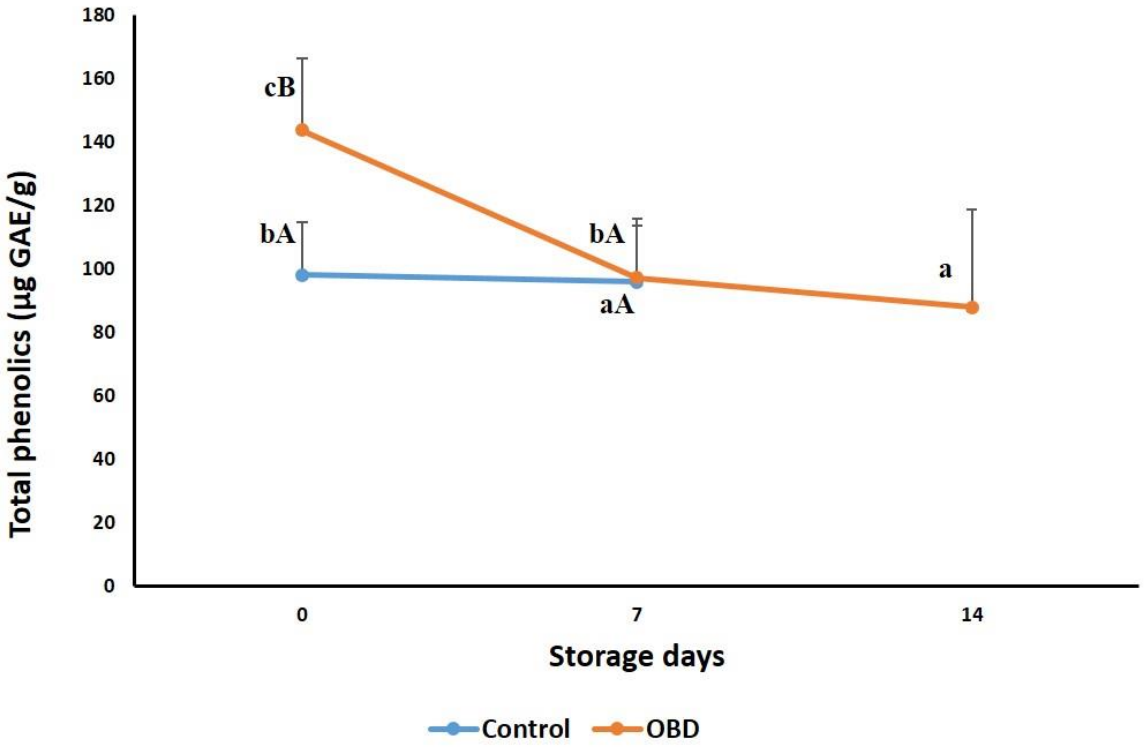
^{AB}Means with different superscript in column differ significantly ($p<0.05$).


DPPH (µmol TE/g)
200
180
160
140
120
100
80
60
40
20
0
bB
aB
a
bA
aA
0
7
14
Storage days
Control
OBD

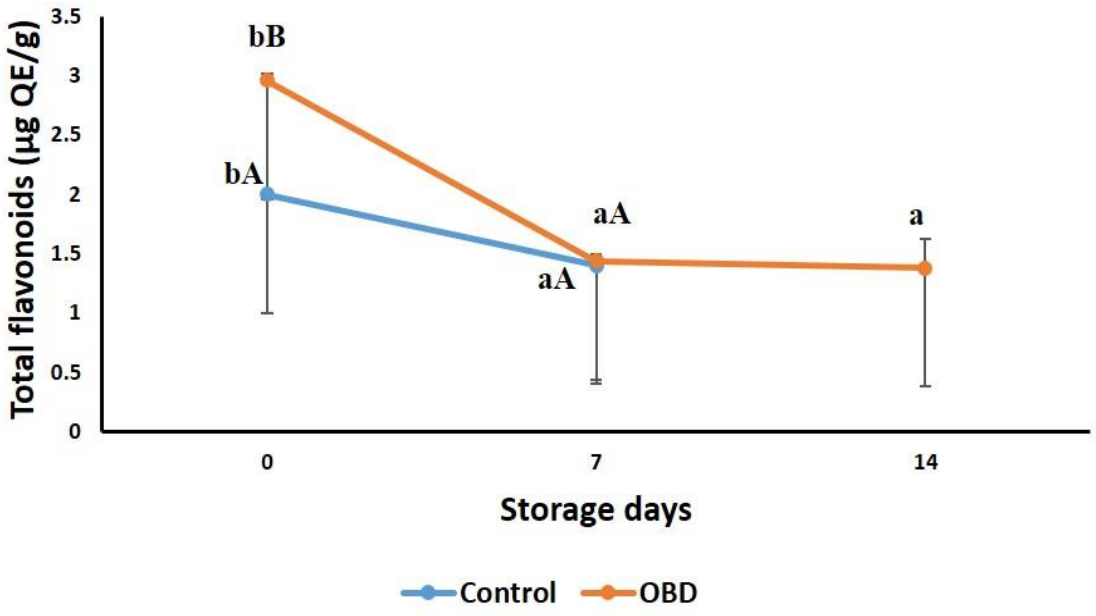
(A)


FRAP (µmol TE/g)
600
500
400
300
200
100
0
cB
bB
a
bA
aA
0
7
14
Storage days
Control
OBD

(B)



(C)



(D)

Figure 4.8 Antioxidant activities (A) DPPH (B) FRAP assay (C) Total phenolic content and (D) Total flavonoid content of non-thermized dessert samples during storage

4.2.1.3 Microbiological characteristics of non- thermized dessert samples during refrigerated storage

Microbiological characteristics of non-thermized control and optimized dessert samples have been presented in the Table 4.13. Total viable counts of control and OBD on 0 day were not significantly different ($p>0.05$) and it increased with storage period for both control and dessert sample however, the increase was found to be significantly different ($p<0.05$). Similar trend was observed by (Amal *et al.,* 2016). Total coliform count and yeast & mold count were found to be absent till 7th day in control sample and 14th day in OBD sample. *Lactobacillus* and *Streptococcus* counts of the OBD samples varied significantly ($p<0.05$) with the progress in storage period. Significantly higher ($p<0.05$) *Lactobacillus* count and lower *Streptococcus count* was observed in control sample than OBD. With progression of storage period, the *Lactobacillus* and *Streptococcus* count was significantly increased ($p<0.05$).

Table 4.13 Effect of refrigerated storage on microbiological characteristics of non-thermized blueberry incorporated goat milk based high protein dessert

Parameters (log cfu/g)	Sample	Day 0	Day 7	Day 14	Day 21
Total viable count	**Control**	6.23 ± 0.84^{aA}	6.54 ± 1.24^{bA}	Spoiled	–
	OBD	6.28 ± 0.28^{aA}	6.36 ± 0.36^{aB}	7.12 ± 1.11^{b}	Spoiled
Coliform count	**Control**	Nil	Nil	Spoiled	–
	OBD	Nil	Nil	Nil	Spoiled
Yeast & mold count	**Control**	Nil	Nil	Spoiled	–
	OBD	Nil	Nil	Nil	Spoiled
Lactobacillus count	**Control**	5.95 ± 0.25^{aA}	6.02 ± 0.56^{aA}	Spoiled	
	OBD	5.84 ± 0.30^{aB}	5.92 ± 1.32^{bB}	6.01 ± 0.65^{c}	Spoiled
Streptococcus Count	**Control**	5.77 ± 0.11^{aA}	5.97 ± 0.76^{bA}	Spoiled	
	OBD	6.30 ± 1.14^{aB}	6.54 ± 0.52^{bB}	6.70 ± 0.28^{c}	Spoiled

* Means±SD from triplicate determinations; OBD: Optimized blueberry crush incorporated goat milk based high protein dessert.
^{abc}. Means with different superscript in row differ significantly ($p<0.05$).
^{AB}. Means with different superscript in column differ significantly ($p<0.05$).

Dimitrellu *et al.,* 2020 reported that increase in *Streptococcus* count with storage could be due to the prebiotic effect produced by the higher phenolic content. Similar findings of *Lactobacillus and streptococcus* count was observed by Demirkol and Tarakci (2018) and the

decrease in the lactobacillus count than control might have been due to the phenolic compounds present in blueberry crush and developed acidity have preventive effect on the growth of *Lactobacillus*. And also the interactions between phenolic compounds and milk proteins or polysaccharides have been reported to effect the growth of microbes.

4.2.1.4 Rheological attributes of non- thermized dessert samples during refrigerated storage

Apparent viscosity of the control and optimized dessert samples were measured using rheometer during storage and are represented in Table 4.14 and Figure 4.8. The apparent viscosity of OBD (12.00 Pa.s) was significantly higher ($p<0.05$) than control (7.03 Pa.s). It was observed that apparent viscosity of both dessert and control samples were increased significantly ($p<0.05$) with increase in storage duration. Significantly higher apparent viscosity ($p<0.05$) of was recorded for OBD (18.46 Pa.s) than control on day 14 of storage. The recorded higher apparent viscosities of optimized product than control samples could be due to the addition of inulin, a prebiotic substance that helps to increase the viscosity and mouth-feel of food products (Chand *et al.,* 2021). In research study of Sengul *et al.* (2012) decreased viscosity was observed with fruit added yoghurt during storage. However, Costa *et al.* (2015) reported the increase in apparent viscosity of yoghurt with storage due to inulin addition.

Table 4.14: Effect of refrigerated storage on apparent viscosity of non-thermized blueberry incorporated goat milk based high protein dessert

Parameter	Sample	Day 0	Day 7	Day 14
Viscosity (Pa.s)	**Control**	7.03 ± 0.86^{aA}	13.00 ± 1.09^{bA}	Spoiled
	OBD	12.00 ± 2.62^{aB}	13.54 ± 0.36^{bA}	18.46 ± 1.00^{c}

* Means±SD from triplicate determinations; OBD: Optimized blueberry crush incorporated goat milk based high protein dessert.
^{abc}Means with different superscript in row differ significantly ($p<0.05$).
^{AB}Means with different superscript in column differ significantly ($p<0.05$)

Frequency dependency data of non-thermized control and OBD samples are presented graphically in Figure 4.9. Compared to control sample, OBD have higher G' and G" values. Storage modulus of both control and OBD samples during storage period were found to be higher than loss modulus indicating that elastic behavior is dominant. Generally, higher G' than G" of product indicates that it more or less likely to behaves as elastic or solid kind of material. On the other hand, higher G'' than G' showed the viscous property of the sample (Paseephol *et al.,* 2008; Suraweera *et al.,* 2020).

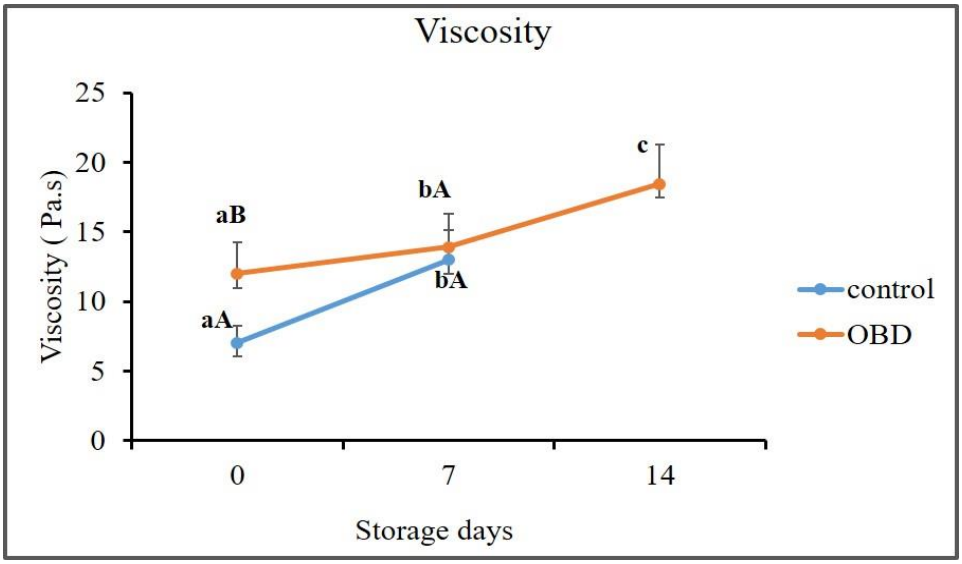


Figure 4.9: Apparent viscosity of non-thermized blueberry incorporated goat milk based high protein dessert

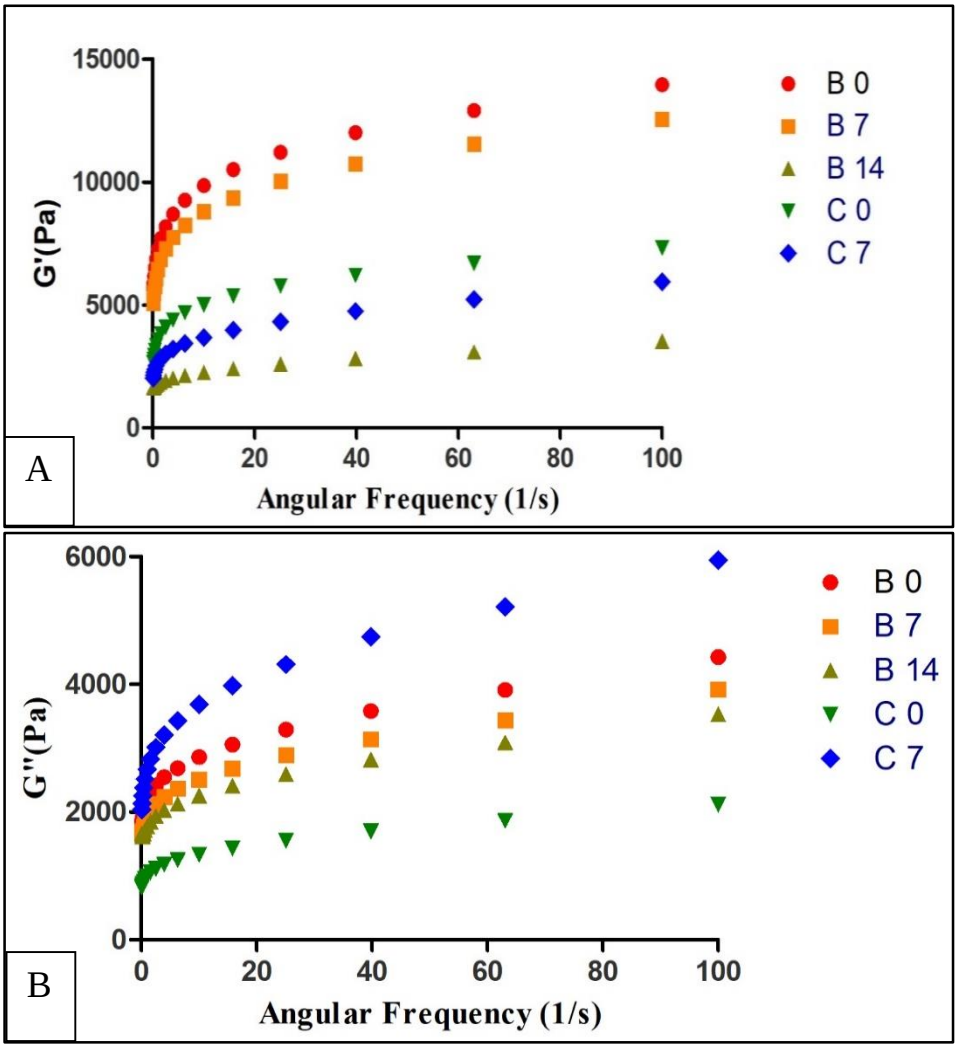


Figure 4.10: (A) Storage modulus and (B) loss modulus of non-thermized blueberry incorporated goat milk based high protein dessert

4.2.1.5 Sensory analysis of non- thermized dessert samples during refrigerated storage

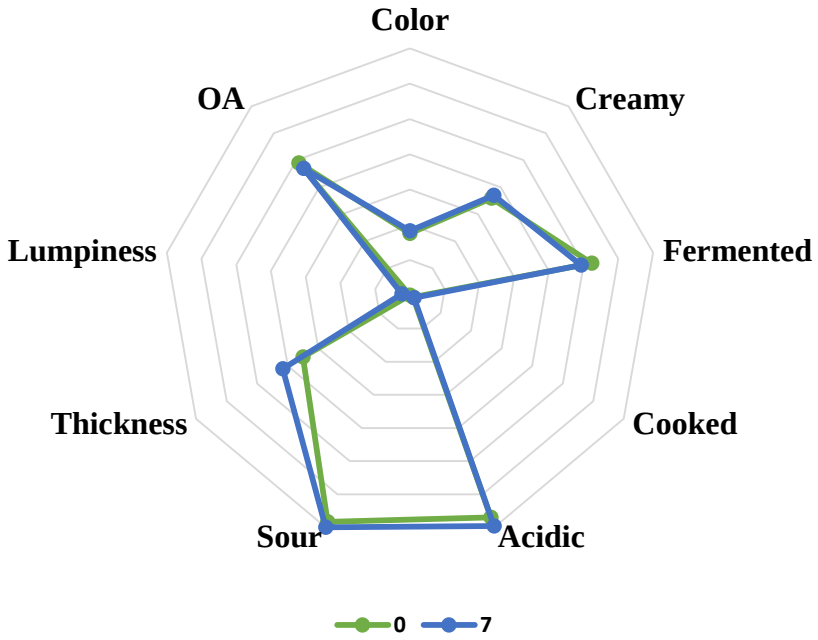
Table 4.15: Effect of refrigerated storage on sensory attributes of non-thermized blueberry incorporated goat milk based high protein dessert

Sensory attributes		Samples	Day 0	Day 7	Day 14
Appearance	**Color**	**Control**	1.76 ± 0.25^{aA}	1.83 ± 0.45^{aA}	Spoiled
		OBD	4.50 ± 0.50^{aB}	5.00 ± 0.20^{cB}	4.66 ± 0.28^{b}
Flavour	**Creamy**	**Control**	3.6 ± 0.61^{aA}	3.70 ± 0.26^{aA}	Spoiled
		OBD	4.70 ± 0.26^{aB}	4.83 ± 0.35^{bB}	4.80 ± 0.20^{b}
	Goaty	**Control**	0.83 ± 0.76^{a}	Nil	Spoiled
		OBD	Nil	Nil	Nil
	Fermented	**Control**	5.23 ± 0.25^{bA}	4.93 ± 0.30^{aA}	Spoiled
		OBD	5.06 ± 0.11^{aB}	5.03 ± 0.25^{aB}	5.20 ± 0.20^{b}
	Cooked	**Control**	0.10 ± 0.10^{aA}	0.13 ± 0.05^{aA}	Spoiled
		OBD	Nil	0.06 ± 0.11^{aB}	0.03 ± 0.05^{a}
	Fruity	**Control**	Nil	Nil	Spoiled
		OBD	4.83 ± 0.28^{b}	4.76 ± 0.25^{a}	4.70 ± 0.36^{a}
	Sweet	**Control**	Nil	Nil	Spoiled
		OBD	5.16 ± 0.28^{b}	4.76 ± 0.25^{a}	4.80 ± 0.20^{a}
	Acidic	**Control**	6.70 ± 0.25^{aA}	6.96 ± 0.05^{bA}	Spoiled
		OBD	5.90 ± 0.36^{bB}	5.63 ± 0.32^{aB}	5.86 ± 0.30^{a}
	Bitter	**Control**	Nil	Nil	Spoiled
		OBD	0.03 ± 0.05^{a}	0.06 ± 0.05^{a}	Nil
	Sour	**Control**	6.83 ± 0.28^{aA}	7.00 ± 0.20^{bA}	Spoiled
		OBD	5.90 ± 0.36^{aB}	5.43 ± 0.40^{aB}	5.76 ± 0.35^{a}
	Astringent	**Control**	Nil	Nil	Spoiled
		OBD	2.90 ± 0.36^{a}	2.83 ± 0.28^{a}	3.06 ± 0.23^{b}
Body & Texture	**Thickness**	**Control**	3.50 ± 0.50^{aA}	4.16 ± 0.28^{bA}	Spoiled
		OBD	6.16 ± 1.04^{aB}	6.76 ± 0.25^{bB}	6.50 ± 0.50^{b}
	Lumpiness	**Control**	Nil	0.23 ± 0.25^{aA}	Spoiled
		OBD	1.16 ± 1.04^{b}	1.50 ± 0.50^{bB}	0.83 ± 0.28^{a}
	Ropiness	**Control**	Nil	Nil	Spoiled
		OBD	Nil	Nil	Nil
Overall acceptability		**Control**	4.90 ± 0.36^{bA}	4.70 ± 0.28^{aA}	Spoiled
		OBD	6.76 ± 0.75^{bB}	5.90 ± 0.36^{aB}	5.43 ± 0.40^{a}

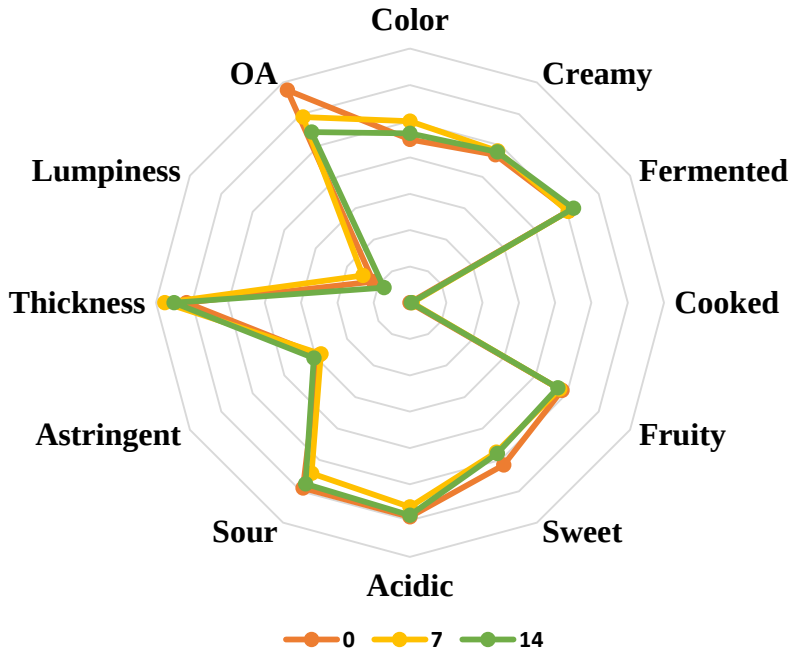
* Means±SD from triplicate determinations; OBD: Optimized blueberry crush incorporated goat milk based high protein dessert.

[abc]. Means with different superscript in row differ significantly ($p<0.05$).

[AB]. Means with different superscript in column differ significantly ($p<0.05$).



(A)



(B)

Figure 4.11: Sensory analysis (spider web diagram) of non-thermized (A) Control and (B) optimized blueberry incorporated goat milk based high protein dessert

Sensory evaluation was performed for both non-thermized control and optimized dessert samples during storage. All the sensory parameters of control and optimized dessert were significantly different ($p<0.05$) on day 0 of storage (Table 4.15). Sensory panel noticed marked difference between control and optimized product in terms of color due to the presence of blueberry crush in optimized product. Huang *et al.* (2012) reported that presence of anthocyanin compounds in blueberry are responsible for intense color visibility. Goaty flavour scores for optimized dessert were found to be zero which indicated that addition of blueberry crush masked the typical goaty flavour of caprine milk. The control dessert samples had shown higher acidic scores ($p<0.05$) than the optimized dessert samples and this could be due to the absence of fruit and sugar in control (Fig 4.10 A & B). It was also observed that addition of blueberry crush, sugar and inulin significantly enhanced ($p<0.05$) the sweetness, and thickness of the optimized dessert samples, respectively. The overall acceptability score of optimized non-thermized dessert samples decreased significantly ($p<0.05$) from 6.76 to 5.43 from day 0 to 14 of storage.

4.2.1.6 Electrophoretic pattern of (Sodium-dodecyl-sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) non- thermized dessert samples during refrigerated storage

Sodium dodecyl sulphate polyacrylamide electrophoresis (SDS-PAGE) is a technique used for separating the proteins based upon their ability to move within an electrical current, which is a function of the length of their polypeptide chains or of their molecular weight (Roy *et al.*, 2014). Non- thermized control and optimized dessert samples were analysed by the SDS-PAGE, for the characterization of protein and estimation of molecular weight. Figure 4.11 Gel bands of SDS-PAGE revealed the presence of protein in the range 10, 13 kDa (β-lactoglobulin and α-lactalbumin) and more bands in the range 29 to 77 kDa belonging mainly to casein protein fractions. Among these, the major fraction was β-casein and the minor was α-casein that can be seen between 29 kDa and 55 kDa at each sample lane. Similar findings of major casein fractions, β-lactoglobulin and α-lactalbumin was studied by (Azhar *et al.*, 2017). Oliveira *et al.* (2015) also observed that major caseins such as κ- casein and β-casein had no differences with and without the addition of fruit.

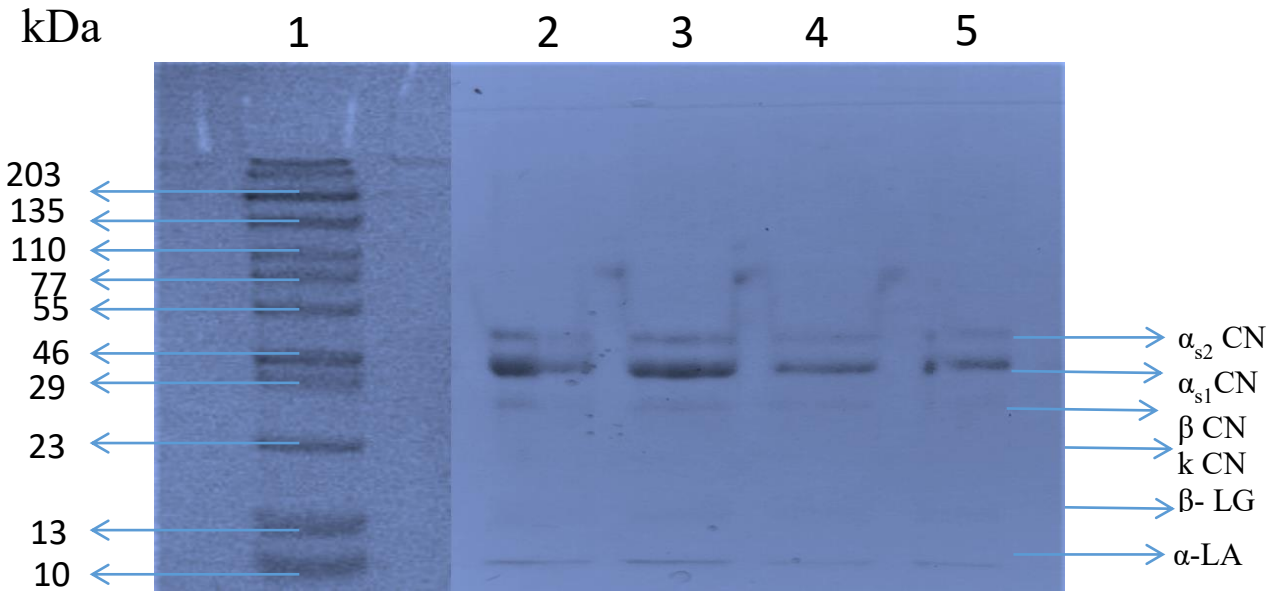


Figure 4.12: Electrophoretic pattern of non-thermized blueberry incorporated goat milk based high protein dessert. The samples from left to right are Standard protein marker (Lane-1), Control (Lane 2), Optimized product-Day 0 of storage (Lane 3), optimized product-Day 7 of storage (Lane -4), optimized product-Day 14 of storage (Lane 5).

4.2.2 Storage stability of thermized blueberry incorporated goat milk based high protein dessert

4.2.2.1 Physico-chemical characteristics of thermized dessert samples during refrigerated storage

Table 4.16 represents the changes in physico-chemical characteristics (pH, acidity and color) of thermized control and optimized samples under refrigerated storage conditions. Alakali *et al.* (2009) studied the physicochemical properties (pH and acidity) of thermized yoghurt wherein, the thermized sample pH and acidity values were 4.5 pH and 0.92% LA, respectively. It is worth mentioning that these findings were comparable with the results of the present study. At day 0, pH, acidity and color values of the control and OBD were significantly different ($p<0.05$). The significant difference in the pH and acidity values might have been due to dissociation of the hydrogen ion and change in equilibrium with heating (Alakali *et al.*, 2009) pH of control and OBD samples decreased ($p<0.05$) while acidity significantly increased ($p<0.05$) with progression in storage period. This could be attributed to the addition of blueberry crush which had the acidity of almost 3.2-3.5 %. Another reason for enhanced acidity of the products with storage duration is reported to be the formation of lactic acid owing to the utilization of lactose of goat skim milk (Sengul *et al.*, 2012). Further, color is an important attribute for the food products' acceptability by the consumer. The color changes in thermized goat milk based high protein dessert samples were measured as *L**, *a**, and *b** values. *L** and

*b** values of optimized samples were significantly lower ($p<0.05$) and *a** value was significantly higher ($p<0.05$) than control. *L** value represents the lightness of the product and the results showed that with the addition of blueberry, *L** and *a** value of optimized sample decreased ($p<0.05$) during storage as compared to the control sample however, *b** value showed significant increase ($p<0.05$) with storage in both control and optimized product. Our results corroborated with Song *et al.* (2018) who also reported that thermization temperature could destruct the anthocyanin due to the hydrolyzation of 3-glycoside structure and resulted in the decreased lightness of the product.

4.2.2.2 Antioxidant properties of thermized dessert samples during refrigerated storage

Antioxidant properties (DPPH and FRAP), total phenolics, total flavonoid content of thermized control and optimized dessert samples have been presented in Table 4.17 and Figure 4.12 A,B,C & D respectively. Results revealed that OBD sample showed higher ($p<0.05$) antioxidant properties, total phenolics and total flavonoid contents than control. Compounds having the antioxidant activity can scavenge or quench the DPPH free radicals by providing hydrogen atom or by donating electron thus, reducing stable DPPH radical to yellow coloured non-radical DPPH (Sharma *et al.*, 2021). In our study, DPPH activity showed significant decrease ($p<0.05$) for control and optimized products with increase in storage duration. Blueberries are rich source of phenolic compounds, particularly anthocyanins. Therefore, total phenol content of both the samples were also analyzed during the storage study and it was observed that the total phenolic content of optimized products was significantly higher ($p<0.05$) than the control throughout the storage period. Similarly, total flavonoid content was also significantly higher ($p<0.05$) for optimized products as compared to control owing to the addition of blueberry crush. Further, FRAP decreased significantly ($p<0.05$) in optimized product from day 0 (424.48) to day 42 (334.08) of storage due to protein-polyphenol interactions. However, it remained significantly higher ($p<0.05$) than control owing to higher anthocyanin content and other phenolic compounds. Reblova (2012) reported that, heating causes acceleration of initiation reactions and oxidation of antioxidants will reduce antioxidant activity. Furthermore, Oliveira *et al.* (2015) reported that the antioxidant activity of the flavonoid compounds rely on chemical structure such as number and arrangement of the hydroxylated groups and 3-hydroxy group in flavonoids are mainly responsible for antioxidant activity. Antioxidant rich components added into the milk could block the 3-hydroxyl group by proteins and this would significantly decrease the antioxidant activity of flavonoids.

Table 4.16 Effect of refrigerated storage on physico-chemical characteristics of thermized blueberry incorporated goat milk based high protein dessert

Parameter	Sample	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
pH	**Control**	4.50 ± 0.00^{dA}	4.38 ± 0.01^{cA}	4.24 ± 0.01^{bA}	4.20 ± 0.00^{bA}	4.17 ± 0.00^{bA}	4.03 ± 0.05^{aA}	3.96 ± 0.05^{aA}
	OBD	4.23 ± 0.00^{fB}	4.03 ± 0.05^{eB}	3.84 ± 0.01^{dB}	3.72 ± 0.03^{cB}	3.66 ± 0.02^{cB}	3.60 ± 0.01^{abcB}	3.58 ± 0.01^{aB}
Acidity (% LA)	**Control**	1.02 ± 0.05^{aA}	1.05 ± 0.05^{aA}	1.14 ± 0.05^{aA}	1.23 ± 0.05^{bA}	1.26 ± 0.09^{cA}	1.32 ± 0.05^{cA}	1.44 ± 0.09^{dA}
	OBD	1.44 ± 0.15^{aB}	1.59 ± 0.05^{abB}	1.74 ± 0.05^{bcB}	1.86 ± 0.05^{cB}	1.95 ± 0.05^{cdB}	2.10 ± 0.05^{dB}	2.34 ± 0.09^{eB}
L*	**Control**	86.61 ± 1.82^{aA}	85.20 ± 0.27^{cA}	83.08 ± 0.58^{bA}	81.70 ± 0.78^{bcA}	77.47 ± 0.23^{aA}	76.23 ± 0.90^{aA}	75.37 ± 1.01^{aA}
	OBD	70.79 ± 0.09^{dB}	70.75 ± 0.12^{dB}	70.20 ± 0.00^{cB}	69.72 ± 0.03^{bB}	69.68 ± 0.15^{bB}	69.56 ± 0.03^{bB}	69.26 ± 0.08^{aB}
a*	**Control**	-1.19 ± 0.06^{aA}	-1.11 ± 0.15^{abA}	-0.92 ± 0.10^{bcA}	-0.79 ± 0.03^{cdA}	-0.81 ± 0.11^{cdA}	-0.51 ± 0.05^{eA}	-0.57 ± 0.09^{deA}
	OBD	3.14 ± 0.06^{abB}	3.10 ± 0.02^{abB}	3.06 ± 0.07^{abB}	3.00 ± 0.02^{bB}	$2..94 \pm 0.02^{abB}$	2.81 ± 0.35^{bB}	2.77 ± 0.49^{aB}
b*	**Control**	6.17 ± 0.41^{aA}	6.33 ± 0.80^{bA}	6.54 ± 0.70^{bA}	7.24 ± 0.17^{cA}	7.72 ± 0.13^{dA}	8.20 ± 0.15^{eA}	8.73 ± 0.14^{eA}
	OBD	4.32 ± 0.05^{aA}	4.33 ± 0.14^{aB}	4.56 ± 0.17^{bB}	5.08 ± 0.57^{cB}	5.13 ± 0.06^{dB}	5.27 ± 0.01^{eB}	5.68 ± 0.26^{fB}

* Means±SD from triplicate determinations; OBD: Optimized blueberry crush incorporated goat milk based high protein dessert.

^{abc}. Means with different superscript in row differ significantly ($p<0.05$).

^{AB}. Means with different superscript in column differ significantly ($p<0.05$)

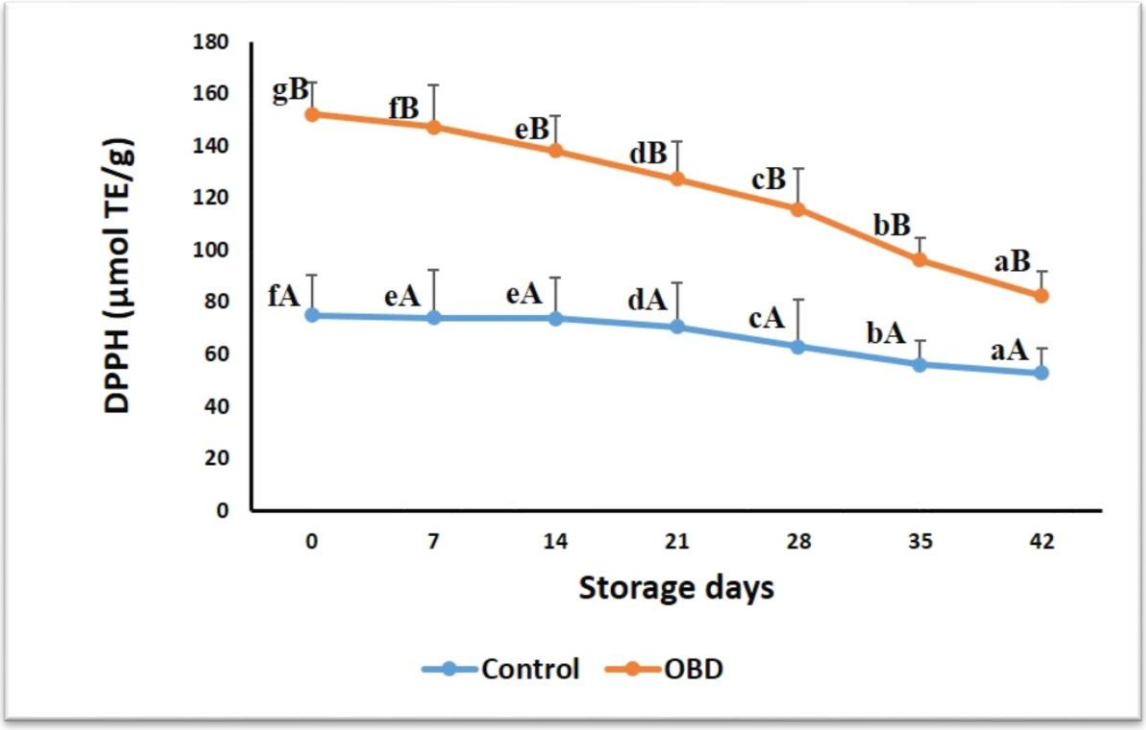
Table 4.17 Effect of refrigerated storage on antioxidant properties of thermized blueberry incorporated goat milk based high protein dessert

Parameter	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
DPPH (µmol TE/g)							
Control	74.82±15.62^{fA}	73.96±18.30^{eA}	73.62±15.75^{eA}	70.39 ±16.79^{dA}	62.85± 18.10^{cA}	55.96± 9.32^{bA}	52.64±9.56^{aA}
OBD	152.00±12.50^{gB}	147.04± 16.00^{fB}	138.04±13.5^{eB}	127.12±14.6^{dB}	115.56±15.9^{cB}	96.00±8.60^{bB}	82.4± 9.20^{aB}
Total phenolic content (µmol GAE/g)							
Control	85.64± 12.41^{fA}	84.96± 15.15^{eA}	84.59± 14.20^{eA}	81.16± 15.64^{dA}	75.04± 17.05^{cA}	68.84± 16.34^{bA}	61.92± 16.52^{aA}
OBD	124.12± 13.25^{eB}	122.16± 15.68^{eB}	119.96± 16.23^{eB}	112.40±17.65^{dB}	102.61± 14.39^{cB}	96.36± 15.05^{bB}	80.96± 15.05^{aB}
Total flavonoids (µmol QE/g)							
Control	2.01± 0.01^{eA}	1.98 ± 0.03^{eA}	1.76± 0.02^{dA}	1.60± 0.01^{cA}	1.52± 0.01^{cA}	1.38± 0.01^{bA}	1.18± 0.08^{aA}
OBD	2.96± 0.02^{gB}	2.74± 0.06^{fB}	2.46± 0.02^{eB}	2.26± 0.00^{dB}	2.02± 0.03^{cB}	1.96± 0.06^{bB}	1.68± 0.02^{aA}
FRAP (µmol TE/g)							
Control	248.96 ±21.70^{fA}	239.04± 25.60^{eA}	232.48±36.9^{eA}	214.24± 33.60^{dA}	205.36± 29.70^{cA}	197.32± 37.5^{bA}	174.32± 35.23^{aA}
OBD	424.48 ±19.34^{fB}	421.88±27.09^{eB}	419.00±24.77^{eB}	410.84±35.45^{dB}	401.36±28.90^{cB}	392.68±33.26^{bB}	334.08±46.13^{aB}

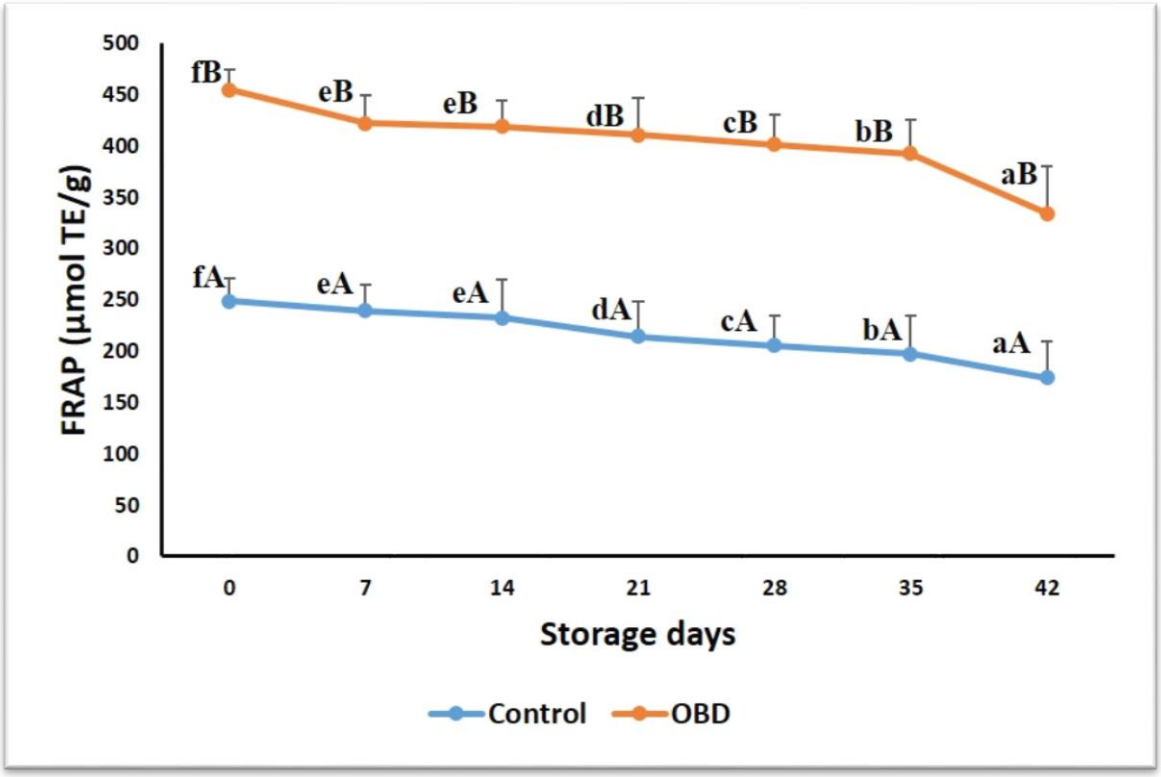
* Means±SD from triplicate determinations; OBD: Optimized blueberry crush incorporated goat milk based high protein dessert

^{abc}. Means with different superscript in row differ significantly ($p<0.05$).

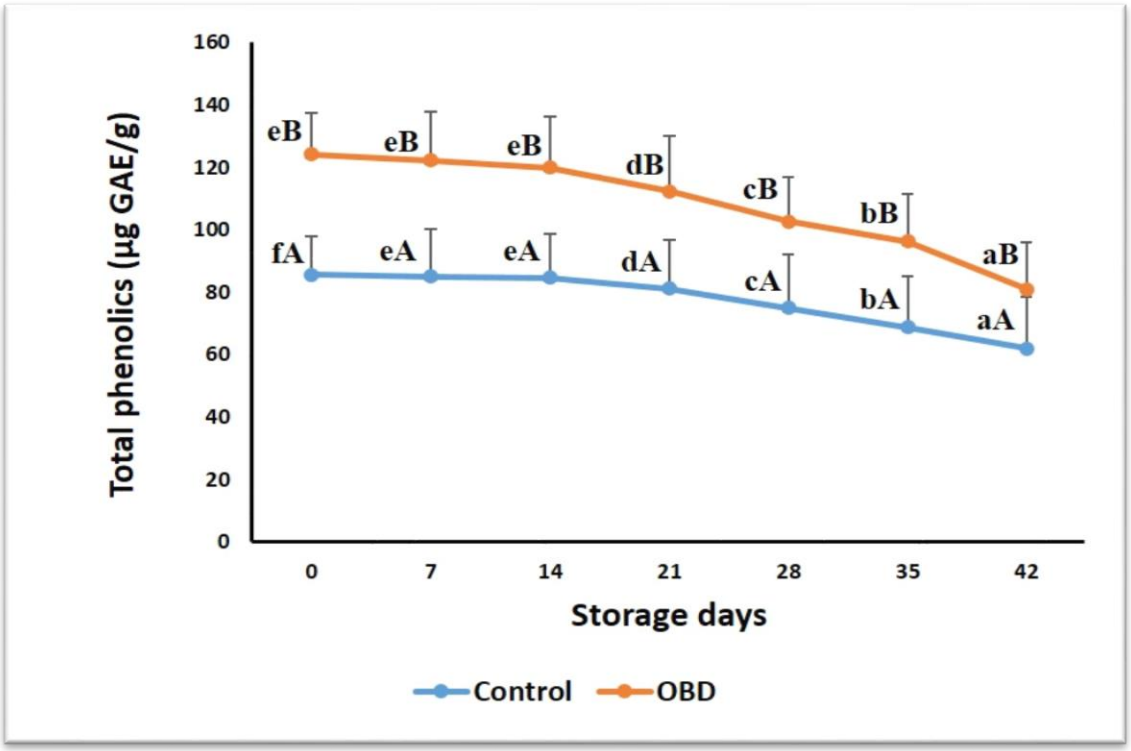
^{AB}. Means with different superscript in column differ significantly ($p<0.05$).


DPPH (µmol TE/g)
180
160
140
120
100
80
60
40
20
0
gB
fB
eB
dB
cB
bB
aB
fA
eA
eA
dA
cA
bA
aA
0
7
14
21
28
35
42
Storage days
Control
OBD

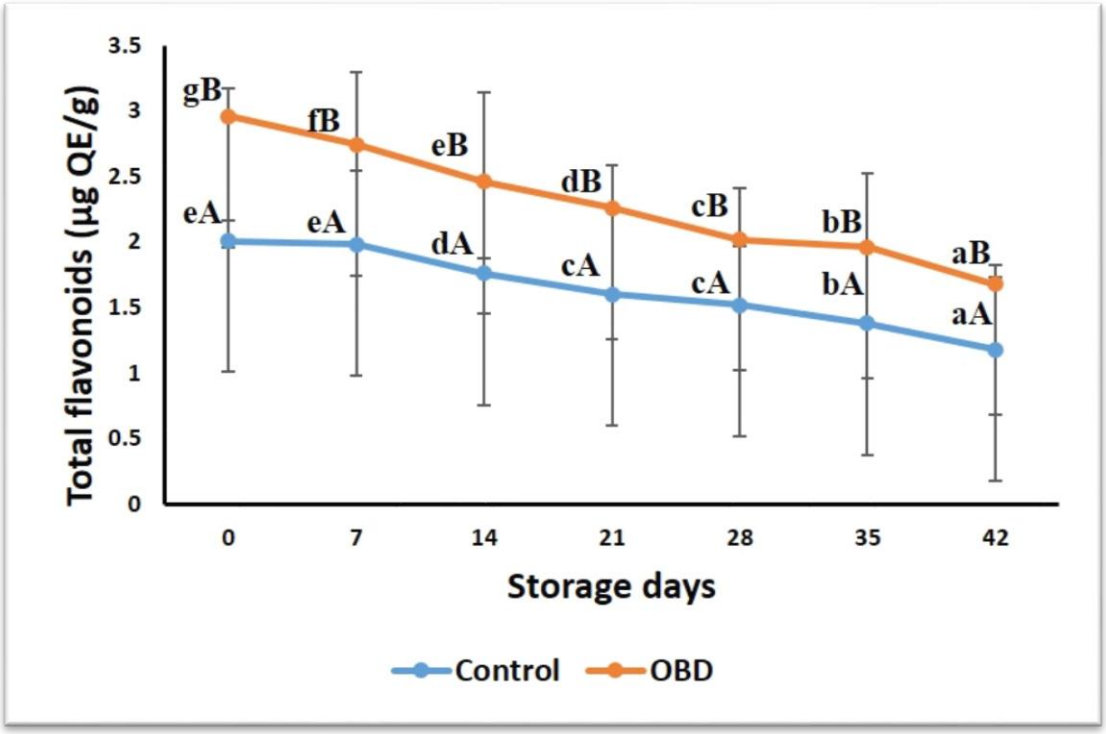
(A)


FRAP (µmol TE/g)
500
450
400
350
300
250
200
150
100
50
0
fB
eB
eB
dB
cB
bB
aB
fA
eA
eA
dA
cA
bA
aA
0
7
14
21
28
35
42
Storage days
Control
OBD

(B)



(C)



(D)

Figure 4.13: Antioxidant activities (A) DPPH (B) FRAP assay (C) Total phenolic content and (D) Total flavonoid content of thermized dessert samples during storage

4.2.2.3 Microbiological characteristics of thermized dessert samples during refrigerated storage

Microbiological characteristics are important in determining the quality of the product. Different microbiological characteristics of thermized control and optimized dessert samples have been depicted in the Table 4.18. Results revealed that viability of microorganisms increased with storage period. The total viable count of both control and optimized products significantly increased ($p<0.05$) with increase in storage duration. Coliforms were found to be absent till 28th day for control and 21st day for OBD. Further, yeast and mold count were observed on day 28th day of storage for both control and OBD. Alakali *et al.* (2008) reported decrease in the microbial load with thermization. *Lactobacillus* and *Streptococcus* counts were significantly ($p<0.05$) increased with storage Thus, it could be interpreted that there was gradual increase of these bacteria during storage. Mixed starter culture comprising of *Lactobacillus spp.* and *Streptococcus thermophilus* was successfully used in goat milk fermentation with high viability (Ranadheera *et al.*, 2012). Decrease in the total viability count in fruit yogurt than control was also observed by (Sarkar, 2019). In addition to this, they also reported decrease in Lactobacillus count with fruit addition which could be due to inhibitory effect on bacterial growth. Lower *Lactobacillus* count than *Streptococcus* might have been found due to the greater sensitivity of the former to oxygen, whereas *Streptococcus* overcome the stress caused by dissolved oxygen (Oleiviera *et al.*, 2015)

4.2.2.4 Rheological characteristics of thermized dessert samples during refrigerated storage

Effect of refrigerated storage on apparent viscosity of control and optimized goat milk based high protein dessert was recorded in the Table 4.19 and Figure 4.13. The apparent viscosity of optimized sample was higher ($p<0.05$) than control and it was observed that during storage, it was increased from day 0 **(**9.57±0.51) to day 21 (17.28±0.51). Further it decreased significantly ($p<0.05$) with storage period. Due to the action of lactic acid bacteria on casein micelle, there might have been a decrease in viscosity of the product during refrigerated storage, however, addition of inulin in the optimized product might have contributed to the enhanced viscosity of the OBD. Costa *et al.* (2015) observed that separation of whey might have resulted in the decreased viscosity in both control and treatment group. Reduction in apparent viscosity might also have been be due to action of bacterial enzymes on casein micelle matrix (Suraweera and Wichchukit, 2020)

Table 4.18 Effect of refrigerated storage on microbiological characteristics of thermized blueberry incorporated goat milk based high protein dessert

Parameter (log cfu/g)	Sample	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Total viable count	**Control**	5.76 ± 1.18^{aA}	5.85 ± 0.25^{bA}	5.96 ± 0.72^{bA}	6.11 ± 0.36^{bA}	6.28 ± 0.11^{cA}	TNTC	TNTC
	OBD	5.63 ± 0.50^{aA}	5.81 ± 0.24^{aB}	5.89 ± 0.45^{bA}	5.98 ± 0.36^{bA}	6.14 ± 0.28^{bB}	TNTC	TNTC
Coliform count	**Control**	Nil	Nil	Nil	Nil	Nil	1.25 ± 1.11^{aA}	1.18 ± 1.02^{aA}
	OBD	Nil	Nil	Nil	Nil	2.84 ± 0.26^{a}	3.90 ± 1.05^{bB}	4.56 ± 1.14^{cB}
Yeast& mold count	**Control**	Nil	Nil	Nil	Nil	2.24 ± 0.21^{aA}	3.30 ± 1.44^{bA}	4.18 ± 0.38^{cA}
	OBD	Nil	Nil	Nil	Nil	2.28 ± 0.26^{aB}	3.11 ± 1.05^{bB}	4.23 ± 1.14^{cB}
Lactobacillus count	**Control**	4.89 ± 0.28^{aA}	4.92 ± 0.75^{bA}	4.96 ± 0.25^{bA}	5.02 ± 1.11^{bA}	5.28 ± 0.32^{cA}	5.42 ± 0.28^{dA}	5.59 ± 0.50^{eA}
	OBD	4.59 ± 1.21^{aB}	4.72 ± 0.02^{bB}	4.84 ± 0.68^{cB}	4.92 ± 0.28^{dB}	5.09 ± 0.40^{dB}	5.16 ± 1.18^{eB}	5.22 ± 0.56^{fB}
Streptococcus count	**Control**	5.31 ± 0.17^{aA}	5.50 ± 0.64^{bA}	5.62 ± 0.41^{cA}	5.81 ± 0.35^{dA}	5.85 ± 0.57^{dA}	5.94 ± 1.05^{eA}	6.23 ± 0.76^{fA}
	OBD	5.52 ± 0.50^{aB}	5.75 ± 0.25^{bA}	5.92 ± 1.18^{cB}	6.12 ± 0.86^{dB}	6.26 ± 1.25^{eB}	6.39 ± 0.36^{fB}	6.55 ± 0.28^{gB}

* Means±SD from triplicate determinations; OBD: Optimized blueberry crush incorporated goat milk based high protein dessert.

^{abc}. Means with different superscript in row differ significantly ($p<0.05$).

^{AB}. Means with different superscript in column differ significantly ($p<0.05$).

Table 4.19 Effect of refrigerated storage on rheological characteristics of thermized blueberry incorporated goat milk based high protein dessert

Parameter	Sample	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Viscosity (Pa.s)	**Control**	7.08 ± 0.15^{aA}	13.20 ± 9.12^{dA}	14.13 ± 3.40^{dA}	13.73 ± 0.70^{dA}	11.55 ± 3.03^{cA}	10.20 ± 4.50^{bA}	10.03 ± 2.81^{aA}
	OBD	9.57 ± 0.51^{aB}	14.43 ± 1.15^{cB}	17.10 ± 0.93^{dB}	17.28 ± 0.51^{dB}	15.06 ± 2.85^{dB}	10.67 ± 1.04^{bA}	9.29 ± 1.93^{aB}

* Means±SD from triplicate determinations; OBD: Optimized blueberry crush incorporated goat milk based high protein dessert.
^{abc}. Means with different superscript in row differ significantly ($p<0.05$).
^{AB}. Means with different superscript in column differ significantly ($p<0.05$).

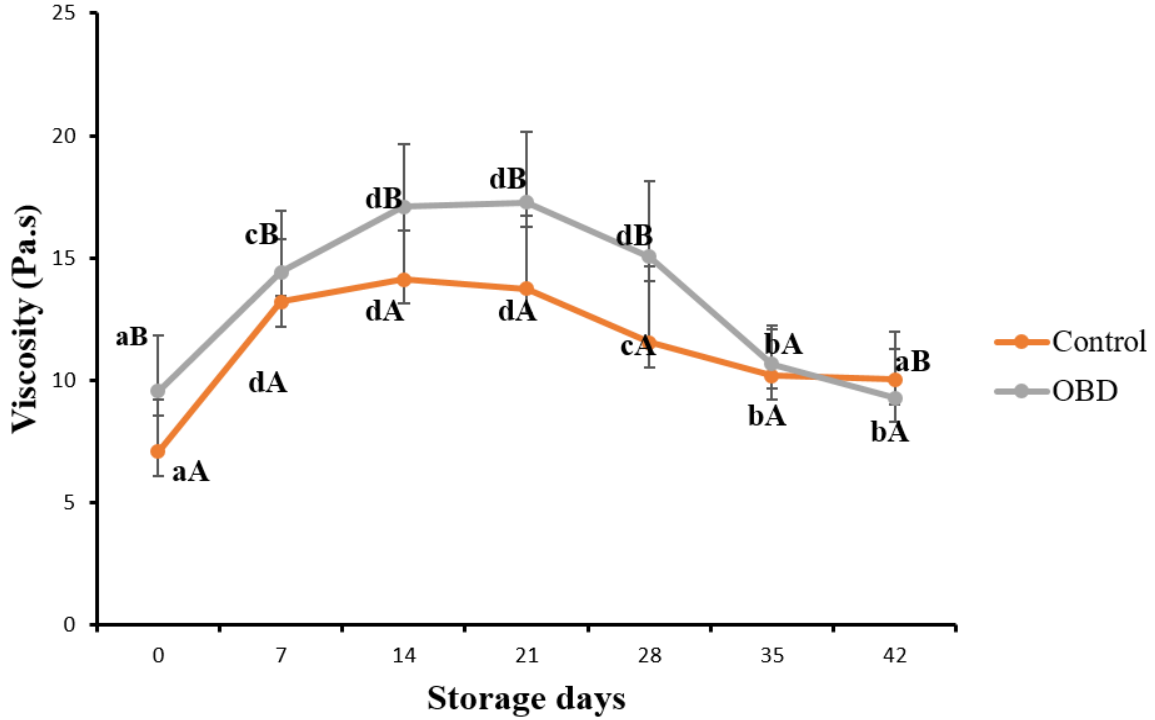


Figure 4.14: Apparent viscosity of thermized control and optimized blueberry incorporated goat milk based high protein dessert

Figure 4.14 represents the Storage modulus (G') and Loss modulus (G") of thermized blueberry dessert and control sample during storage. From the graph, the results revealed that G' was higher than G" which indicates the elasticity of the sample. Generally, higher G' than G" of product indicates that it is more or less likely to behave as elastic or solid kind of material. On the other hand, higher G'' than G' also revealed the viscous property of the sample (Paseephol *et al.*, 2008; Suraweera *et al.*, 2020).

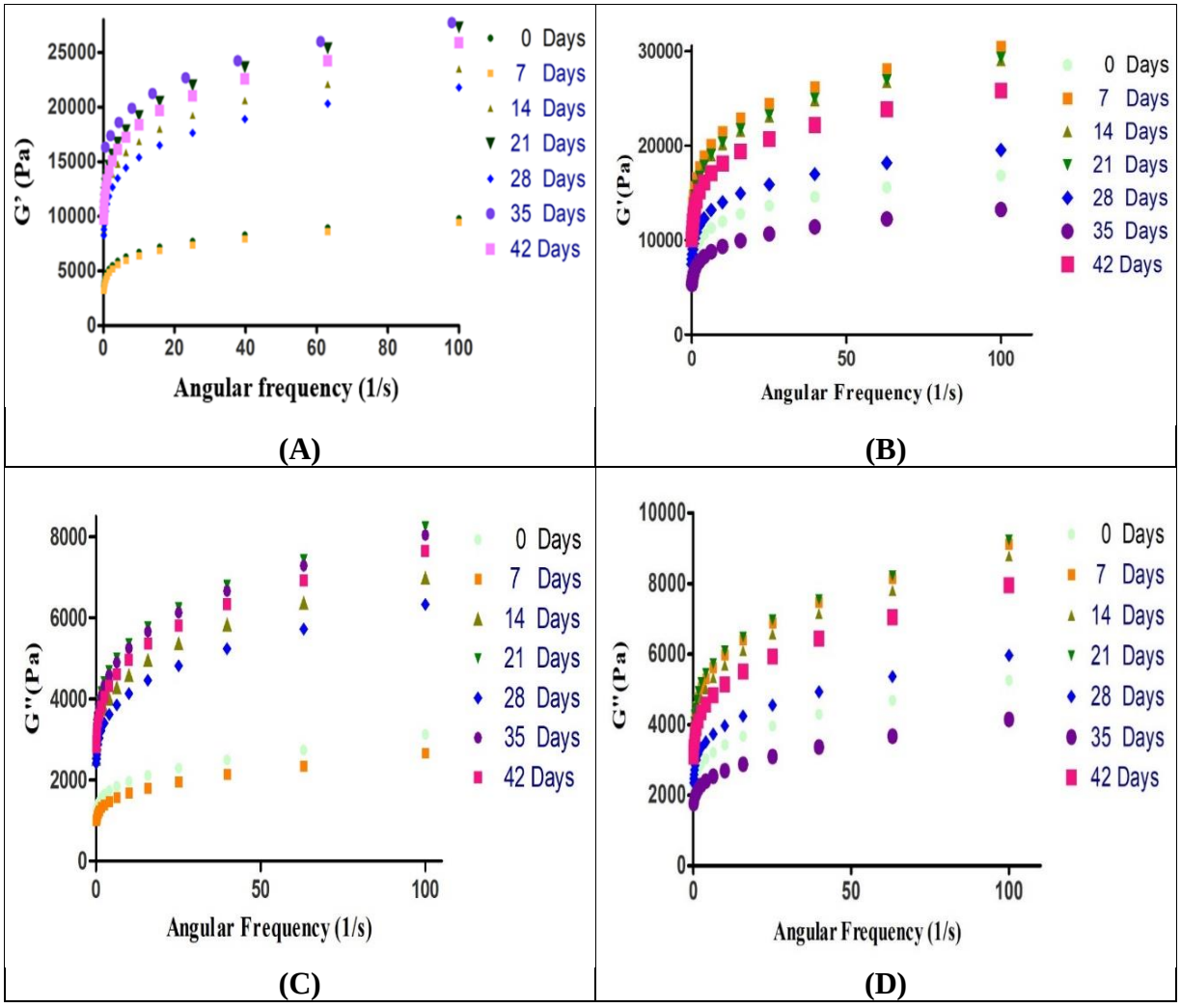
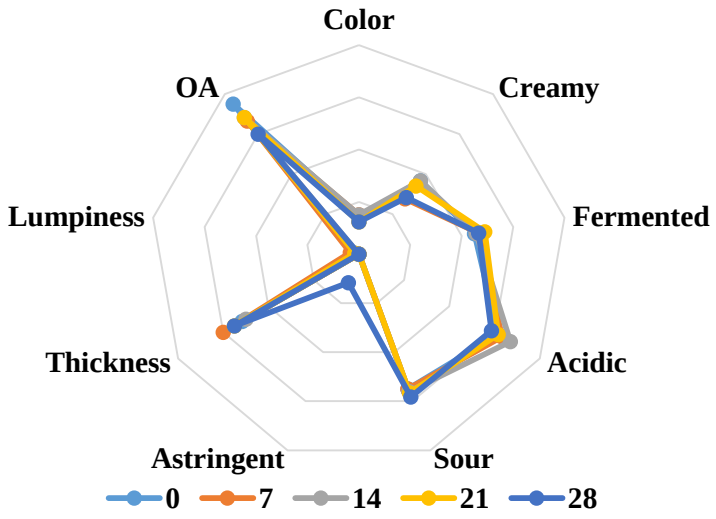


Figure 4.15: Storage modulus and loss modulus of control (A & C, respectively) and optimized (B & D, respectively) thermized blueberry incorporated goat milk based high protein dessert

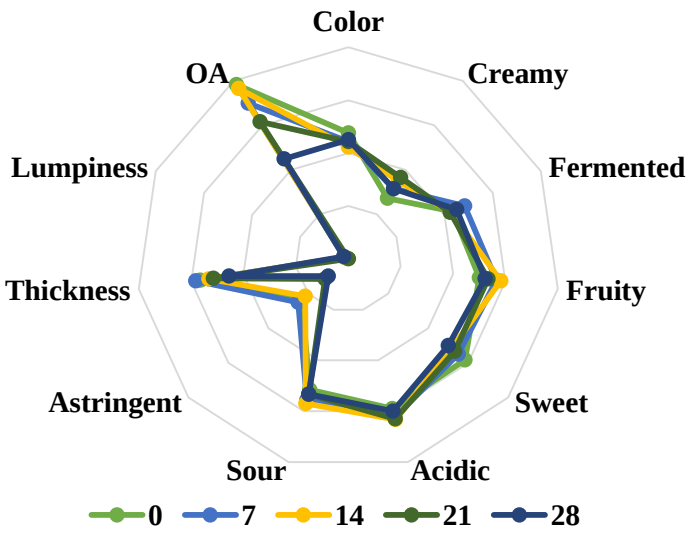
4.2.2.5 Sensory analysis of thermized dessert samples during refrigerated storage

Sensory evaluation of both control and optimized products was carried out and the results revealed that the optimized product had highest score in appearance ($p<0.05$) owing to the addition of blueberry crush. Color showed significant difference ($p<0.05$) between control and optimized product, while there was no significant difference ($p>0.05$) with increase in

storage days. Regarding flavour, control sample was observed with goaty flavour during initial days of storage however, it was decreased with increase in storage period. Optimized goat milk based high protein dessert was not reported with any goaty flavour. Further, fruity flavour and sweetness had also been significantly enhanced with blueberry addition ($p<0.05$). Moreover, fruit addition also increased the astringency of the optimized product. Thickness of optimized was significantly higher ($p<0.05$) as compared to control sample which might be due to inulin addition however, it was decreased in both the samples with increase in storage period. Further, the overall acceptability of both control and optimized products also decreased ($p<0.05$) with storage.



(A)



(B)

Figure 4.16: Sensory analysis (spider web diagram) of thermized (A) Control and (B) optimized blueberry incorporated goat milk based high protein dessert

Table 4.20 Effect of refrigerated storage on sensory attributes of thermized blueberry incorporated goat milk based high protein dessert

Parameter		Sample	0	7	14	21	28
appearance	**Color**	**Control**	1.40±0.52^{aA}	1.5±0.50^{aA}	1.50±1.32^{aA}	1.23±0.25^{aA}	1.23±0.75^{aA}
		BD 80:20	4.76±0.25^{aB}	4.43±0.58^{aB}	4.23±0.25^{aB}	4.43±0.66^{aB}	4.50±0.30^{aB}
Flavour	**Creamy**	**Control**	3.66±0.28^{aA}	2.76±0.75^{aA}	3.66±0.28^{aA}	3.40±0.17^{aA}	2.83±0.28^{aA}
		BD 80:20	2.73±0.64^{aA}	3.16±0.76^{aA}	3.50±0.30^{aA}	3.66±0.41^{aA}	3.16±0.35^{aA}
	Goaty	**Control**	0.50±0.50^{a}	0.16±0.28^{a}	Nil	Nil	Nil
		BD 80:20	Nil	Nil	Nil	Nil	Nil
	Fermented	**Control**	4.50±0.50^{aA}	4.66±0.57^{aA}	4.56±0.40^{aA}	4.90±0.36^{aA}	4.66±0.28^{aA}
		BD 80:20	4.33±0.28^{aA}	4.83±0.28^{aA}	4.26±0.46^{aA}	4.23±0.25^{aB}	4.50±0.50^{aA}
	Cooked	**Control**	0.10±0.17^{aA}	0.10±0.17^{a}	0.10±0.10^{a}	Nil	Nil
		BD 80:20	0.03±0.28^{aA}	Nil	Nil	Nil	Nil
	Fruity	**Control**	Nil	Nil	Nil	Nil	Nil
		BD 80:20	5.00±0.50^{a}	5.66±0.28^{a}	5.83±0.28^{a}	5.33±0.28^{a}	5.23±0.25^{a}
	Sweet	**Control**	Nil	Nil	Nil	Nil	Nil
		BD 80:20	5.83±0.28^{a}	5.50±0.50^{a}	5.16±0.28^{a}	5.33±0.28^{a}	5.00±0.00^{a}
	Acidic	**Control**	6.16±0.28^{aA}	6.33±0.41^{aA}	6.70±0.17^{aA}	6.16±0.57^{aA}	5.86±0.11^{aA}
		BD 80:20	5.90±0.36^{aA}	6.23±0.25^{aA}	6.33±0.28^{aA}	6.30±0.17^{aA}	6.00±0.20^{aA}
	Bitter	**Control**	Nil	Nil	Nil	Nil	Nil
		BD 80:20	Nil	Nil	Nil	Nil	Nil
	Sour	**Control**	5.50±0.50^{aA}	5.50±0.50^{aA}	5.60±0.17^{aA}	5.66±0.28^{aA}	5.83±0.28^{aA}
		BD 80:20	5.16±0.28^{aA}	5.56±0.60^{aA}	5.70±0.17^{aA}	5.33±0.40^{aA}	5.33±0.28^{aA}
	Astringent	**Control**	Nil	Nil	Nil	Nil	1.16±0.28^{aA}
		BD 80:20	2.33±0.57^{a}	2.50±0.86^{a}	2.16±0.28^{a}	1.16±0.28^{a}	1.00±0.86^{aA}
Body & Texture	**Thickness**	**Control**	5.16±1.25^{aA}	6.00±0.50^{aA}	5.00±0.50^{aA}	5.50±0.50^{aA}	5.50±0.50^{aA}
		BD 80:20	5.66±0.76^{aA}	5.83±0.76^{aA}	5.33±0.41^{aA}	5.16±0.28^{aA}	4.56±0.40^{aB}
	Lumpiness	**Control**	0.16±0.28^{aA}	0.33±0.57^{aA}	Nil	0.16±0.28^{a}	Nil
		BD 80:20	0.10±0.17^{aA}	0.16±0.28^{aA}	0.16±0.28^{a}	Nil	0.20±0.26^{a}
	Ropiness	**Control**	Nil	Nil	Nil	Nil	Nil
		BD 80:20	Nil	Nil	Nil	Nil	Nil
Overall acceptability		**Control**	7.50±0.50^{aA}	6.66±0.76^{aA}	6.83±0.28^{aA}	6.83±0.76^{aA}	6.00±0.50^{aA}
		BD 80:20	7.83±0.28^{cA}	7.00±0.50^{cA}	7.66±0.57^{cA}	6.16±0.28^{bA}	4.50±0.50^{aB}

* Means±SD from triplicate determinations.

^{abc}. Means with different superscript in row differ significantly ($p<0.05$).

^{AB}. Means with different superscript in column differ significantly ($p<0.05$).

4.2.2.6 Electrophoretic pattern of (Sodium-dodecyl-sulfate-polyacrylamide gel electrophoresis, SDS-PAGE) thermized dessert samples during refrigerated storage

Sodium dodecyl sulphate polyacrylamide electrophoresis (SDS-PAGE) is a technique used for separating the proteins based upon their ability to move within an electrical current, which is a function of the length of their polypeptide chains or of their molecular weight (Roy *et al.*, 2014). Thermized control and optimized dessert samples were analysed by the SDS-PAGE for the characterization of protein and estimation of molecular weight. It was observed from figure 4.16, the gel bands of SDS-PAGE for thermized sample that showed the presence of protein in the range 10, 13 kDa and more bands in the range 29 to 77 kDa. Three major band which present were α-casein, β-casein and κ-casein. The lower two minor band 10-13 kDa were identified as β-lactoglobulin and α-lactalbumin. The major fraction is β-casein and the minor is α-casein that can be seen between 29 kDa and 55 kDa at each sample lane. Similar findings of major casein fractions, β-lactoglobulin and α-lactalbumin was studied by (Azhar *et al.*, 2017). Oliveira *et al.* (2015) observed that major caseins such as κ- casein and β-casein had no differences with and without the addition of fruit.

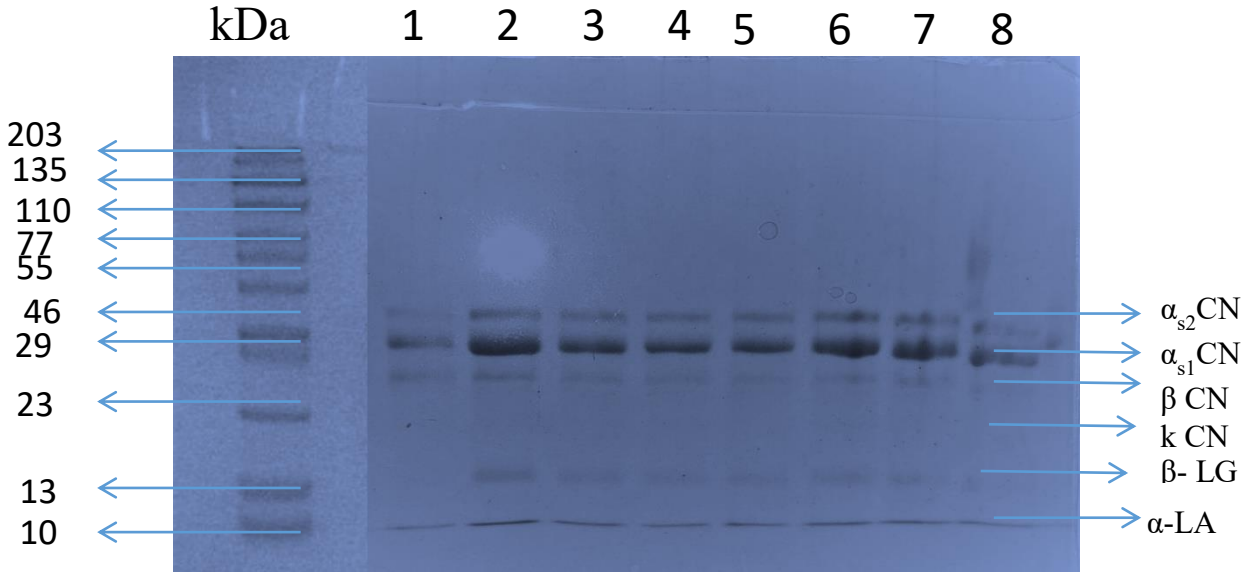


Figure 4.17 Electrophoretic pattern of thermized blueberry incorporated goat milk based high protein dessert.

(The samples from left to right are Standard protein marker (Lane-1), Control (Lane 2), Optimized product-Day 0 of storage (Lane 3), optimized product-Day 7 of storage (Lane -4), optimized product-Day 14 of storage (Lane 5), optimized product-Day 21 of storage (Lane 6), optimized product-Day 28 of storage (Lane 7), optimized product-Day 35 of storage (Lane 8), optimized product-Day 42 of storage (Lane 9).

CHAPTER-5

Summary and Conclusion

SUMMARY AND CONCLUSION

The summary of the present investigation of **Technology of development of goat milk based high protein dessert** and the conclusion derived are presented in this chapter.

5.1 OPTIMIZATION OF PROCESSING CONDITIONS AND FORMULATION FOR BLUEBERRY INCORPORATED GOAT MILK BASED HIGH PROTEIN DESSERT

5.1.1 Process optimization of blueberry incorporated goat milk based high protein dessert

5.1.1.1 Effect of Heating (90⁰ C/20 min and 30 min) on physicochemical and rheological properties of goat skim milk

- With increase in duration of heat treatment (at 90ºC) i.e., from 20 to 30 minutes, there was a significant ($p<0.05$) increase in acidity and total solids.
- Slight increase in protein content ($p>0.05$) of the GSM samples was recorded with increase in duration of heat treatment.
- Apparent viscosity of the GSM heated at 90ºC for 20 and 30 minutes showed decrease in viscosity with increase in shear rate while, GSM heated at 90ºC for 30 minutes showed higher viscosity.

5.1.1.2 Effect of ultrafiltration on physicochemical and rheological properties of goat skim milk retentate

- Goat skim milk subjected to ultrafiltration of 3 different volume concentrations (1X, 2X and 3X) were analyzed.
- pH of GSM retentates were found to be decreased and acidity was increased with increase in concentration.
- Total solids and protein significantly increased ($p<0.05$) with the increase in the GSM concentration.
- The apparent viscosity of GSM retentates also decreased with increase in shear rate and the maximum apparent viscosity was observed in GSM retentate of 3X concentration.

5.1.1.3 Physicochemical analysis of whey and protein curd mass obtained by fermentation of goat skim milk retentate

- The higher acidity (0.19% LA) and lactose (5.08%) were observed in 3X whey samples.
- The higher pH (4.53) and lower acidity (0.15% LA) values in 1X whey could be attributed to its lower protein, total solids and mineral content.

- Significantly higher and lower pH values ($p<0.05$) were observed in 1X PCM (4.56) and 3X PCM (4.51), respectively.
- With increase in concentration of GSM base, acidity and protein content of PCM samples markedly increased ($p<0.05$).
- Significantly higher ($p<0.05$) protein (20.25%) and acidity (1.53% LA) were exhibited by 3X PCM sample than 1X PCM samples.
- The storage modulus of PCM was found to be higher than loss modulus indicating elastic behavior.

5.1.2 Formulation optimization of blueberry incorporated goat milk based high protein dessert

5.1.2.1 Optimization of blueberry crush, sugar and inulin levels for preparation of goat milk based high protein dessert

- For the formulation optimization of blueberry dessert, in the first stage sensory analysis of 3 different ratios of BC:PCM 10:90, 20:80, 30:70 were done by sensory score card.
- Based on the different sensory attributes, blueberry dessert with ratio BC:PCM 20:80 was selected for further study.
- Further, the selected BC: PCM 20:80 was incorporated with 3 levels of sugar 5%, 10%, 15% and sensory analysis was done and 10% sugar level was optimized based on sensory evaluation.
- No significant change in sensory attributes ($p>0.05$) was observed with inulin addition, therefore optimization of inulin level was done on basis of rheological analysis.
- The apparent viscosity of blueberry dessert with 4% of inulin was found to have higher viscosity.
- Finally, blueberry dessert with PCM: BC 80:20, 10% sugar, 4% inulin was optimized for further characterization and storage studies.

5.1.3 Characterization of physicochemical and rheological properties of blueberry incorporated goat milk based high protein dessert

- The pH, acidity (% LA), fat (%), protein (%), ash (%) and total solids (%) of optimized blueberry dessert was 4.23, 1.37, 1.01, 13.55, 0.93 and 48.79, respectively.
- Significantly ($p<0.05$) higher pH and TS in the optimized product than control samples.

- Higher *L** value (87.20) was observed for control sample, *L** and *b** values of the optimized product significantly ($p<0.05$) decreased with the blueberry addition while, *a** values significantly ($p<0.05$) increased. Higher *a** value of dessert sample than control was observed.
- The apparent viscosity of the optimized blueberry crush incorporated goat milk based high protein dessert was significantly higher ($p<0.05$) (12 Pa.s) than the control dessert (7.03Pa.s).
- The antioxidant activities DPPH, total phenolics, total flavonoids and FRAP of blueberry incorporated goat milk based high protein dessert were significantly higher ($p<0.05$) than control dessert samples due to addition of blueberry crush.
- The DPPH and FRAP activity of blueberry incorporated goat milk based high protein dessert were observed to be (169.85 µmol TE/g) and (447.32 µmol TE/g) respectively.
- Significantly higher ($p<0.05$) total phenolics (143.64 µg GAE/g) and flavonoids (2.96 µg QE/g) were observed for blueberry incorporated goat milk based high protein dessert than control dessert.
- Coliform and yeast mold count in both optimized and control dessert samples were found to be absent. Significantly higher ($p<0.05$) *Streptococcus* count and lower *Lactobacillus* counts were observed in optimized product than control.
- Significantly higher sweetness and fruity flavor ($p<0.05$) was found to be in optimized product than control.

5.2 DETERMINATION OF SHELF LIFE OF GOAT MILK BASED HIGH PROTEIN DESSERT

5.2.1 Storage stability of non-thermized blueberry incorporated goat milk based high protein dessert

- With increase in storage period, pH values decreased and acidity values were increased in both control and optimized dessert samples.
- All color attributes (*L**, *a** & *b**) of control and optimized desserts differed significantly ($p<0.05$) throughout the storage.
- On 0^{th} day, significantly ($p<0.05$) higher DPPH (169.85 µmol TE/g) and FRAP (447.32 µmol TE/g) were recorded for optimized non-thermized dessert than control samples.
- The total flavonoid content of optimized dessert decreased significantly ($P<0.05$) from 2.96 to 1.38 from 0^{th} day to 14^{th} day. Higher phenolic content of 143.64 µg GAE/g was

noticed in optimized non-thermized dessert on 0^{th} day storage and it was significantly higher ($p<0.05$) as compared to control (98.84 µg GAE/g).

- Total viable counts increased with storage period for control and dessert sample. Coliform and yeast and mold counts were found to be absent.
- Similarly, increase in *Lactobacillus* and *Streptococcus* counts were observed with storage.
- Apparent viscosity of both dessert and control samples increased significantly ($p<0.05$) with progressing storage period.
- Storage modulus of both dessert samples during storage was found to be significantly higher ($p<0.05$) than loss modulus indicating dominant elastic behavior.
- Further, sensory attributes such as overall acceptability found to be decreased with storage days while acidic and sourness were found to be increased.
- SDS-PAGE for non-thermized sample showed the presence of protein in the range 10, 13 kDa and more bands in the range of 29 to 77 kDa. Three major band present were α-casein, β-casein and κ-casein. The lower two minor band (10-13 kDa) were identified as β-lactoglobulin and α-lactalbumin.

5.2.2 Storage stability of non-thermized blueberry incorporated goat milk based high protein dessert

- The pH of the control and dessert decreased while acidity increased with storage.
- With the addition of blueberry, *L** value decreased with storage as compared to the control sample. Yellowness (*b**) value increased significantly with storage in both control and blueberry dessert.
- DPPH activity showed significant decrease ($p<0.05$) in antioxidant activity with storage for blueberry dessert and control samples.
- Total flavonoid content was higher for dessert than control by the addition blueberry and FRAP have been decreased significantly ($p<0.05$) from 0^{th} day (424.48) to 42^{nd} day (334.08) in blueberry dessert with storage.
- The total viable count of both sample and control have been increased with storage. Yeast and mold count was found to be present on 28^{th}, 35^{th} and 42^{nd} days of storage.
- Apparent viscosity of the blueberry dessert was significantly ($p<0.05$) than control and it increased from 0^{th} to 21^{st} day for both the samples and thereafter, it decreased.
- Sensory analysis data showed that there was significant difference ($p<0.05$) in the color of control and optimized dessert.

- The overall acceptability score was found to be decreased with storage whereas acidic and sourness increased with storage days.
- SDS-PAGE for thermized sample showed the presence of protein in the range 10, 13 kDa and more bands in the range 29 to 77 kDa. However, no significant difference was observed in the protein pattern of control and optimized sample.

5.3 Conclusion

- UF GSM (ultrafiltrated goat skim milk) 3X had highest protein and 3X PCM (protein curd mass) was used for preparation of blueberry incorporated goat milk based high protein dessert.
- Formulation of PCM: BC 80:20 with 10% sugar and 4% inulin was most accepted on the basis of sensory scores and rheological properties.
- Optimized formulation had pH, acidity (% LA), fat (%), protein (%), ash (%) and total solids (%) with the value corresponding to 4.23, 1.37, 1.01, 13.55, 0.93 and 48.79, respectively.
- Storage studies indicated decreasing trend in pH, antioxidant activity ($p<0.05$) but acidity increased ($p<0.05$) with storage duration.
- Based on various physico-chemical, microbiological and sensory attributes, non-thermized control and optimized formulation were acceptable upto day 7 and day 14, respectively. While, both control and optimized thermized samples were acceptable upto 28 days of refrigerated storage.

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ANNEXURE

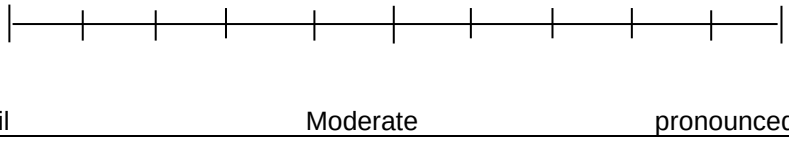
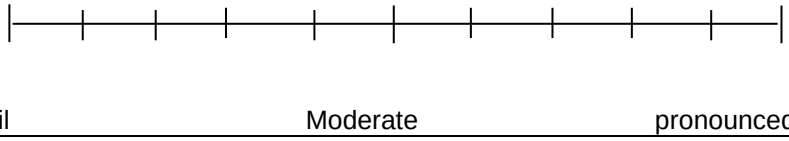
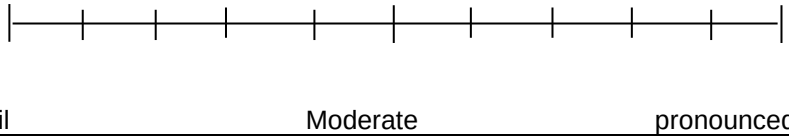
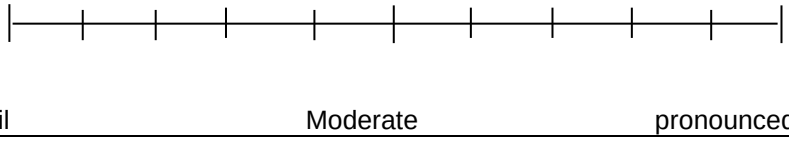
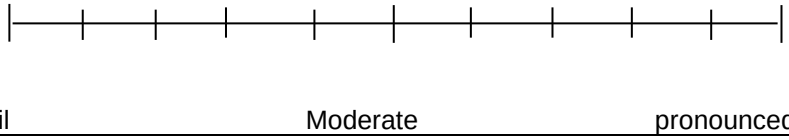
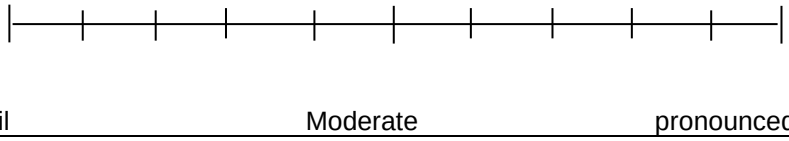
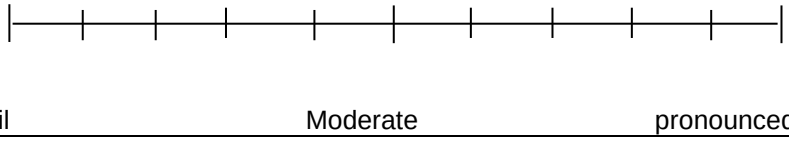
ANNEXURE

Sensory Evaluation Card for Goat milk based Dessert

Sample:_____ **Date:___/___/20___**

Kindly evaluate the given samples for the following sensory attributes using the intensity scales. To indicate your judgment, write the sample code in respective cell.

Property	Intensity
Appearance	
Free whey	Nil — Moderate — pronounced
Color	Dull — Moderate — Dark
Flavour	
Creamy	Nil — Moderate — pronounced
Goaty	Nil — Moderate — pronounced
Fermented	Nil — Moderate — pronounced
Cooked	Nil — Moderate — pronounced
Fruity	Nil — Moderate — pronounced
Sweet	Nil — Moderate — pronounced
Acidic	Nil — Moderate — pronounced

Bitter	
Sour	
Astringent	
Body and Texture	
Lumpiness	
Thickness	
Ropiness	
Overall Acceptability	

Remarks, if any:

Signature

Name