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M.Sc. in Food Engineering

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**CHARACTERIZATION OF FUNCTIONAL PROPERTIES OF
OAT PROTEIN AND ENRICHMENT OF KEFIR WITH OAT
PROTEIN**

**M.Sc. THESIS
IN
FOOD ENGINEERING**

**BY
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**M.Sc. Thesis
in
Food Engineering
Gaziantep University**

**Supervisor
Prof. Dr. Emine ERÇELEBİ**

**by
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January 2023**



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ABSTRACT

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In this study, it was aimed to investigate the functional properties of oat protein, and to improve nutritional value of kefir by adding oat protein. For this purpose, water holding capacity, oil holding capacity, foaming properties, protein solubility and emulsion properties of oat protein were determined. Then, 0.5%, 1% and 2% (w/v) oat protein and 30% (w/v) kefir grains were added to cow's milk to produce four different types of kefir and physicochemical, sensory and microbiological properties of kefir were examined. Oat protein added to kefir had a significant effect on pH, titration acidity, serum separation, viscosity and total phenolic substance values and amount of *Lactococcus* depending on protein concentration ($p<0.05$). During the storage period, an increase was observed in the titration acidity, viscosity and total phenolic substance values of the kefir samples. Addition of oat protein reduced serum separation. Minimum serum separation was observed at 2% oat protein concentration. Addition of oat protein to kefir has been shown to promote the replication of *Lactococcus*. At the end of storage, the amount of lactic acid bacteria increased in kefir with 1% oat protein concentration. According to the results, it was observed that the addition of oat protein increased the amount of *Lactococcus* of kefir, improved its physicochemical properties and did not adversely change its sensory properties. Based on the data obtained, it has been concluded that adding oat protein can give kefir a functional property, and considering the sensory properties, it can be recommended to use 2% oat protein in kefir production.

Key Words: Functional Food, Kefir, Oat, Protein, Probiotic.

ÖZET

YULAF PROTEİNİNİN FONKSİYONEL ÖZELLİKLERİNİN BELİRLENMESİ VE KEFİRİN YULAF PROTEİNİYLE ZENGİNLEŞTİRİLMESİ

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Bu çalışmada, yulaf proteininin fonksiyonel özelliklerini araştırmak, kefire yulaf proteini ekleyerek kefirin besin değerinin artırılması amaçlandı. Bu amaçla, yulaf proteinin su tutma kapasitesi, yağ tutma kapasitesi, köpürme özellikleri, protein çözünürlüğü ve emülsiyon özellikleri belirlendi. Daha sonra inek sütüne ağırlıkça % 0,5, %1 ve %2 yulaf proteini ve %30 kefir daneleri ilave edilerek dört farklı kefir elde edildi ve kefirin fizikokimyasal, duyuşal ve mikrobiyolojik özellikleri incelendi.

Kefire eklenen yulaf proteininin protein konsantrasyonuna bağlı olarak pH, titrasyon asitliği, serum ayrılması, viskozite, toplam fenolik madde değerleri ve *Lactococcus* miktarı üzerine önemli etkisi oldu ($p<0.05$). Depolama süresi boyunca kefir örneklerinin titrasyon asitliği, viskozitesi ve toplam fenolik madde değerlerinde artış gözlemlendi. Yulaf proteininin eklenmesi serum ayrılmasını azalttı. Minimum serum ayrılması %2 yulaf protein konsantrasyonunda gözlemlendi. Yulaf proteininin kefire eklenmesinin laktik asit bakterilerinin çoğalmasını desteklediği görüldü. Depolama sonunda, %1 yulaf protein konsantrasyonuna sahip kefirde *Lactococcus* miktarı arttı. Elde edilen sonuçlara göre yulaf proteini ilavesinin kefirin *Lactococcus* miktarını arttırdığı, fizikokimyasal özelliklerini iyileştirdiği ve duyuşal özelliklerini olumsuz yönde değiştirmediği görüldü. Elde edilen verilerden yola çıkılarak yulaf proteini ilavesinin kefire fonksiyonel özellik kazandırabileceği, duyuşal özellikleri dikkate alındığında ise kefir üretiminde %2 oranında yulaf proteini kullanılması önerilebilir.

Anahtar Kelimeler: Fonksiyonel Gıda, Kefir, Yulaf, Protein, Probiyotik.

‘Dedicated to my mother, Sema Karakaş’

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TABLE OF CONTENTS

	Page
ABSTRACT	v
ÖZET.....	vi
ACKNOWLEDGEMENTS.....	viii
TABLE OF CONTENTS.....	ix
LIST OF TABLES	xii
LIST OF FIGURES	xiv
LIST OF SYMBOLS/ABBREVIATIONS.....	xv
CHAPTER I: INTRODUCTION	1
CHAPTER II: LITERATURE SURVEY	6
2.1 Proteins.....	6
2.1.1 Plant Proteins	7
2.1.1.1 Oat Protein	9
2.2 Probiotics And Prebiotics	13
2.2.1 Use Of Probiotic Microorganisms	14
2.2.2 Probiotic Foods	15
2.3 Kefir.....	16
2.3.1 Definition and History of Kefir	16
2.3.2 Kefir Grains	18
2.3.3 Kefir Production	19
2.3.3.1 Kefir Production Process	20
2.3.4. Sensory Properties of Kefir.....	23
2.3.5. The Effect of Kefir on Health	23
2.4. Studies on the Addition of Plant Protein to Dairy Products.....	24
CHAPTER III: MATERIALS AND METHODS.....	27
3.1 Materials.....	27
3.2 Methods	28
3.2.1 Analysis Of Oat Protein.....	28

3.2.1.1 Water Holding Capacity (WHC).....	28
3.2.1.2 Oil Holding Capacity (OHC)	28
3.2.1.3 Foaming Properties	29
3.2.1.4 Solubility of Protein	29
3.2.1.5 Emulsion Properties	30
3.2.1.6 Emulsion Creaming Index	31
3.2.2 Analysis Of Kefir.....	31
3.2.2.1 Kefir Production.....	31
3.2.2.2 pH.....	32
3.2.2.3 Titratable acidity	32
3.2.2.4 Serum Separation	32
3.2.2.5 Viscosity.....	32
3.2.2.6 Total Phenolic Substance	33
3.2.2.7 Determination of <i>Lactococcus</i> Bacteria.....	33
3.2.2.8 Sensory Analysis of Kefir	35
3.2.2.9 Statistical Analysis	34
CHAPTER IV: RESULTS AND DISCUSSION	36
4.1 Results of Oat Protein.....	36
4.1.1 Water Holding Capacity (WHC)	36
4.1.2. Oil Holding Capacity (OHC).....	36
4.1.3 Foaming Properties	37
4.1.4 Solubility of Protein.....	37
4.1.5. Emulsion Properties.....	39
4.1.6. Emulsion Creaming Index	41
4.2 Results of Kefir Analysis	44
4.2.1 pH.....	44
4.2.2 Titratable Acidity	46
4.2.3 Serum Separation.....	47
4.2.4 Viscosity	50
4.2.5 Total Phenolic Substance.....	52
4.2.6 Determination of <i>Lactococcus</i> Bacteria.....	54
4.2.7 Sensory Analysis.....	55
4.2.7.1. Appearance.....	56

4.2.7.2 Odor	57
4.2.7.3 Taste	57
4.2.7.4 Consistency	57
4.2.7.5 Overall Acceptability	57
CHAPTER V: CONCLUSION	58
REFERENCES.....	60
APPENDIX	78
CURRICULUM VITAE (CV)	106



LIST OF TABLES

	Page
Table 2.1 Protein content of some plants.....	8
Table 2.2 Chemical composition of oat.....	12
Table 2.3 Vitamin and mineral content of oat.....	12
Table 2.4 Probiotic microorganisms inside dairy products.....	15
Table 2.5 Chemical composition of kefir.....	22
Table 2.6 Chemical composition of kefir - Essential amino acids.....	22
Table 2.7 Vitamin and mineral content of kefir.....	22
Table 3.1 Experimental design and progress of the thesis work.....	27
Table 4.1 The solubility of oat protein.....	38
Table 4.2 The results of emulsion activity index and emulsion stability index of oat protein.....	39
Table 4.3 The results of creaming index of oat protein stabilized emulsion.....	42
Table 4.4 The homogeneous subsets of creaming index of oat protein stabilized emulsion.....	42
Table 4.5 The pH changes of kefir samples with oat protein.....	44
Table 4.6 The homogeneous subsets of pH changes.....	45
Table 4.7 The titratable acidity changes of kefir samples with oat protein.....	46
Table 4.8 The homogeneous subsets of titratable acidity changes.....	46
Table 4.9 The serum separation changes of kefir samples with oat protein.....	48
Table 4.10 The homogeneous subsets of serum separation changes.....	48
Table 4.11 The viscosity changes of kefir samples with oat protein.....	50
Table 4.12 The homogeneous subsets of viscosity changes.....	50
Table 4.13 The total phenolic substance changes of kefir samples with oat protein.....	52

Table 4.14	The homogeneous subsets of total phenolic substance changes.....	53
Table 4.15	The amount of <i>Lactococcus</i> changes of kefir samples with oat protein.....	54
Table 4.16	The results of taste parameters.....	56
Table 4.17	The results of sensory analysis.....	56



LIST OF FIGURES

	Page
Figure 2.1 The photograph of kefir grains.....	19
Figure 2.2 Traditional kefir production.....	20
Figure 2.3 Industrial kefir production.....	21
Figure 3.1 Sensory analysis evaluation form.....	34
Figure 4.1 The graph of oat protein solubility.....	38
Figure 4.2 The graph of emulsion activity index and emulsion stability index of oat protein.....	40
Figure 4.3 The graph of creaming index of oat protein stabilized emulsions.....	43
Figure 4.4 The graph of pH changes.....	45
Figure 4.5 The graph of titratable acidity of kefir with oat protein.....	47
Figure 4.6 The graph of serum separation of kefir with oat protein.....	49
Figure 4.7 The graph of viscosity of kefir with oat protein.....	51
Figure 4.8 The graph of total phenolic substance of kefir with oat protein.....	53
Figure 4.9 The graph of the amount of <i>Lactococcus</i> of kefir with oat protein.....	54

LIST OF SYMBOLS/ABBREVIATIONS

A	Absorbance
A₀	Absorbance of the emulsion at 0 min
A₁₀	Absorbance of the emulsion at 10 min
EAI	Emulsion activity index
ESI	Emulsion stability index
WHC	Water holding capacity
OHC	Oil holding capacity
OPC	Oat protein concentrate
OPI	Oat protein isolate
SMP	Skim milk powder
LAB	Lactic acid bacteria
CFU	Colony forming unit,
GAE	Gallic acid equivalents
h	Hour
SDS	Sodium dodecyl sulfate
v/v	Volume per volume
w/v	Weight per volume
%	Percent
N	Dilution factor;
c	The weight of protein per unit volume (g/ml);
φ	The oil volumetric fraction
CI	Creaming Index
H_s	The height of the serum
H_t	The total height of emulsions,

FC	Folin CioCalteu
cP	Centipoise



CHAPTER I

INTRODUCTION

Nutrition is defined as the consumption of nutrients necessary for the maintenance of life, growth and development, improvement of health, protection, repair and exchange of body tissues for humans and all creatures (Eren, 2018). The feeding process begins in the mother's womb and is directly related to the individual's body functions throughout life. The most important factor for individuals to live their lives healthy is their adequate and balanced diet (Kocatepe, 2015).

Adequate and balanced nutrition is the use of all nutrients in the body to ensure that people live their lives in a healthy way. A healthy, adequate and balanced diet is necessary to protect yourself from physical fitness, health and risk of disease (Demirezen, n.d.; Mukudi, 2003).

Modern life caused by advances in science, technology and industrialization have changed the living conditions, as well as the culture of nutrition, the content of foods and the way they are produced. These changes, especially in convenience of foods, affect the biochemical structure of the human body (Seçkin, 2011). As a result of malnutrition habits, serious chronic diseases such as obesity, cardiovascular diseases and cancer develop (Kapil, 2014).

Pre-processed ready-made foods such as canned meat, vegetables, vegetable oils, refined grains are increasingly taking place in people's diet lists (Brückner-Gühmann et al., 2019). Thus, the human diet is very rich in carbohydrate sources, animal products, saturated fat and cholesterol. This situation has caused many nutritional disorders to enter our lives (Kocatepe, 2015).

Nowadays, these conditions have led consumers to focus on nutrition and food production. Researchers are interested in the production of foods that have high nutritional value and positive effects on human health. Foods that have this property are called as functional foods (Liutkevičius et al., 2015).

Functional foods, beyond providing nutrients, also provide additional health benefits to the consumer. This food group includes many different foods and food components, from minerals to fibers. Among these, probiotic foods have a special importance due to the increasing consumer demand due to their health effects (Güneş, 2018).

Although our society has a significant difficulty in changing our eating habits, the technological developments in the food industry have been effective in accepting newer and more effective forms of our traditional foods (Liutkevičius et al., 2015). Functional foods are defined as a food or food ingredient that, in addition to its nutritional value, has a positive effect on an individual's health, physical performance or mental state (Frewer et al., 2003). Thereby preventing disease such as cardiovascular diseases, cancer, high blood pressure, cholesterol, sugar, ulcers and diarrhea (Liutkevičius et al., 2015). In order for a food to be functional, it must have factors such as bioactive compounds, probiotic microorganisms and prebiotic substances, and these factors must be adequately sent to the relevant area of the body (Göncü, 2016).

There is considerable interest in the consumption of functional foods not only in our country but also in other countries. Functional food demand is great especially in developed countries of the world because it is effective in preventing common diseases (Liutkevičius et al., 2015). The food industry aims to develop functional foods and functional food ingredients (for example, probiotics and plant-derived proteins) that have positive effects on consumers' health and well-being (Gibson, 1994). For this reason, the development, production and presentation of new products containing biologically active substances are particularly important for consumers' choice (Goncu, 2017).

Different products can be made into functional foods but dairy products attract the attention of producers and consumers due to their positive effects on human health. For this reason, the production of these products is increasing day by day in the food industry.

The main purpose of functional food production is to provide one or more benefits on health. For this purpose, the producer (Jaskari et al., n.d.) realizes functional food production by enriching, supplementing, changing or improving the existing

nutritional value and options with detailed or living organisms (Gülbandilar et al., 2017). Especially, probiotic microorganisms used in dairy products are made by enriching them with the addition of a new nutrient that is not found in foods (Kadioğlu, 2017).

Studies on functional dairy products have an important place in studies on functional food products. It is quite convenient to turn milk and dairy products into functional products, both due to their health effects and the fact that they are included in the daily diet of almost every individual (Türkmen, 2017).

Increasing the functional properties of milk and dairy products will have an important place both commercially and in terms of nutrition of our people (Seçkin, 2011). Cereals are becoming increasingly important as additives that form the basis for the improvement of the functional aspects of traditional natural foods such as vegetables, fruits, vegetable oils and skimmed milk. In recent years, in order to develop functional foods, the use of cereals in various food formulations and the development of cereal-based functional foods have been the subject of many researches (Charalampopoulos et al., 2002; Kurtuldu, 2012; Mälkki, 2004; Trepel, 2004).

Cereals are stated to be an important source of dietary fiber and contain 50% (w/w) fiber (Skendi et al., 2003). As a result of clinical studies, it is stated that dietary fibers have protective and even therapeutic effects against diseases such as intestinal syndrome, diverticulosis, colorectal cancer, diabetes, hypercholesterol, osteoporosis, heart attack and obesity (Gibson, 1994).

In addition, it is emphasized that fibers have benefits such as blood glucose-lowering effects as well as blood sugar in the human body (Charalampopoulos et al., 2002; Mårtensson et al., 2002). In recent years, attention has been paid to the importance of consumption in terms of reducing and preventing the effects of hypercholesterol and gastrointestinal disorders (Staffolo et al., 2004).

Fibers are different types of carbohydrates derived from the plant cell wall and cannot be hydrolyzed by human digestive enzymes. Special colon bacteria, such as *Bifidobacterium* and *Lactobacillus*, can easily ferment certain special forms of dietary fiber, synthesizing short-chain fatty acids, such as butyrate, acetate, propionate, that

respond to the metabolic energy needs of the host organism and improve their vitality and activities (Jaskari et al., 1998; Sghir et al., 1998; Vasiljevic et al., 2007).

It is thought that the best way to get the most health benefits from dairy products is using nutritional fibers (Göncü, 2016). In recent years, the number of studies on the addition of dietary fiber to fermented milk products has increased with the increasing variety in the field of functional foods. Water-soluble fibers are mostly used in dairy products. Apart from this, dietary fiber is used as stabilizer, total dietary fiber content enhancer and fat substitute. The usage areas of dietary fibers in dairy products are yogurt, ice cream, cheese and fermented milk products (Seçkin, 2011). The usage of dietary fiber in the production of foods provides positive contributions both in terms of technology and health. Dietary fibers can reduce the risk of chronic diseases such as colon and breast cancer. Dairy products containing low calorie nutritional fiber can help control weight (Akin, 2016).

In addition, adding nutritional fiber to dairy products is important in eliminating the negative effects caused by the reduction of fat. With the usage of nutritional fibers, the variety of dairy products and the consumption of dairy products will increase and make a positive contribution to the country's economy (Göncü, 2016).

Due to changing living conditions and intense work tempo, people do not pay attention to healthy nutrition. Due to being fast and practical, processed products have started to be consumed frequently, and the tendency towards natural and fresh foods has decreased. This situation brings together diseases such as cardiovascular diseases, cancer, hypertension, high cholesterol, diabetes, ulcers, diarrhea and obesity (Saygın et al., 2011).

Oat has recently attracted attention due to its rich fiber content, β -glucan and amino acid diversity, unsaturated fatty acids, vitamins and minerals, and high protein content compared to other grains. However, lysine, an essential amino acid, is limited in oats, as in other grains. In contrast, milk proteins provide a good amount of essential amino acids. Kefir is a fermented dairy product that contains many beneficial yeasts and bacteria, making it an important probiotic-based functional food. The combination of kefir and oat protein is suitable and increases the nutritional value of the resulting product (Başçam, 2014). However, there are few reports in the literature. From this

point of view, in our study, it was aimed to investigate the enrichment of kefir with oat protein.

The following goals were targeted:

- To investigate the functional properties of oat protein,
- To increase the nutritional value by adding oat protein to kefir, which is a fermented milk product and already high in nutritional value,
- To contribute to people's healthy diet and keeping their weight under control by obtaining a fast, practical and healthy food product,
- To investigate effect of oat protein on the amount of *Lactococcus* in kefir,
- To increase the nutritional value of kefir and to develop a new functional product,
- To investigate the sensory properties of kefir with added oat protein.

CHAPTER II

LITERATURE SURVEY

2.1 Proteins

Proteins are essential components of all living organisms. Proteins are biopolymers made up of units known as amino acids, with molecular weights ranging from 5000 to several million daltons. The word protein is derived from the ancient Greek word *proteios* meaning “first come”, “first in line” (Damodaran et al., 2007). Proteins are basically compounds that contain 50-55% carbon, 6-7% hydrogen, 20-23% oxygen, 12-19% nitrogen and 0.2-3.0% (w/w) sulfur and are synthesized only in ribosomes. Different proteins contain different numbers and types of amino acids, and since the amino acids that make up the structure are linked to each other by peptide bonds, they are in a polypeptide structure. Proteins can be formed by a single polypeptide or by combining several polypeptides. Each polypeptide chain, or protein in general, has a certain amino acid number, sequence, a certain molecular weight, chemical content and a three-dimensional structure (Saldamli, 2007).

Proteins used in food production can be roughly divided into two groups as animal origin and vegetable origin proteins. Many plant-derived proteins are attracting attention as a low-cost protein source in human nutrition (Ogunwolu et al., 2009).

The relationship between protein and health was first raised in the 2010 Dietary Guidelines Advisory Board's evidence review. The 2010 Dietary Guidelines for Americans recommended first shifting food intake patterns to a more plant-based diet, with an emphasis on beans/legumes, whole grains, nuts/seeds, and vegetables, all of which are vegetable sources of protein (McGuire, 2011).

The general perception of plant protein is beginning to improve. Although once perceived as less nutritious and an incomplete source of essential amino acids, plant protein is now seen as a healthy option to meet protein needs/recommendations (Richter et al., 2015).

Functional properties of proteins are important in food processing and product formulations. Some of the functional properties are water retention, oil binding, emulsion and foaming. These functional properties are affected by internal factors such as molecular structure and weight, and external factors such as the isolation method of proteins, pH, ionic strength and the presence of other components. Which of the functional properties is more important depends on the food material for which the protein concentrate or isolate will be used. For example, high water and oil holding capacity is desired in sausages, breads and cakes, while high emulsifying and foaming properties are preferred for salad dressings, sausages, soups, confectionery, frozen desserts and cakes (Ahmedna et al., 1999; Kinsella, 1979).

2.1.1 Plant Proteins

Currently, functional properties of some plant proteins have been used in food formulations and processing, and scientific studies on plant proteins have increased in recent years (Çetiner, 2018). According to the literature, plant proteins are a powerful alternative to whey isolate, which is frequently used commercially in terms of some functional properties. There is a wide variety of sources in the production of plant protein isolates (Shen et al., 2008).

During the development of new methods, it is important to preserve the nutritional value, sensory and functional properties of the products. In studies related to some functional properties of protein isolates obtained from various sources belonging to grain, legume and oilseed product groups such as solubility, emulsification, foam binding and gelling; in terms of these properties, it was found to be equivalent to commercial isolates (Çetiner, 2018). The protein content of some plants is given Table 2.1 (Ogawa et al., 2000).

Table 2.1 Protein content of some plants

Oilseeds		Legumes		Cereals	
Raw material	Protein Content (% w/w)	Raw material	Protein Content (% w/w)	Raw material	Protein Content (% w/w)
Soybean	40	Pea	23-31	Wheat	9-14
Flax seed	18-25	Lentil	21-31	Rice bran	9-15
Rapeseed /Canola seeds	17-36	Chickpea	20	Oat	12-25

Having 15 major allergen proteins, primarily gluten, in soybean and its products is a negative feature. In addition, the inhibition of iron absorption due to phytate in the content of soy proteins restricted the use of soy and products and caused the search for other protein sources. Although flax seed is a good source of protein, these proteins' usage in food formulations is limited due to its low functional properties (Ogawa et al., 2000).

In a study, it was found that the canola meal emulsion, gel formation and foaming properties are better in soy and flax seed meal (Khattab, 2009).

Rapeseed / canola seeds contain mostly tannins and phenolic compounds such as phytic acids. These components limit the use of rapeseed / canola seeds in food formulations by negatively affecting their physicochemical properties, digestibility and functional properties (Çetiner, 2018).

The most commonly used plant protein in legumes is pea protein. Peas are rich in protein and carbohydrates, but contain a relatively high concentration of insoluble dietary fiber and low levels of fat. Legumin and visilin are two main storage proteins found in peas and show high emulsifying properties. Due to its amino acid composition and acceptable functional properties, peas are an alternative source of plant protein that can be used to enrich food products (Shen et al., 2008).

Lentils are another legumes important for human nutrition with their high protein and rich nutritional content. Being rich in phenolic and flavonoid content, lentil also has beneficial effects on human health such as lowering cholesterol, colon cancer and risk of type 2 diabetes (Jarpa-Parra, 2018).

Chickpeas contain high levels of protein, low levels of fat and sodium. However, chickpeas with dietary fiber and complex carbohydrates also contain high levels of vitamins and minerals (calcium, phosphorus, iron, magnesium) (Roy et al., 2010). Chickpea has high nutritional value and has functional properties such as superior emulsifier and oil binding.

Wheat proteins are examined in three groups as gluten, gliadin, albumin / globulin. Studies on wheat protein are mostly aimed at reducing the allergenic effect of gluten, with the recent occurrence of celiac disease, gluten allergy and gluten-related diseases (Çetiner, 2018).

Oat proteins consist of four fractions: albumin, glutenin, prolamine and globulin. Oat protein, which is newly produced commercially, is a quality plant protein with its nutrient components (Kumar et al., 2021).

Even though some plants have more the protein content than oat, this study focuses on oat for various reasons. First only few studies have been conducted on the usage of oat protein to produce functional foods. Second oat protein's functional properties such as its emulsion and gel stability and its foam binding capacities have a high potential for improvement. Because of that more studies on functional properties and oat protein applications should be done (Yavuz, 2016).

2.1.1.1 Oat Protein

Oat (*Avena sativa*) is a genus found in the Aveneae tribe of the Grammineae family. The oat grain consists of 20-30% (w/w) husk, approximately 75% (w/w) endosperm and approximately 5% (w/w) embryo. In addition, it contains 40% (w/w) starch (Yürür, 1998). Oat, which is an important raw material in the animal feed industry, is a cereal plant whose uses in human nutrition (bread, biscuits, probiotic drinks, baby food and oat flakes thanks to its satiating feature) are increasing day by day. Because

oat grain is a good source of protein, fiber and minerals (Karaman et al., 2020; Peterson et al., 2005).

Oat is a type of cereal that has functional importance that enriches the product to which it is added in terms of nutritional and sensory properties and must be kept in the daily diet against diseases such as cholesterol, diabetes, cardiovascular diseases, obesity and cancer (Yaver, 2014).

Recently, oats have become a sought-after product in human nutrition. Oat, which is more nutritious than many other grains due to dietary fiber, lipids, vitamins and antioxidants, is a suitable protein source. Especially, the fact that β -glucan has a cholesterol-lowering effect and reduces the risk of chronic diseases such as type 2 diabetes and heart disease increases the importance of oats in human nutrition (Yaver, 2014).

β -glucan is one of the most important dietary fibers found in cereal grains, especially oats and barley (Özcan et al., 2013). It is stated that β -glucan in barley and oats reduces blood cholesterol level and risk of heart-related diseases (Tudorica et al., 2004).

Oats attract attention in research and commercial field due to their high β -glucan content and compounds with antioxidant effect. Oat protein in the form of an oat protein concentrate is obtained from traditional β -glucan production as a protein-rich by product. In addition, during the industrial production of β -Glucan, the recovery of the protein-rich by-product as oat protein concentrate (OPC) results in a source of grain protein for food and improves the overall economy of the process (Brückner-Gühmann et al., 2019).

Functioning of the protein-rich oat-fraction and its implementation as a valuable food ingredient will raise the maintainability of the process. Oat protein concentrate (OPC) can ensure an alternative to skim milk powder (SMP) and traditional thickeners. As a retainable ingredient, OPC is a good source of health supplementing valuable components such as dietary fiber, protein and bioactive compounds. In addition, it can also act as a functional ingredient in semi-solid foods such as acid-derived gels due to its high protein and starch content (Brückner-Gühmann et al., 2019; Vasiljevic et al., 2007).

The extraction of oat protein isolate (OPI) from oats will be of great importance in providing potential value-added products for the food sector (Liu et al., 2009). There are reports showing that OPI has good emulsifying activity and binding properties (Ma & Khanzada, 1987; Wu et al., 1977). In one study, it was said that deamide and succinylated oat protein isolate improved water solubility and emulsification activity compared to their natural forms (Mirmoghtadaie et al., 2009). It can be thought that the emulsifying activity of oat protein isolate, its secondary structure, and the formation of the protein adsorption layer at the air/water interface may be related to its self-assembly properties. However, it is known that no research has been done on the secondary structure and morphology of the oat protein isolate (Liu et al., 2009).

The amount of protein in oats varies between 10.24-24.0% (w/w) depending on the variety and environmental conditions (Pomeranz et al., 1971). 50-80% (w/w) of oat protein is globulin and 10-20% (w/w) is prolamin (Peterson, 1976).

Globulins are water-soluble compounds while prolamins are neither water-soluble nor insoluble compounds. Avenin, the secondary protein of oats, is a prolamine. Oat is the only type of cereal that is rich in protein content, 80% (w/w) of the storage protein is avenalin, and contains globulin or legume-like proteins. Prolamins are the most general group of cereal proteins. Oat prolamine is avenin. The quality of oat protein is similar to soy protein, which is equivalent to milk, meat and egg protein, according to the World Health Organization (Singh et al., 2013).

It has been reported that the nutritional value and usefulness of oat protein vary between 90.3-94.2%, its biological value is between 74.5-79.6%, net protein use is between 69.1-72.4% and protein availability rate is between 2.25-2.38% (Thompson et al., 1991).

Since the gluten content in oat grains is lower than other grains, it is considered as a gluten-free food. It has been suggested that there is no harm when using oat food in patients with gluten sensitivity (celiac disease) (Picarelli et al., 2001). The definitive use of oats for people with celiac disease in the United States has not been confirmed. However, it is suggested that it can be used by limiting the amount of consumption (about half a glass of dry whole grain oatmeal per day) (Thompson, 2003).

In addition, oat, which has the highest protein content among all grains, has also been a highly consumed source of protein, since it does not contain non-nutritious ingredients. However, lysine that is an essential amino acid, is found in oats limited (Çetiner, 2018). On the contrary, milk proteins provide a good essential amino acid (Seçkin, 2011). Milk proteins contain essential amino acids, and 19 essential amino acids, except hydroxyproline, are included in the composition of milk proteins (Metin, 2005). Therefore, a combination of milk and oat proteins provides a good source of this essential amino acid. In addition, studies reveal that thanks to the development of microbial balance in the environment of oats, more favorable conditions are provided for the gastrointestinal tract, especially the development of lactic acid bacteria and *Bifidobacterium* and *Lactobacillus* species are supported (Ryhänen et al., 1996). A combination of fermented milk and oat protein is suitable and increases the nutritional value of the product obtained. In the context of plant-derived proteins, oats have a suitable composition and provide valuable nutrients for human consumption.

Table 2.2 and 2.3 show the nutritional value of oats.

Table 2.2 Chemical composition of oat for 100grams (Dursun Şirin, 2021)

Energy	1469 kJ (351 kcal)
Fat, total	5.80 g
Protein	11.35 g
Fiber, total diet	12.24 g
Water	10.66 g
Carbohydrate	52.27 g
Starch	54.59 g

Table 2.3 Vitamin and mineral content of oat (Dursun Şirin, 2021)

Vitamins (mg) 100g		Mineral content (g) 100g		Trace elements	
B1	0.391 mg	Calcium	72 mg	Iron (mg)	5.91 mg
B2	0.171 mg	Phosphor	320 mg	Zinc (mcg)	2.31 mg
B6	0.240 mg	Magnesium	131 mg		
Niacin	1.033 mg	Potassium	468 mg		
		Salt	56 g		

2.2 Probiotics And Prebiotics

Probiotic dairy products are known as functional nutrients (Öncebe, 2019). Probiotics are living microorganisms that mean \"for life\" in two parts as \"pro\" and \"biota\", and prebiotics are digestible carbohydrates that increase the number and activity of colon bacteria and the effect of probiotics (Taşdemir, 2017). Probiotics can be defined as live microbial food supplements that benefit the host by improving the microbial balance of the gut (Demirgöl, 2018).

Studies conducted in recent years show that probiotics may be effective on gastrointestinal system diseases. By stimulating the proliferation of beneficial microorganisms in prebiotics, it has been shown that probiotic effect is helpful. (Taşdemir, 2017).

Lactic acid bacteria constitute the most important group of probiotic microorganisms. Among them, *Bifidobacterium* and *Lactobacillus* species are the most widely used probiotic microorganisms (Uymaz, 2010). Probiotics increase beneficial bacteria (*Lactobacilli*, *Bifidobacteria*, etc.) in the intestinal environment, reduce pathogenic bacteria (*Clostridium*s, *Bacteroides*) and help maintain and renew the natural balance in the microbiota (Kalip, 2018). The presence of probiotic bacteria in the diet has beneficial effects such as lowering serum cholesterol levels, preventing cancer, regulating intestinal microbial flora, improving calcium absorption and lactose utilization (Kizilaslan, 2016).

In order for probiotic microorganisms to achieve the expected beneficial effect, they must be taken into the body in numbers of 10^6 - 10^7 cfu/ml or more and remain viable during the production and shelf life of the food they are in (Vasiljevic, 2008).

These microorganisms are non-pathogenic, non-toxin-producing, anta-gonistic effects against pathogens, able to pass to the intestinal system by showing resistance to acid and bile salts, to form an antimicrobial compounds, to stabilize the intestinal microbiota, to maintain its vitality in storage (Gülbandilar et al., 2017). These microorganisms are mostly consumed in the form of yogurt, fermented beverages and kefir (Akpınar et al., 2019).

In order to encourage the development of probiotic microorganisms, prebiotics are used. Prebiotics are also defined as food ingredients that can be fermented but not digested, which promote the development and activities of certain bacteria in the gut (Holzapfel, 2002; Klaenhammer, 2000; Palframan et al., 2002).

Recently, a new trend has been introduced as a new concept in dairy products, defined as a mixture of probiotics and prebiotics and defined as synbiotics that improve the health of the host organism by promoting the implantation and survival of live microbial diet additives in the gastrointestinal tract (Gibson, 1994). Consumers are demanding foods with increasing features such as pleasant flavor, low calorie value or low fat content and beneficial health effects (Goncu, 2017).

2.2.1 Use Of Probiotic Microorganisms

Probiotic bacterial cultures are marketed directly or indirectly with milk and dairy products. Functional food is used for probiotic microorganisms due to its health effects (Seçkin, 2011). Milk and dairy products are foodstuffs that can be considered as a suitable medium to transport beneficial bacteria to the intestinal system. Dairy products such as yogurt, cheese, and kefir can play a positive role in promoting the viability and development of probiotic cultures. In this context, nowadays probiotic products are mostly limited to yogurt and fermented milk beverages that contain probiotic cultures that are beneficial to health.

Lactobacilli and *Bifidobacteria* are the most widely used microorganisms in the probiotic milk and dairy market, and products made using these microorganisms are the best and best-known products on the market (Bengmark, 2001; Guarner et al., 2005).

In recent years, probiotic cultures have been added to the composition of fermented milk products, and the product has been given extra physiological effect and nutritional value (Gülbandilar et al., 2017).

Probiotic microorganisms found in dairy products were given in Table 2.4.

Table 2.4 Probiotic microorganisms inside dairy products
(Gülbandilar et al., 2017; Tomar et al., 2017)

Product	Probiotic microorganism
Cheese	• <i>Bifidobacteria</i> • <i>Lactobacilli</i> • <i>Enterococci</i>
Yogurt	• <i>Lactobacillus acidophilus</i> • <i>Bifidobacterium subsp. vela.</i>
Kefir	• <i>Lactobacillus,</i> • <i>Lactococcus,</i> • <i>Leuconostoc genus</i>

2.2.2 Probiotic Foods

Fermentation is the process of converting simple raw materials into a more valuable product as a result of the activities of various microorganisms (Kurniati et al., 2016). While fermentation makes foods more durable, it also contributes positively to the formation of aroma, odor and taste (Songun, 2016). Meat, milk, cereals, various fruits and vegetables can be fermented into more diverse, delicious, nutritious and long-lasting products (Atamer et al., 1988).

Although yogurt and fermented milk products constitute the majority of probiotic products today, the trend towards non-dairy probiotic foods is increasing day by day (Antunes et al., 2013). Fruit juices, desserts and cereal-based products containing probiotics are also suitable environments for probiotics. During the production of probiotic beverages, fruits, vegetables and cereals can be used as substrate or prebiotic (Betoret et al., 2003).

Whole grain products are increasingly becoming the most advantageous probiotic carrier. The main reason for this is the probiotic content and useful components such as indigestible carbohydrates, soluble fiber, phytochemicals and bioactive components that whole grain offers to consumers (Marquart, 2005). Oat, barley and malt substrates enable *Lactobacilli* to tolerate harsh environmental conditions and support the development of pure and mixed probiotic cultures (Herrera-Ponce et al., 2014). It is stated that with the development of probiotic microorganisms in cereal substrates, chronic diseases such as obesity, cardiovascular diseases, type 2 diabetes and some types of cancer can be reduced (T. Wang et al., 2014). Although probiotic-containing

approaches in meat products are not entirely new, few manufacturers consider fermented meat products as carriers for probiotics (De Vuyst et al., 2008; Rivera-Espinoza, 2010).

The low and absence of heat treatment in fermented meat products provides the necessary environment for the development of probiotics. In order to benefit from the beneficial effects of probiotics, the viability and number of probiotic microorganisms are optimal must be kept at a level. Therefore, the ripening period should not exceed 1 month, the pH should not be lower than 5, excessive drying or heat treatment should be avoided (De Vuyst et al., 2008; Khan et al., 2011).

Fermented dairy products are products with different flavors and consistencies obtained as a result of fermentation of milk by various microorganisms. These microorganisms are usually lactic acid bacteria (Atamer et al., 1988). When it comes to fermented milk product, the first products that come to mind are; cheese, yogurt, ayran, butter, kefir and kumiss. Kefir, one of these products, has therapeutic effects together with its high nutritional value and antioxidant properties (Gaware et al., 2011).

2.3 Kefir

2.3.1 Definition and History of Kefir

Kefir is a fermented milk product that contains many useful yeasts and bacteria which makes it an important probiotic based functional foods (Köroğlu et al., 2015). Although there is not much information about the history of kefir making, it is stated that the origin of the making of kefir drink emerged thanks to the shepherds in the Caucasus Mountains (Koroleva, 1988). Kefir, whose origins are northern Caucasus, is a beverage that local people produce incidentally using cow and goat milk and is consumed for cooling. The importance of kefir was understood in the 1920s when Russian scientists researched probiotic bacteria on kefir research and realized that the number of probiotic bacteria in the yogurt was 25-30 in kefir (Aghatabay, 2005). The morphological structure of kefir culture is similar to cauliflower (Sarkar, 2008). These kefir grains, which carry out the fermentation of milk, are small microorganism clusters held together by the polysaccharide matrix called kefiran. (Lopitz-Otsoa et al., 2006).

Lactic acid bacteria (LAB) and yeasts in the structure of kefir grain ferment milk in homofermentative and heterofermentative ways, resulting in lactic acid, carbon dioxide (CO₂), small amounts of alcohol and aromatic molecules. All of these molecules contribute to the development of kefir's unique sensory properties. The most important feature that distinguishes kefir from other fermented milk products is; The fact that lactic acid and ethyl alcohol fermentation occurs in the same environment as a result of the bacteria and yeasts in the kefir grain working in a symbiotic way (Ünlütürk, 1998; Yilmaz et al., 2006).

It is not known how and where the kefir grains used in the production of the product are produced (Koroleva, 1988). Later, the consumption of this drink, which spreads to many parts of the world and which people enjoy, has increased gradually. Kefir has traditionally been fermented with milk kefir grains; in industrial production, it is produced by using microorganisms obtained or isolated from grains as starter culture (Tomar et al., 2017). Kefir is a fermented and carbonated refreshing milk with a slightly acidic aromatic taste and creamy foam composition containing *Lactobacillus*, *Leuconstoc*, *Lactococcus* and acetic acid bacteria, and many types of bacteria.

Besides milk components such as protein, fat and lactose, it contains a small amount of lactic acid, ethanol, carbon dioxide, acetic acid, acetaldehyde, acetoin and diacetyl, which affect its taste and smell (Kadioğlu, 2017). While milk is most commonly used for kefir production, kefir can also be produced from milk of different animals such as goats, sheep, camels, or plant sources such as soy, rice, coconut milk. Kefir can be prepared from full-fat, semi-skimmed or non-fat pasteurized milk. The main raw material used in the production of kefir is cow milk; however, kefir can also be produced from caprine or ovine milk (Karatepe, 2014).

Kefir is an important beverage with its functional and probiotic properties. Kefir is a source of vitamins B1, B2, B6, vitamin B12, folic acid, vitamin K and biotin. It contains calcium, phosphorus, potassium, magnesium, zinc, copper, manganese, iron and cobalt (Ahmed et al., 2013; Çağındı, 2003; Esmek, 2015).

Microorganisms in the structure of kefir make it easier to digest with the changes they create in milk. Thus, the nutrients in kefir are easily absorbed by the body. Especially

with the conversion of lactose in milk to lactic acid, kefir is easily consumed by people who are lactose intolerant (Kurniati et al., 2016).

It is becoming increasingly popular as a result of new research. Since it includes nutritional and growth factors, kefir can be consumed at any age and it is safe for pregnant and lactating women (Kadioğlu, 2017).

2.3.2 Kefir Grains

Kefir grains are irregularly shaped, yellow-white elastic structures with the appearance of small cauliflower or popcorn. It is a symbiotic fermented milk product mediator that contains bacteria and yeast in a polysaccharide structure (Loretan et al., 2003; Plessas et al., 2007). Kefir grain sizes vary from 1-2 mm to 3-6 mm (Wang et al., 2008). Kefir grain contains polysaccharides, a small amount of oil and casein (Magalhaes et al., 2011). Microorganisms live in the grain in a symbiotic state. Microorganisms from these grains added to milk pass into the milk environment and perform fermentation. In addition to lactic acid bacteria, acetic acid bacteria and yeasts are also present in kefir grains (Özden, 2008). Kefir grains contain *Lactobacillus*, *Leuconostoc*, *Lactococcus* and acetic acid bacteria, and many types of yeasts (Kadioğlu, 2017).

Besides milk components such as protein, fat and lactose, it contains a small amount of lactic acid, ethanol, carbon dioxide, acetic acid, acetaldehyde, acetoin and diacetyl, which affect its taste and smell (Kadioğlu, 2017). These bacteria and yeasts are embedded in a gel-like polysaccharide matrix called “kefiran” (Wang et al., 2008).

In kefir fermentation, lactic acid and alcohol fermentations run simultaneously, and yeasts and lactic acid bacteria are responsible for fermentation in a symbiotic relationship (Zhou et al., 2009). Lactic acid bacteria mainly produce lactic acid; yeasts, on the other hand, produce ethanol and carbon dioxide, creating the sharp acidic sour taste, which is the typical aroma of kefir, and the stinging feeling of carbon dioxide on the tongue (Irigoyen et al., 2005).

Kefir grains, which contain a complex microflora, give kefir its own unique properties. The diversity, type and number of the population in the microflora of kefir grains, as well as the biochemical properties and microbiological profile of the milk used, or the physical or geographical differences regarding the method used in production lead to

differences in the color, taste, odor, texture or chemical or microbial composition of the final product (Witthuhn et al., 2005). Photograph of kefir grains was given in Figure 2.1.



Figure 2.1 The photograph of kefir grains

2.3.3 Kefir Production

Both traditional and industrial methods are used for kefir production but they share basically the same principles (Çağındı, 2003). The traditional method of making kefir is by adding kefir grains directly to the milk. Traditional kefir can be prepared at home using kefir grains with fresh or pasteurized milk (John, 2000).

In recent years, different methods are being developed in kefir production. It has been determined that with the combination of milk powder and milk powder-buttermilk, a kefir production close to the desired properties can be made (Kadioğlu, 2017).

The use of whey powder has led to the occurrence of flavor differences in the proportions, and it has not been found suitable for kefir production. Studies such as the use of whey powder and buttermilk in the production of kefir, known as milk powder and dairy residues instead of milk, are generally aimed at increasing the nutritional value of kefir and reducing its cost (Kocatepe, 2015).

The composition of kefir was found to be 0.5-1.5% ethyl alcohol, about 0.7% milk acid and 3.2% fat. A good kefir should be of fluent consistency, homogeneous and

bright appearance. A good kefir should contain 0.6-0.9% lactic acid, 0.6-0.8% alcohol and 50% CO₂ (v/v) (Yüksekdağ, 2003).

2.3.3.1 Kefir Production Process

Kefir can be produced traditionally in homes and industrially in plants. The flow charts of the two methods are given below (Shen et al., 2018):

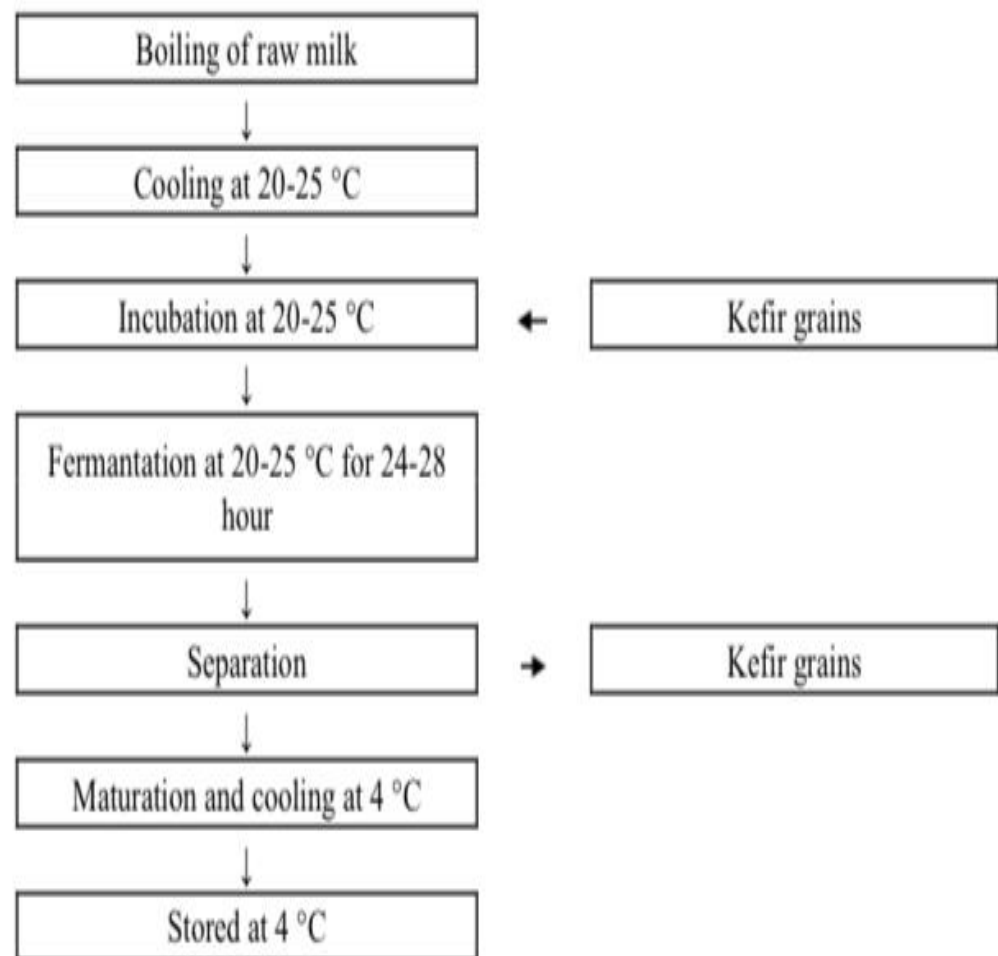


Figure 2.2 Traditional Kefir Production

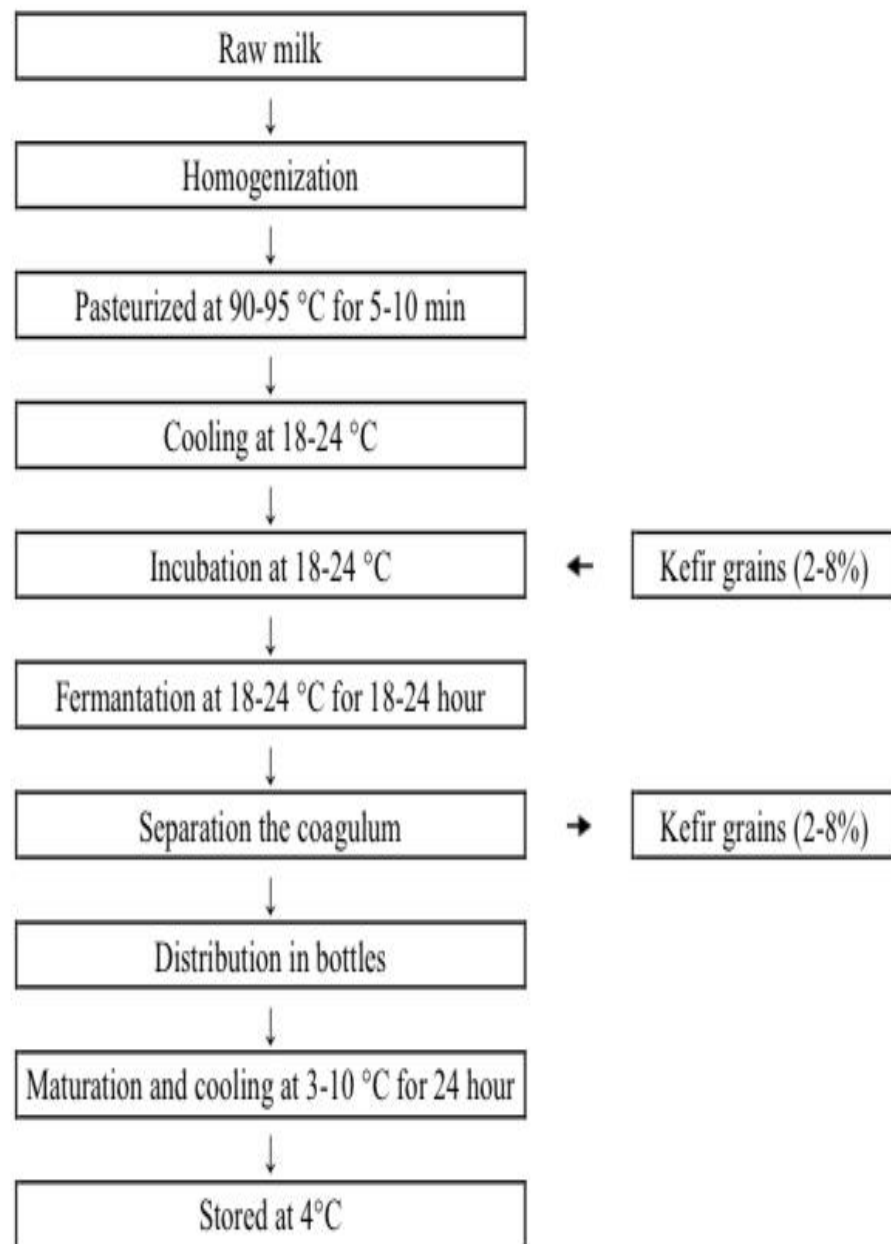


Figure 2.3 Industrial Kefir Production

Kefir is a very rich product in terms of nutritional value. Table 2.5, 2.6 and 2.7 shows the nutritional value of kefir.

Table 2.5 Chemical Composition of Kefir for 100 grams (Kadioğlu, 2017)

Energy	65kcal
Fat (%) (w/w)	3.5
Protein (%) (w/w)	3.3
Lactose (%) (w/w)	4.0
Water (%) (w/w)	87.5
Milk Acid (g)	0.8
Ethyl Alcohol (g)	0.9
Lactic Acid (g)	1.0
Cholesterol (mg)	13.0

Table 2.6 Chemical Composition of Kefir for 100 grams - Essential amino acids (g)

Lysine	0.27
Leucine	0.34
Isoleucine	0.21
Methionine + cystine	0.12
Phenylalanine + tyrosine	0.35
Valin	0.22
Tryptophan	0.05
Threonine	0.17

Table 2.7 Vitamin and Mineral Content of Kefir for 100 grams (Kadioğlu, 2017)

Vitamins (mg)		Mineral content (g)		Trace elements	
A	0.06	Calcium	0.12	Iron (mg)	0.05
Carotene	0.02	Phosphor	0.10	Copper (mcg)	12.0
B1	0.04	Magnesium	12	Molybdenum (mcg)	5.5
B2	0.17	Potassium	0.15	Manganese (mcg)	5.0
B6	0.05	Sodium	0.05	Zinc (mcg)	0.36
B12	0.5	Chlorine	0.10		
Niacin	0.09				
C	1.0				
D	0.08				
E	0.11				

2.3.4 Sensory Properties of Kefir

The sensory properties of kefir are determined by lactic acid, oxalic acid, some volatile fatty acids, small amounts of CO₂ and LAB, and some aromatic components (acetaldehyde and acetoin) produced by yeasts. Thousands of flavoring substances produced during fermentation affect the taste of kefir. A good kefir should have a flowing consistency, homogeneous and shiny appearance. A lumpy structure is considered a defect. It has a slightly sour taste and the fermented taste should be felt when drunk (Guzel-Seydim et al., 2011).

2.3.5 The Effect of Kefir on Health

Epidemiological studies have shown that there is a relationship between nutrition and diseases. In studies using kefir-added diets, it has been stated that there are anti-carcinogenic, antibacterial, immunological, hypocholesterolemic, gastrointestinal and metabolic benefits (Tomar et al., 2017). It is stated that kefir gives good results in anorexia, insomnia, tuberculosis and kidney diseases, biliary disorders, jaundice, various infections and eczema (Yüksekdağ, 2003). It is noteworthy that there are no diseases such as tuberculosis, cancer and digestive disorders in the Caucasus, where kefir is recognized as a youth drink and it is drunk instead of water, and the average human life reaches 110-130 years (Yüksekdağ, 2003).

In the researches, it has been determined that kefir plays an important role in this issue and it can be used successfully in the treatment of some diseases. As a result of a study on the therapeutic properties of kefir, 100 people between 25- 65 years of age had a 100% improvement in intestinal disorders, 60-70% in insomnia, 50-80% in nervous depressions, 30-50% in high blood pressure. It has been explained that 70% of those who use kefir stop taking other medicines they use. There is a direct relationship between nutrition and the immune system. It has been shown in both human studies and animal experiments that kefir has a regulatory effect on the immune mechanism (Esmek, 2015).

Today, the positive effects of bioactive peptides in fermented milk and dairy products on human health are well known. Findings obtained in a study with 18 healthy volunteers have been interpreted as the use of kefir may enable the immune response

in the lumen of the gastrointestinal tract to work more effectively (Adiloğlu et al., 2013).

It is stated that kefir also has anti-inflammatory effect. Also it has an antibacterial effect against pathogenic bacteria such as *Salmonella enteritidis*, *Staphylococcus aureus* and *Escherichia coli*. The beneficial bacteria that are taken into the body together with kefir when they are drunk continuously, especially lactobacilli, settle in the intestines, correct the microflora there and eliminate the bacteria that cause disease with the acid compounds they produce (Guzel-Seydim et al., 2011; John, 2000; Karatepe, 2014).

Today, the new trend consists of producing milk and yoghurts containing prebiotics and probiotics and presented to consumers' attention, and their benefits for health are emphasized. In addition, kefirs containing prebiotic fiber are also available in markets. It is known that the popularity of kefir also increases in popularity with its beneficial microflora in the gastrointestinal tract (Kadioğlu, 2017).

2.4 Studies on the Addition of Plant Protein to Dairy Products

In a study made by adding 1-3% (w/w) pea flour and 1-3% (w/w) milk powder, microbiological and physical properties of probiotic fermented milk drinks were examined. During 28 days of storage, an increase in acid production and microbial development (colony forming unit, CFU) of probiotic fermented milk drinks with 3% (w/w) pea flour added was observed. After 28 days of storage, the average pH of all samples decreased from 4.50 to 4.04. Serum separation was significantly reduced in probiotic fermented milk drinks with 1-3% (w/w) pea flour added compared to the control group and milk powder-added probiotic fermented milk drinks. The addition of pea flour reduced serum separation in fermented milk drinks, improving the stability of the gel structure and viscoelastic properties of fermented beverages. Pea flour is stated as a good prebiotic source thanks to its high fiber and protein content and as an additive that improves texture in fermented products (Zare et al., 2013).

In another study, changes in the physico-chemical and sensory properties of fat-free fermented milk drinks with soy protein isolate, pea protein isolate, wheat gluten and rice protein during the storage period were investigated. Fermented milk beverage produced by adding 0.5% (w/v) plant proteins to skim milk was stored. The viscosity,

amino acid and sensory properties of the product have been determined. The lowest viscosity values were measured in the rice protein sample, the highest viscosity values were measured in the wheat gluten samples. When the protein and amino acid contents of fermented milk drinks are examined, it can be concluded that the highest protein value is found in the pea protein isolate sample, the soy protein isolate sample has a high essential amino acid content, and the plant proteins increase the amino acid content of the fermented milk beverage. In sensory analysis, fermented milk drinks with soy protein isolate, pea protein isolate and wheat gluten added are more liked in terms of taste (Başçam, 2014).

In another study, the possibility of using lentil flour as a prebiotic for *Lactobacillus casei* in probiotic yogurts was investigated. For this purpose, three different probiotic yoghurts were produced by adding 0.5% (w/w) and 0.1% (w/w) lentil flour to cow's milk. The sensory and microbiological properties of yoghurts were investigated during the daily storage period (1st, 10th and 20th days). According to the results, it was determined that the addition of lentil flour had a prebiotic effect for *Lactobacillus casei*, but negatively affected the sensory properties. In the light of the data obtained, it was concluded that by adding lentil flour, it was concluded that yoghurt could gain a functional feature, and it was suggested to use 0.5% (w/w) lentil flour in yoghurt production considering the sensory properties (Akın et al., 2019).

In another study, it was observed that when pumpkin seed flour, pea and hemp proteins were used as additives in the production of classical yogurt, it not only increased the nutritional value of the final product, but also improved its sensory and rheological properties (Dabija et al., 2018).

In another study, yogurt was enriched with two oat protein products, one concentrate (OPC) and one isolate (OPI). Structural parameters and sensory properties were evaluated and compared with traditional yoghurt enriched with skimmed milk powder (SMP). In fermentation experiments with OPI, it was observed that the compatibility of highly purified isolated oat protein with milk proteins was low. In contrast, OPC was found to be a good substitute for SMP, resulting in improved product quality in syneresis and mouthfeel. It has been stated that OPI can be considered for its nutritional benefits rather than its functional properties, while OPC is a suitable

component for the application of nutritious oat protein due to the excellent techno-functional properties of oat starch it contains (Brückner-Gühmann et al., 2019).

Another study evaluated the sensory properties and consumer acceptance of low-fat unsweetened yogurt enriched with insoluble triticale, wheat or oat fibers at 15 and 30 g/kg levels. Addition of insoluble triticale fiber caused yellowish-brown color, grainy flavor and distinctive grittiness in fortified yoghurts. The products were classified in the 'very good' quality category, despite the low quality scores given to the 30 g/kg fiber-fortified yoghurts. It has been noted that insoluble dietary fiber from triticale shows promising potential for use as a fortifying ingredient in the production of fiber-enriched fermented milk products such as yogurt (Tomic et al., 2017).



CHAPTER III

MATERIALS AND METHODS

Experimental design and progress of the thesis work was presented in Table 3.1.

Table 3.1 Experimental design and progress of the thesis work

I	Supply of raw materials, equipment and reagents
II	Investigation of properties of oat protein isolate
	Determination of • water holding capacity • oil holding capacity • foaming properties (foam capacity and foam stability) (UV-Spectrophotometer) • protein solubility (UV-Spectrophotometer) • emulsion properties (emulsion activity index and emulsion stability index) (UV-Spectrophotometer) • emulsion creaming index
III	Preparation of kefir samples with 0%, 0.5%, 1% and 2% (w/v) oat protein
IV	Analysis of 0%, 0.5%, 1% and 2% (w/v) oat protein added kefir samples • pH • titratable acidity • serum separation • viscosity (viscometer) • total phenolic substance (UV-Spectrophotometer) • <i>lactococcus</i> bacteria • sensory analysis
V	Evaluation of the effect of adding oat protein to kefir

3.1 Materials

Raw cow milk used for kefir production was obtained from Gaziantep city center in March 2022. Kefir grains were obtained from an online store (Büşra Şafak, Konya). Oat protein isolates were sent as gifts by Lantmännen Functional Foods (Stocholm, Sweden). Soybean oil was purchased from the local market (Arıca Doğal Ürünler, İzmir). Sodium hydroxide (NaOH), hydrochloric acid (HCl), bovine serum albumin (BSA), folin ciocalteu reagent, sodium carbonate (Na₂CO₃), sodium dodecyl sulfate (SDS) and gallic acid were purchased from Sigma Chemical Company (St. Louis, MO,

USA). Copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), potassium sodium tartrate ($\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$), peptone were purchased from Merck Chemical Company (Darmstadt, Germany). Phenolphthalein was purchased from Riedel-de Haën, Honeywell (Charlotte, North Carolina, US). M17 agar was purchased from Biolife (Milano, Italy).

3.2 Methods

3.2.1 Analysis of Oat Protein

3.2.1.1 Water Holding Capacity (WHC)

1 gram of samples were dispersed in 20 ml distilled water with a vortex mixer for 1 minute in graduated centrifuge tubes (Karaman et al., 2014). After that the dispersions were centrifuged (Centrifuge 5810R, Eppendorf AG, Germany) at 2000g for 30 min (Guan et al., 2007). These procedures were repeated three times and the average was calculated. The amount of the supernatants was determined and WHC was expressed as g of water held per g of sample (Özdemir, 2021).

$$\text{WHC (g/g)} = (W_2 - W_1) / W_0 \quad (4.1)$$

WHC: g of water / g of protein

W_2 : Tare of tube + wet sample weight (g)

W_1 : Tare of tube + weight of dry sample (g)

W_0 : Dry sample weight (g)

3.2.1.2 Oil Holding Capacity (OHC)

Five hundred milligrammes of samples were added to 5 ml soybean oil in graduated centrifuge tubes, and mixed for 30 s with a vortex mixer. The oil dispersions were centrifuged (Centrifuge 5810R, Eppendorf AG, Germany) at 2000g for 30 min. These procedures were repeated three times and the average was calculated.

The amount of oil separated was determined and OHC was expressed as g of oil held per g of sample (Özdemir, 2021).

$$\text{OHC (g/g)} = (V_1 - V_2) / W_0 \quad (4.2)$$

OHC: g of oil / g of protein

V_1 : The initial amount of oil (g)

V_2 : The amount of supernatant (g)

W_0 : The amount of dry matter (g)

3.2.1.3 Foaming Properties

Foaming properties were determined by modifying to the method of Özdemir (2021). 100 mg of the sample was weighed and dissolved in 10 mL of distilled water. The resulting solutions were incubated for 2 min at room temperature. It was homogenized at room temperature in a homogenizer (IKA T-18 digital Ultraturrax, Germany) with a 12.7 mm dispersing tool (S 18 N-19 G) at 20,000 rpm for 3 minutes. The whipped samples were immediately transferred to a 25 mL cylinder and the total volume was read after 30 s. These procedures were repeated three times and the average was calculated. The foam capacity was calculated according to the following equation:

$$\text{Foam Capacity (\%)} = \frac{A-B}{B} * 100 \quad (4.3)$$

The mixture obtained after foam capacity analysis was kept at 20 °C for 30 minutes. After resting, the final volume was recorded as C value. Thus, the foam stability value was calculated with the help of the following equation:

$$\text{Foam Stability (\%)} = \frac{C-B}{B} * 100 \quad (4.4)$$

A: Volume after whipping (mL) for 30 s.

B: Volume before whipping (mL).

C: Volume after whipping (mL) for 30 min.

3.2.1.4 Solubility of Protein

500 mg of oat protein was dissolved in 40 ml of distilled water. Three different samples at pH 5, 7 and 9 were prepared using 0.1 N NaOH and 0.1 N HCl. The samples were stirred at room temperature for 30 minutes on a magnetic stirrer. It was centrifuged at

4000g for 30 minutes. The supernatant was filtered with filter paper and the protein solubility was determined by the biuret method (Morr et al., 1985).

To prepare Biuret reagent, 1.5 g of copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) and 6 g of Potassium Sodium Tartrate ($\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$) were dissolved in 500 ml of distilled water. 300 ml of 10% w/v sodium hydroxide (NaOH) solution was added to the first solution with continuous stirring and it was completed to 1 L with distilled water.

1 ml protein solution was put in tube for each sample. 4ml Biuret reagent was added and waited 30 minutes at room temperature. After that the absorbance was read at 540 nm using UV VIS spectrophotometer (SP-3000nano, OPTIMA, Tokyo, Japan) and the protein concentration was determined using standard curve.

The solubility of the protein was calculated as (Brückner-Gühmann et al., 2018) :

$$\text{Protein Solubility(\%)} = \frac{\text{protein in 100 ml supernatant (g)}}{\text{protein in 100ml sample taken (g)}} * 100 \quad (4.5)$$

3.2.1.5 Emulsion Properties

Emulsion activity index (EAI) and emulsion stability index (ESI) were determined by modifying the turbidimetric method of Pearce and Kinsella (1978).

Six types of 30 ml samples were prepared with oat protein concentrations of 0.2%, 0.5%, 1%, 5%, 10%, and 20% (w/v) for the water phase. 0.06 gram protein for 0.2% oat protein concentration, 0.15 gram protein for 0.5%, 0.3 gram protein 1%, 1.5 gram protein for 5%, 3 gram protein for 10% and 6 gram protein for 20% were weighed. Then pure water was added to each sample so that the total solution amount was 30 ml and mixed well. 10 ml of soybean oil was added to each sample. Emulsions were homogenized at room temperature in a homogenizer (IKA T-18 digital Ultraturrax, Germany) with a 12.7 mm dispersing tool (S 18 N-19 G) at 1 min at a speed of 10,000 rpm. Afterwards 50 μl emulsions were drawn from the bottom of the vessel, and diluted with 10 ml 0.1% (w/v) SDS solution. The absorbance A_0 was measured at 500 nm at 0 min and the absorbance A_{10} was measured at 500 nm in the UV VIS spectrophotometer (SP-3000 nano, OPTIMA, Tokyo, Japan) at 10 min after emulsion

formation. The emulsion activity index (EAI) and the emulsion stability index (ESI) were calculated as follows (Guan et al., 2007):

Emulsion Activity Index:

$$EAI \left(\frac{m^2}{g} \right) = \frac{2 * 2.303 * A_0 * N}{c * \phi * 10000} \quad (4.6)$$

Emulsion Stability Index:

$$ESI \text{ (min)} = \frac{A_0 * 10}{A_0 - A_{10}} \quad (4.7)$$

where N represents a dilution factor; c is the weight of protein per unit volume (g/ml); and ϕ is the oil volumetric fraction (0.25).

3.2.1.6 Emulsion Creaming Index

Emulsions with oat protein concentrations of 0.2%, 0.5%, 1%, 5%, 10% and 20% (w/v) were prepared. Ten millimeters (10 ml) of each emulsion was filled into capped centrifuge tubes and then stored vertically at room temperature. The height of the serum (H_s) and the total height of emulsions, (H_t) were recorded after storage up to 48 hours at room temperature for different period times. The CI% was reported as (Shao, 2014):

$$CI(\%) = (H_s/H_t) * 100 \quad (4.8)$$

3.2.2 Analysis Of Kefir

3.2.2.1 Kefir Production

1 liter of raw cow's milk was boiled at 96°C for 10 minutes. After cooling to 20-25°C oat protein was added to kefir at a concentration of 0%, 0.5%, 1% and 2% (w/v) and mixed well. Then, 25-30 grams of kefir grains were added and incubated in a sealed glass bottle at 25°C until the pH reached 4.6 and coagulation occurred. After fermentation, it was filtered through a plastic strainer. Kefir grains were separated and stored, and kefir was left to mature for 24 hours at 4°C. On the 1st, 7th and 14th days

of the fermentation, pH, titratable acidity, serum separation, viscosity, total phenolic substance, lactic acid bacteria, and sensory analyzes were performed.

3.2.2.2 pH

The pH values of kefir samples were measured in pH meter (Hanna precision pH meter, pH 211) (Başçam, 2014).

3.2.2.3 Titratable acidity

10 grams of kefir sample was weighed. 10 ml of distilled water was added. 2-3 drops of 1% phenolphthalein were added and titrated with 0.1 N NaOH until a permanent light pink color was obtained (Göncü et al., 2017).

Titratable acidity was calculated as a percentage.

$$\% \text{ Titratable acidity (lactic acid)} = \frac{\text{Volume of titrant} \times N \times 90 \times 100}{\text{weight of sample} \times 1000} \quad (4.9)$$

N = normality of titrant

90 = Equivalent weight for lactic acid

3.2.2.4 Serum Separation

Kefir placed in a 100 ml measuring graduated cylinder was stored at +4°C in refrigerator. The amount of separated serum was determined in ml at the end of the 1st, 7th, and 14th days. The result was written in ml / 100 ml (Lucey et al., 1998).

3.2.2.5 Viscosity

Kefir viscosity was tested using a viscometer (Brookfield RVT, United States of America). 250 ml sample was put into a beaker glass beaker. Sample viscosity was measured at room temperature (25.0± 2°C) using a spindle no. 2 at 125 rpm speed. The result was recorded as kefir viscosity at m.Pa.s (Setyawardani et al., 2020).

3.2.2.6 Total Phenolic Substance

Total phenolic content was determined by the Folin Cio Calteu (FC) method. Folin Ciocalteu reagent was diluted with water at a ratio of 1:10 (v/v). Then, sodium carbonate was prepared with water as 75g/L.

100 µL of kefir and 200 µL of Folin Ciocalteu reagent were placed in a beaker and waited for 2 minutes. Then, 800 µL of sodium carbonate was added to each beaker. It was kept in a the dark place for 2 hours at room temperature. And the absorbance was read at 765 nm in the UV VIS spectrophotometer (SP-3000 nano, OPTIMA, Tokyo, Japan) by using glass cuvettes of 1 cm path-length (~24°C). Gallic acid was used as a standard. Total phenolic substance was expressed in mg gallic acid equivalents (GAE) per liter. A mixture of distilled water and reagents was used as a blank. (Albayrak et al., 2010).

3.2.2.7 Determination of *Lactococcus* Bacteria

25 grams of kefir samples were taken and mixed in 225 ml of 0.1% (w/v) sterile peptone water. Each sample was diluted 1/10 sequentially in tubes containing 0.1% sterile peptone water. For each kefir sample, 0.2 ml sample was taken and inoculated on M17 agar for *Lactococcus* enumeration using spread plate method, and plates were incubated at 37 °C for 48 hours under anaerobic conditions. Number of microorganisms were calculated using following formula and reported as colony forming units (cfu) per g sample (Erdoğan, 2020):

$$\# \frac{\text{microorganisms}}{\text{g of kefir}} = \frac{\text{Count of microorganisms in one petri}}{\text{inoculum amount in one petri}} * \text{Dilution Factor} \quad (4.10)$$

3.2.2.8 Sensory Analysis of Kefir

Sensory analyzes were performed by 10 volunteer panelists. The panelists independently evaluated the samples named A, B, C, and D. The parameters based on sensory evaluation were detailed with a minimum of 1 and a maximum of 5 points, and a table was created and the appearance in kefir samples; serum separation, shine, opacity, uniform structure, odor; taste; sourness, sweetness, bitterness, pungency, astringency, aroma intensity; consistency properties were evaluated. The sensory analysis evaluation form was given in Figure 3.1.

SENSORY ANALYSIS																					
Please give a score from 1 to 5. (1 = unacceptable, 5 = excellent)																					
Sample name		A					B					C					D				
Scale		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Appearance																					
Serum Separation																					
Shine																					
Opacity																					
Uniform Structure																					
Odor																					
Taste																					
Sour																					
Sweet																					
Bitter																					
Sharp																					
Astringency																					
Aroma Intensity																					
Consistency																					
Overall acceptability																					

Figure 3.1 Sensory analysis evaluation form

3.2.2.9 Statistical Analysis

Three repetitions of each test were carried out to characterize the functional properties of oat protein. The data obtained were subjected to one-way analysis of variance (ANOVA). For kefir analysis, in the trials, 4 different products were produced, including the control group, and all analyzes were performed in 3 parallels. In the study, the differences in the oat concentrate and storage time in kefir samples were determined by applying a randomized plot design, and accordingly analysis of variance (ANOVA) was applied. The comparison of the significant differences between the averages was performed with the LSD test ($p < 0.05$) by using SPSS version 26 (SPSS Inc., Chicago, IL, USA).

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Results of Oat Protein

4.1.1 Water Holding Capacity (WHC)

WHC is water that can be held by a protein matrix under especial terms which avoids its renunciation from the three-dimensional structure. It depends on the microstructure of the matrix consisted of by protein. WHC is an important property, especially in solid products. Low WHC often causes dryness, astringency and affects the flavor of the product. There are few studies reporting the effect of modifications of oat proteins on WHC (Mirmoghtadaie et al., 2009; Nieto et al., 2016; Nieto-Nieto et al., 2014, 2015).

In this study, the average water holding capacity of oat protein was found to be 1.95g/g (Appendix A.1). WHC content of oat protein in literature have been reported as 1.94g/g, 2.00g/g, 1.27g/g and 2.90g/g, respectively (Guan et al., 2007; Ma, 1984; Mirmoghtadaie et al., 2009; Prosekov et al., 2018). Our result are consistent with the result of Guan et al., 2007.

4.1.2. Oil Holding Capacity (OHC)

OHC is the determination of the absorption of fat or oil by the weight of the protein molecules. It is an important property for preserving taste and texture while food processing applications. (Kumar et al., 2021).

In this study, the average fat holding capacity of oat protein was determined as 2.63 g/g (Appendix A.2). OHC content of oat protein in literature, respectively; 2.50 g/g, 2.10 g/g (Guan et al., 2007; Ma, 1984). Our result are consistent with the result of Guan et al., 2007.

4.1.3 Foaming Properties

In the manufacture of various food products based on their whipping or foaming properties, the inclusion of gas or air is necessary to create a stable foam. Protein molecules are handily absorbed at the interface to generate stable film or foam. Foaming properties are excessively connected with pH and treatment during processing (Kumar et al., 2021). Foam formation and its properties are affected by protein type, preparation method, composition, solubility and concentration, ambient pH, presence of salts, and hydrophobic interactions. Interactions and molecular changes resulting from different applications determine foam behavior (Massoura et al., 1998).

In this study, the average foam capacity value of oat protein was determined as 87.4%, and the average foam stability value was determined as 67.2% (Appendix A3). Our result are consistent with the result of Ma (1984). In that study, the foaming capacity of oats had been reported as 85% and the foam stability for 30 minutes as 70%.

4.1.4 Solubility of Protein

Solubility is an important protein property in the application as a functional ingredient in different food products, especially semi-solid and liquid products. Soluble proteins have the ability to provide homogeneous dispersibility in colloidal systems and improve interfacial properties. Therefore, solubility is a critical property for protein use in food applications. Although the solubility of oat protein is similar to other plant globulins, it is generally lower than the others (Mäkinen et al., 2017). Oat globulins include glutamine-rich fractions that become fewer hydrophilic than other plant globulins by exposure to solvent at the surface. Factors affecting protein solubility are, different fractions of protein, heat treatment, oat pretreatment (oil removal), extraction method and especially pH (Kumar et al., 2021; Ma, 1984).

The solubility of oat protein values was given in Table 4.1 and the solubility of oat protein graph was given in Figure 4.1.

Table 4.1 The solubility of oat protein

pH	Solubility of oat protein (%)
5	18.46±0.17 ^a
7	71.91±0.64 ^b
9	81.55±0.3 ^c

*Values marked with different letters are statistically different ($p < 0.05$).

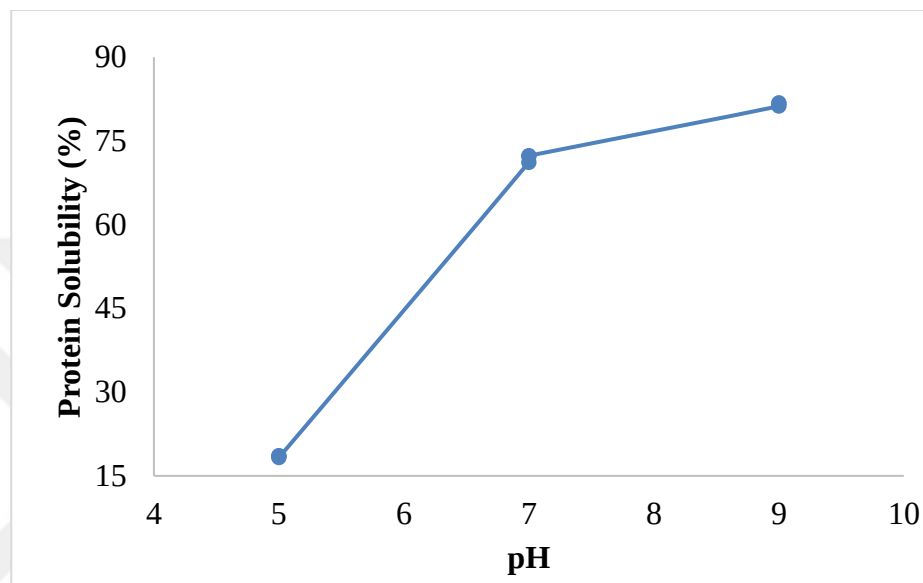


Figure 4.1 Oat protein solubility

From Figure 4.1, it was seen that the solubility increased as the pH value increased. The lowest solubility was observed at pH=5, while the highest solubility was observed at pH=9. Proteins show the lowest solubility at the isoelectric point (pI) (Yavuz, 2016). For oat protein isolate, this value is 4.9 (Brückner-Gühmann et al., 2018).

In this study, the oat protein solubility value was measured as 18.46% for pH 5, 71.91 % for pH 7, and 81.55% for pH 9. In a study, the solubility value of oat protein was measured as 17.6% for pH 4 and 73.3% for pH 7 (Brückner-Gühmann et al., 2018). Our result are consistent with the result of Brückner-Gühmann (2018).

Measurements were made at pH 5, 7 and 9 for solubility of oat protein. Considering the protein solubility according to the pH values, a significant difference was observed at the $p < 0.05$ level (Appendix A5).

4.1.5 Emulsion Properties

The emulsification properties of proteins are determined by the emulsion activity index (EAI) and emulsion stability index (ESI). Emulsion activity demonstrate the capacity of surfactant proteins to cover oil at the interface that can be emulsified per unit protein. The higher the emulsifying activity, the lower the interfacial tension. Emulsion stability demonstrate the ability of the emulsion to withstand changes such as agglomeration, creaminess, and coalescence over a period of time. The emulsifying properties of oat proteins depend on solubility, which changes with pH values (Spaen & Silva, 2021).

This study was carried out at pH=7 at room temperature. EAI and ESI values were given in the Table 4.2. And the graph of EAI and ESI were given in Figure 4.2

Table 4.2 Emulsion activity index and emulsion stability index of oat protein

Concentration (%)	EAI (m ² /g)	ESI (min)
0.2 %	70.08 ^a	61.66 ^a
0.5 %	48.88 ^b	62.38 ^a
1 %	8.35 ^c	69.51 ^{ab}
5 %	4.79 ^d	85.85 ^{ab}
10 %	4.28 ^d	92.92 ^b
20 %	2.64 ^e	142.78 ^c

*Values marked with different letters are statistically different (p<0.05).

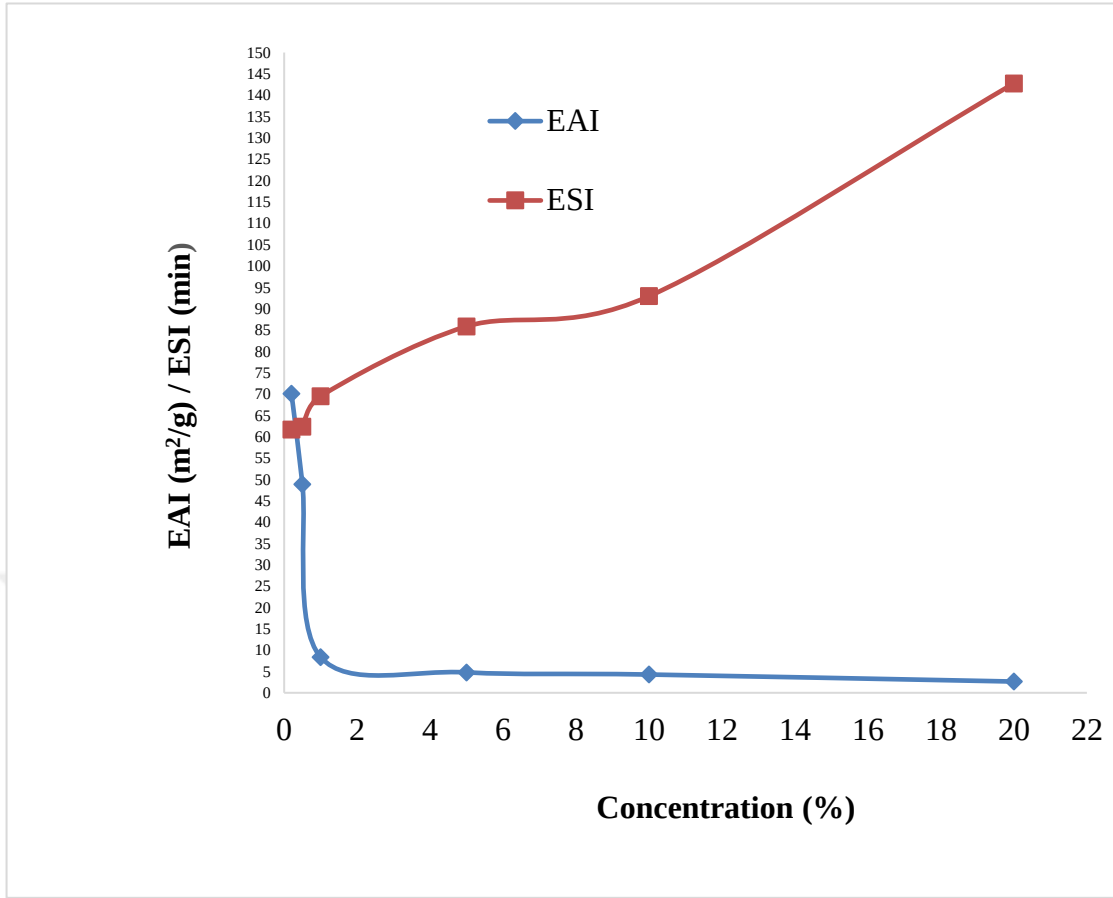


Figure 4.2 Emulsion activity index and emulsion stability index of oat protein

In this study, EAI values were found between 2.64 and 70.08 m²/g. As shown in Table 4.2, the highest EAI value for oat protein was observed at the lowest concentration of 0.2%. It was observed that the EAI value decreased as the oat protein concentration increased. This is because the protein rapidly migrates to the interfacial layer, where it exhibits higher EAI values in the presence of lower protein concentrations. The activation energy barrier, on the other hand, limits protein migration to the interfacial layer at higher protein concentrations (Guo & Mu, 2011).

For 0.5% oat protein concentration at pH=7, in this study EAI value was 48.88 m²/g. At the same conditions, in Mirmoghtadaie's study, the EAI value was 49.0 m²/g (Mirmoghtadaie et al., 2009). The values appear to be compatible.

Considering the variance analysis results, a significant difference at p<0.05 level was observed between the concentrations for the EAI values of oat protein (Appendix A5).

In this study, ESI values between 61.66 and 142.78 min. As shown in Table 4.2, the lowest ESI value for oat protein was observed at the lowest concentration of 0.2%. It was observed that the ESI value increased as the oat protein concentration increased. As the concentration increases, the flow rate slows down and the viscosity increases. It is more difficult for the droplets to aggremeration and the stability of the emulsion increases. The study of Guo and Mu (2011) confirms that the ESI value increases as the concentration increases.

For 0.5% oat protein concetration at pH=7, in this study ESI value was 62.38 min. At the same conditions, in Mirmoghtadaie's study (2009), the ESI value was 62.6 min. The values appear to be compatible.

Considering the variance analysis results, a significant difference at $p < 0.05$ level was observed between the concentrations for the ESI values of oat protein (Appendix A5).

4.1.6. Emulsion Creaming Index

Creaming stability is investigated to evaluate the relative stability of oil-in-water emulsions (Keowmaneechai, 2002). Creaming Index (CI %) was calculated by visual monitoring of emulsions. The CI of the emulsions was measured for up to 48 hours for emulsions stored at 25°C. The results of creaming index of oat protein stabilized emulsions were given in Table 4.3. Homogeneous subsets of creaming index of oat protein stabilized emulsions were given in Table 4.4. And the graph of creaming index of oat protein stabilized emulsions were given in Figure 4.3.

Table 4.3 Creaming index of oat protein stabilized emulsions

Time (h)	Concentration (%)					
	0.20%	0.50%	1%	5%	10%	20%
0	0	0	0	0	0	0
8	0.25	0.21	0.17	0.011	0.011	0
16	0.41	0.36	0.325	0.044	0.011	0
24	0.39	0.37	0.33	0.111	0.011	0
32	0.39	0.38	0.35	0.144	0.017	0
40	0.4	0.38	0.36	0.222	0.017	0
48	0.41	0.38	0.36	0.277	0.022	0

Table 4.4 Homogeneous subsets of creaming index of oat protein stabilized emulsions

Oat Protein Concentration (%)	Creaming Index
0.20%	0.3214 ^a
0.50%	0.2971 ^b
1%	0.2707 ^a
5%	1.0429 ^a
10%	0.1143 ^a
20%	0.0000 ^a

*Values marked with different letters are statistically different ($p < 0.05$).

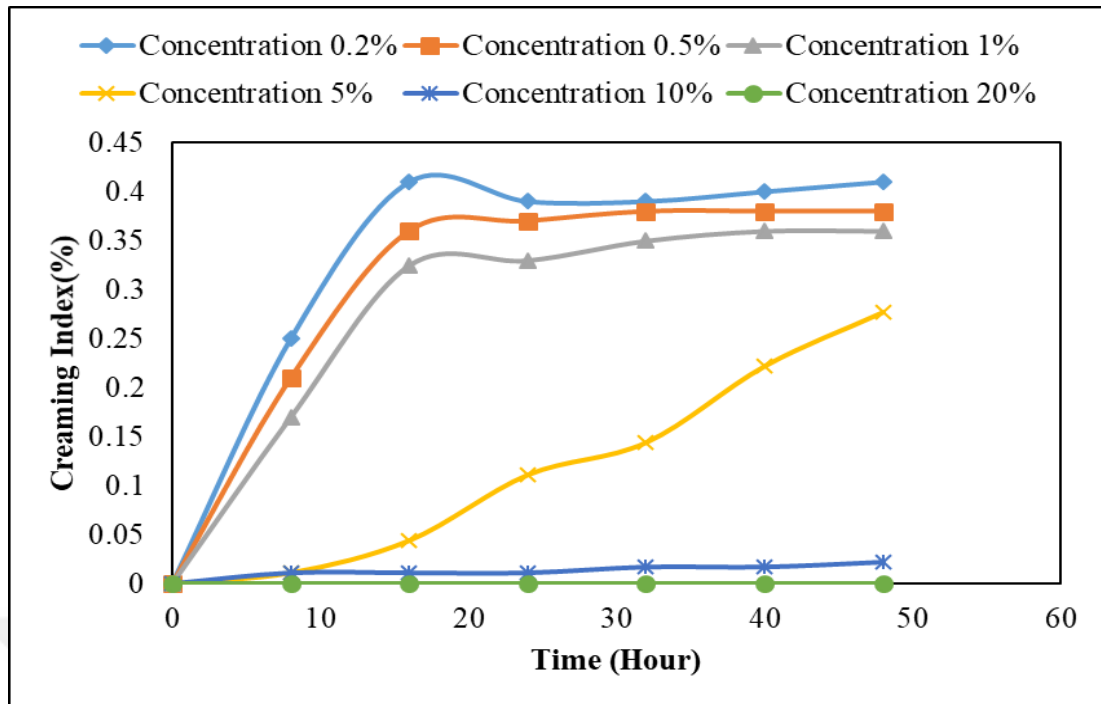


Figure 4.3 Creaming index of oat protein stabilized emulsions

Creaming is considered as the movement of droplets that are not the same size. The movements affect each other and the droplets deform (Güngör et al., 2013). In this study, the highest creaming index value was observed at 0.20% oat protein concentration, while the lowest creaming index was observed at 20% oat protein concentration. It was observed that the CI% decreased with increasing oat protein concentration. The rheological properties of products are affected by the total solids concentration. The viscosity increases as the total solids concentration increases (Keser, 2020). According to Stokes' law, creaming rate is inversely proportional to viscosity and emulsion stability. As the emulsion stability increases, the creaming index for gravity separation decreases. In parallel with the increase in viscosity, the physical stability of the emulsion increases. Creaming index can indicate the stability of the emulsion formulation (Güngör et al., 2013; Khan et al., 2008; Koroleva et al., 2015; Shi et al., 2010). The highest emulsion stability and lowest creaming index at 20% oat protein concentration confirm this.

Considering the results of analysis of variance, a significant difference at $p < 0.05$ level was found between time and between concentrations (Appendix A6).

4.2 Results of Kefir Analysis

4.2.1 pH

The pH value gives information about the acidity of the product during the storage of kefir and is an important parameter in determining its quality (Delikanlı, 2012; Donkor et al., 2006; Mulvihill, 1995).

In this study, the pH values of kefir samples varied between 3.95 and 4.68. The lowest pH value was observed in kefir with 2% oat protein added on the 14th day, and the highest pH value was observed on the 1st day in kefir with 0% oat protein added (without the addition of oat protein). The average pH values obtained as a result of the analysis of kefir samples with oat protein and the results of homogeneous subsets were given in Table 4.5 and Table 4.6. And the graph of pH changes of kefir with oat protein was given in Figure 4.4.

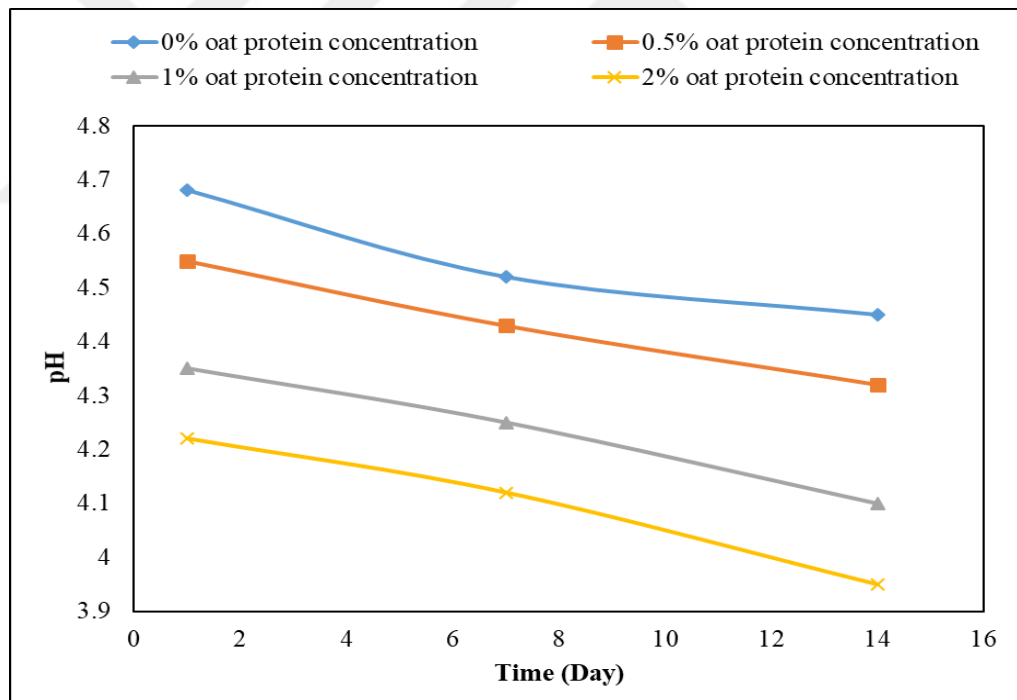
Table 4.5 The pH changes of kefir samples with oat protein

Oat protein concentration (%w/v)	pH during storage		
	Day 1	Day 7	Day 14
0.00%	4.68	4.52	4.45
0.50%	4.55	4.43	4.32
1%	4.35	4.25	4.10
2%	4.22	4.12	3.95

Table 4.6 Homogeneous subsets of pH

		pH
Oat Protein Concentration of Kefir (%)	0%	4.55 ^a
	0.50%	4.43 ^b
	1%	4.23 ^c
	2%	4.09 ^d
Storage Time	Day 1	4.45 ^a
	Day 7	4.33 ^b
	Day 14	4.2 ^c

*Values marked with different letters are statistically different ($p < 0.05$).

**Figure 4.4** pH changes of kefir with oat protein

In a study, pH values of kefir samples varied between 4.39 and 4.73 during 30 days of storage at 4°C (Arslan, 2015). In another study, the pH levels of the samples were found to be similar to each other in the range of 4.45 and 4.62 values (Songun, 2016). Our results were consistent with results of these studies.

Considering the variance analysis results for pH were examined, a significant difference was observed for oat protein concentration and storage time at $p < 0.05$ level (Appendix A7).

4.2.2 Titratable Acidity

The degree of fermentation of lactose is one of the important parameters that determine the titratable acidity in fermented milk products (Krasaekoopt et al., 2006). In the Turkish Food Codex Communique on Fermented Milk, it is stated that the titratable acidity of kefir should be at least 0.6% as % lactic acid. The average titratable acidity values obtained as a result of the analyzes made on kefir samples with oat protein and the results of homogeneous substance were given in Table 4.7 and 4.8. And the graph of titratable acidity changes of kefir with oat protein was given in Figure 4.5.

Table 4.7 The titratable acidity changes of kefir samples with oat protein

Oat protein concentration (%w/v)	Titratable acidity (%) during storage		
	Day 1	Day 7	Day 14
0.00%	0.765	0.882	0.909
0.50%	0.846	1.017	1.008
1%	0.959	1.026	1.053
2%	1.017	1.044	1.134

Table 4.8 Homogeneous subset of titratable acidity

		Titratable Acidity
Oat Protein Concentration (%)	0%	0.85 ^a
	0.50%	0.95 ^b
	1%	1.01 ^{bc}
	2%	1.06 ^c
Storage Time	Day 1	0.89 ^a
	Day 7	0.99 ^b
	Day 14	1.02 ^b

*Values marked with different letters are statistically different ($p < 0.05$).

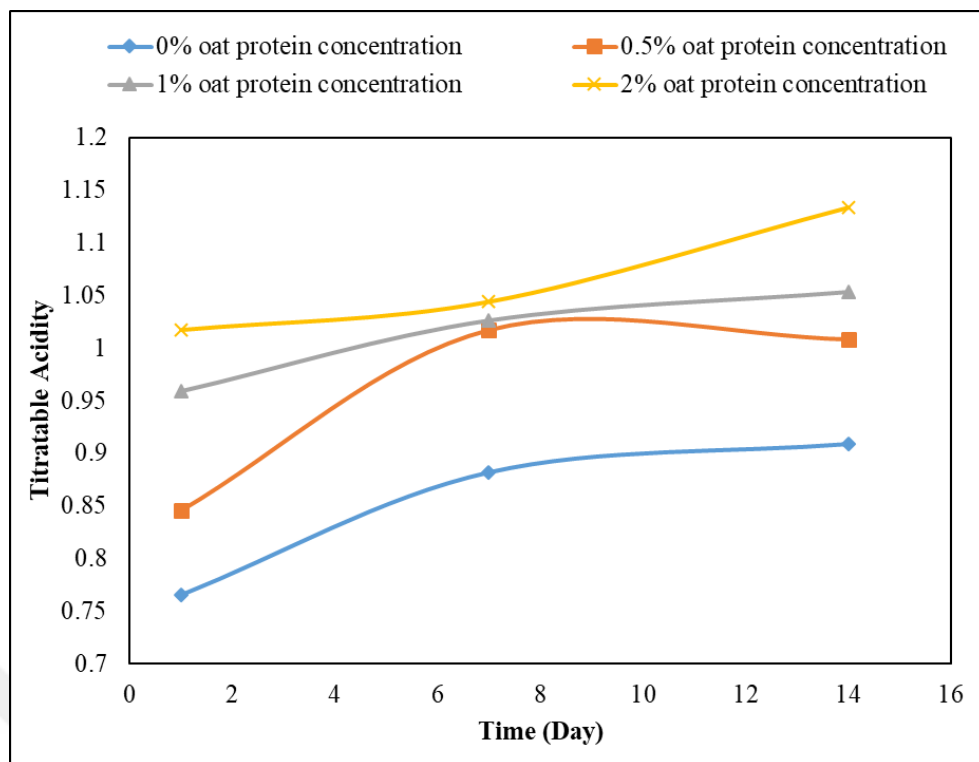


Figure 4.5 Titratable acidity changes of kefir with oat protein

The titratable acidity values of the samples varied between 0.765 and 1.134%. When the average titratable acidity of kefir samples was examined, it was seen that all values were in accordance with the Turkish Food Codex Communiqué on Fermented Milk.

During the storage period, the lowest titration acidity value was determined on the 1st day, and the highest titration acidity value was determined on the 14th day. Researches confirm that the titration acidity increases due to the activities of the starter cultures and the enzymes produced by these cultures during the storage period (Atamer et al., 1988; Barrantes et al., 1994; Delikanlı, 2012).

Considering the variance analysis results for titratable acidity were analyzed, a significant effect was observed at $p < 0.05$ level for oat protein concentration and storage time (Appendix A8).

4.2.3 Serum Separation

Serum separation is an important parameter in the determination of clot stability and expresses an inhomogeneous structure/high gel instability in fermented milks (Lucey et al., 1998). The average serum separation values obtained as a result of the analysis

performed on kefir samples with oat protein and the results of homogeneous subsets were given in Table 4.9 and Table 4.10. And the graph of serum separation changes of kefir with oat protein was given in Figure 4.6.

Table 4.9 The serum separation changes of kefir samples

Oat protein concentration (%w/v)	Serum separation (ml/100ml) during storage		
	Day 1	Day 7	Day 14
0.00%	0	0.06	0.11
0.50%	0	0.04	0.06
1%	0	0.02	0.05
2%	0	0	0.02

Table 4.10 Homogeneous subsets of serum separation

		Serum separation
Oat Protein Concentration (%)	0%	0.0567 ^a
	0.50%	0.0333 ^a
	1%	0.0233 ^a
	2%	0.0067 ^a
Storage Time	Day 1	0.0000 ^a
	Day 7	0.0300 ^{ab}
	Day 14	0.0600 ^c

*Values marked with different letters are statistically different ($p < 0.05$).

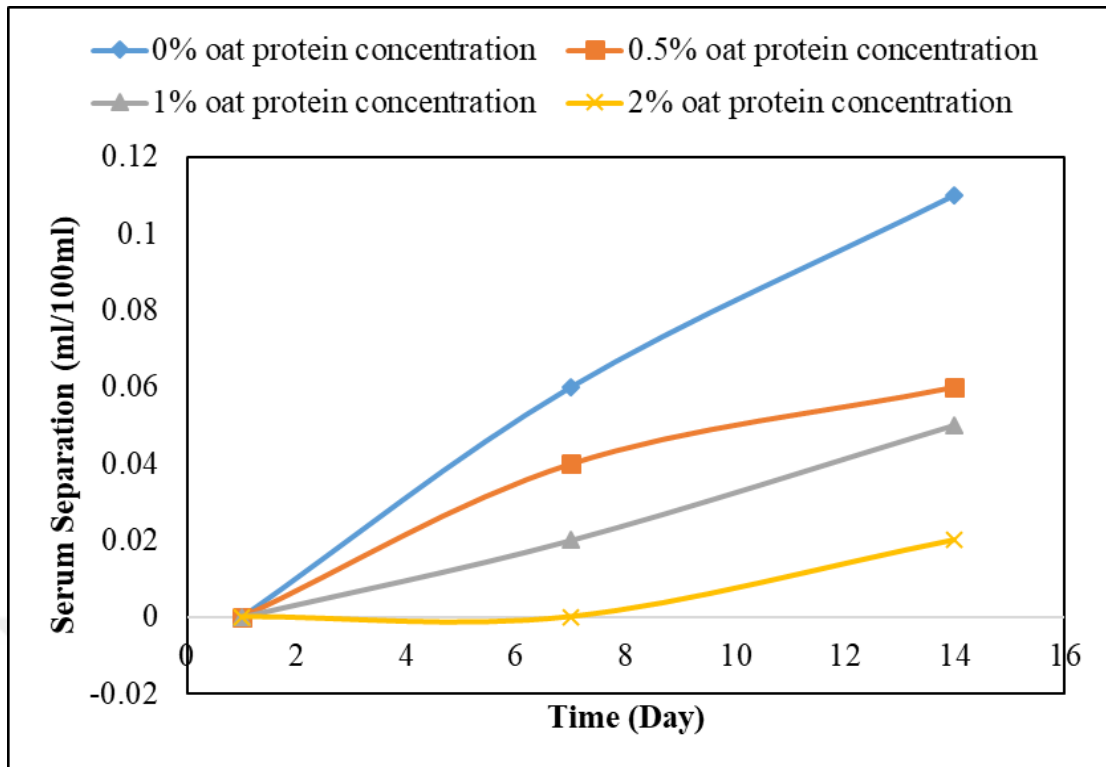


Figure 4.6 Serum separation changes of kefir with oat protein

In this study, serum separation values in the samples ranged from 0 to 0.11 mL/100 mL. It was observed that serum separation decreased as the oat protein concentration increased. It is known that an increase in the amount of protein causes an increase in gel strength (Faergemand et al., 1999; Russell et al., 2006). Studies have shown that protein supplementation reduces water release values during storage, depending on the water binding capacity of proteins (Akalin, 2008; Augustin et al., 2003). By binding long-chain polysaccharides to the casein molecules, the micelle structure is formed and the water holding capacity of the curd in kefir increases. During storage, the weakening of the clot causes the structure to release water after a while and serum separation occurs (Vasiljevic et al., 2007).

Considering the variance analysis results for serum separation were analyzed, a significant effect was observed at $p < 0.05$ level for oat protein concentration and storage time (Appendix A9).

4.2.4 Viscosity

Texture is important for the quality of fermented milk products and encompasses all sensory, structural and rheological properties (Ozcan, 2013). Viscosity is defined as the resistance of the food material against flow. During the production of fermented milk products, a viscous structure emerges as a result of gelation of protein molecules in the structure of milk with the effect of heat and pH. Viscosity value of fermented milk products is an important criterion used to determine the curd structure. In addition, in sensory analysis, it is stated that viscosity is an important quality control criterion that determines consistency (Brennan, 2005; Domagała et al., 2006).

The average viscosity value obtained as a result of the analysis of kefir samples and the results of homogeneous subsets were given in Table 4.11 and Table 4.12. And the graph of viscosity of oat protein added kefir samples was given in Figure 4.7.

Table 4.11 The viscosity changes of kefir samples

Oat protein concentration (%w/v)	Viscosity (cP) during storage		
	Day 1	Day 7	Day 14
0.00%	149	156.15	166.1
0.50%	176	191.2	172.6
1%	180.2	195.3	188
2%	187	198	205

Table 4.12 Homogeneous subsets of viscosity

		Viscosity
Oat Protein Concentration (%)	0%	157.0833 ^a
	0.50%	179.9333 ^{ab}
	1%	187.8333 ^b
	2%	196.6667 ^b
Storage Time	Day 1	175.9750 ^a
	Day 7	179.5375 ^a
	Day 14	185.6250 ^a

*Values marked with different letters are statistically different ($p < 0.05$).

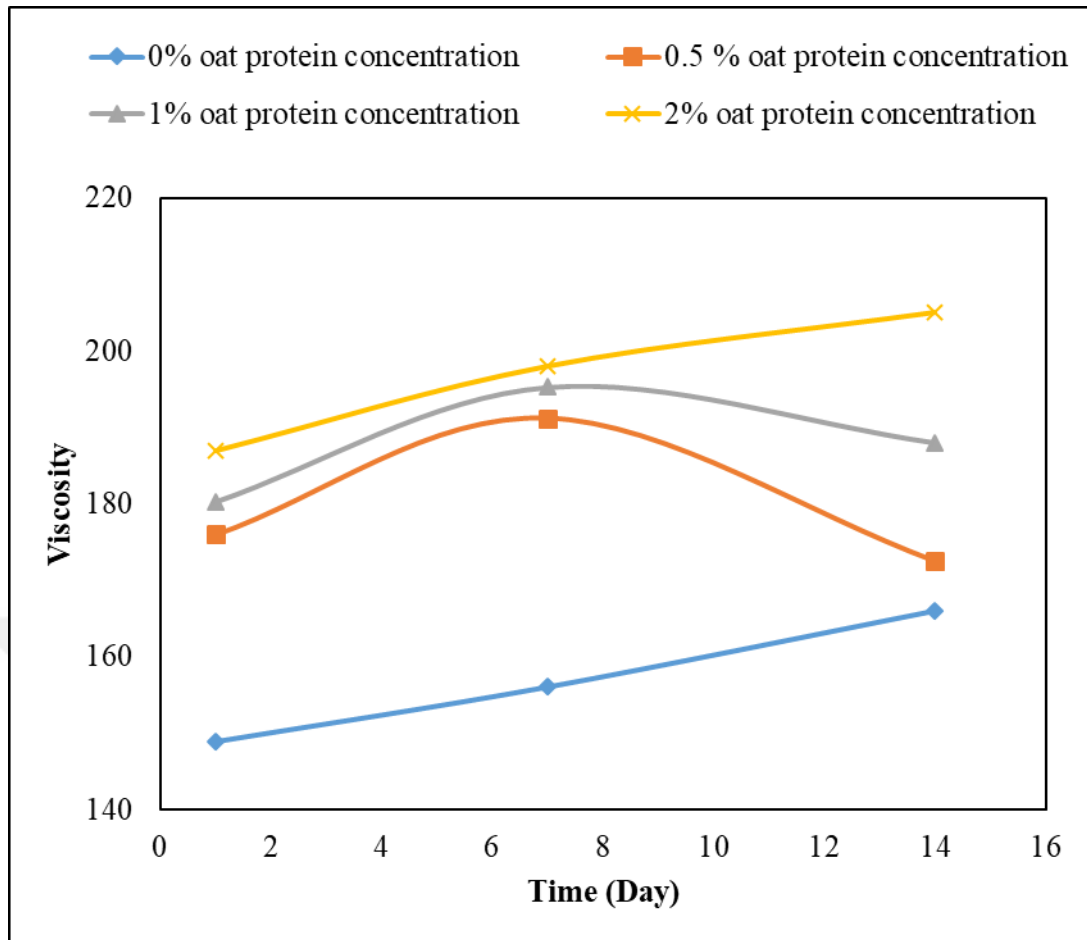


Figure 4.7 Viscosity of oat protein added kefir samples

Viscosity values in kefir samples ranged from 149 cP to 205 cP at 125 rpm, room temperature. It was observed that the addition of oats increased the viscosity of kefir. The lowest viscosity value was observed in the 0% oat protein added kefir on the 1st day, and the highest pH value was observed on the 14th day in the 2% oat protein added kefir.

There are significant differences between the viscosity values reported by different researchers. Ünal et al. (2020) determined while the average viscosity value of kefir samples at 50 rpm was 318.46 cP, the average value at 100 rpm was 196.36 cP. In her study, Songun (2016) found the viscosity values of the control, 1% (w/v) and 2% (w/v) inulin added kefir samples to be between 365.8-488.7cP. There is no viscosity standard for kefir samples. However, it is known that the viscosity increases as the protein concentration increases (Keser, 2020). Proteins used in the enrichment of fermented milk products with high nutritional value and digestibility are among the most preferred additives (Delikanlı, 2012; Havea, 2006).

In the study of Dimitreli et al., (2013) it was reported that the viscosity values of kefir samples to which whey concentrate was added increased. This result are consistent with our results.

Considering the variance analysis results for viscosity were examined, a significant difference was observed for oat protein concentration while there was no difference for storage time at $p < 0.05$ level (Appendix A10).

4.2.5 Total Phenolic Substance

Determining the total amount of phenolic substances in foods is important in terms of giving an idea about the hydroxyl groups that provide antioxidant activity (Huang et al., 2005). The Folin Ciocalteu (FC) Method was used for the measurement of total phenolic substances of kefir samples (Prior et al., 2005). Standard curve of gallic acid was used for total phenolic substance results was given in Appendix A18.

The average total phenolic substance of samples and the results of homogeneous subsets were given in Table 4.13 and Table 4.14. And the graph of total phenolic substance of kefir samples was given in Figure 4.8.

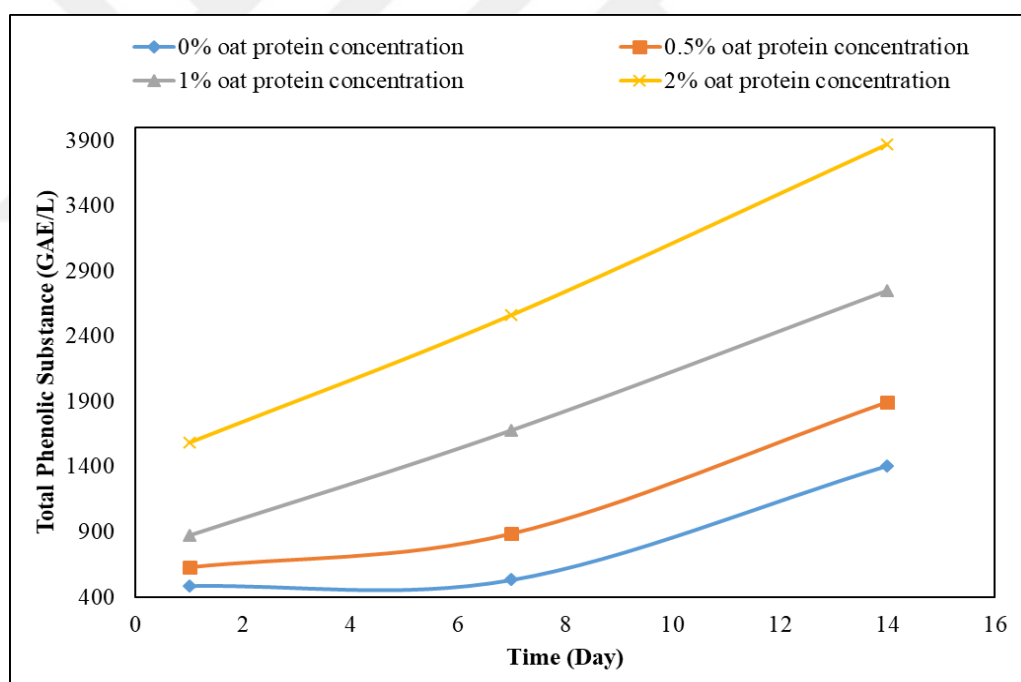
Table 4.13 The total phenolic substance changes of kefir samples

Oat protein concentration (%)	Total phenolic substance (mg gallic acid equivalents (GAE/L)) during storage		
	Day 1	Day 7	Day 14
0.00%	487	533	1410
0.50%	628	886	1896
1%	875	1682	2754
2%	1589	2567	3875

Table 4.14 Homogeneous subsets of total phenolic substance

		Total phenolic substance
Oat Protein Concentration (%)	0%	810.0000 ^a
	0.50%	1256.6667 ^a
	1%	2738.0000 ^b
	2%	3567.0000 ^b
Storage Time	Day 1	1712.2500 ^a
	Day 7	1942.0000 ^{ab}
	Day 14	2624.5000 ^b

*Values marked with different letters are statistically different.

**Figure 4.8** Total phenolic substance of kefir samples

The total phenolic substance values of the samples varied between 487 and 3875 mg gallic acid/L. Yirmibeşoğlu ve Öztürk (2020) found the total phenolic content of kefir obtained from cow's milk using local kefir grain was 516.61 mg GAE/ml, in their study.

Considering the variance analysis results for total phenolic substance were analyzed, a significant effect was observed at $p < 0.05$ level for oat protein concentration and storage time (Appendix A11).

4.2.6 Determination of *Lactococcus* Bacteria

In this study, only the number of *Lactococcus*, which is a group of lactic acid bacteria species, was determined to investigate the effect of oat protein on the number of microorganisms in kefir. The amount of *Lactococcus* of samples was given in Table 4.15 during storage. And the graph of amount of *Lactococcus* of kefir with oat protein was given in Figure 4.9.

Table 4.15 The amount of *Lactococcus* changes of kefir samples

Oat protein concentration (%)	The amount of <i>Lactococcus</i> (x 10 ⁷) (cfu/ml) during storage		
	Day 1	Day 7	Day 14
0.00%	2.02	3.72	0.09
1%	1.8	5.3	3.4

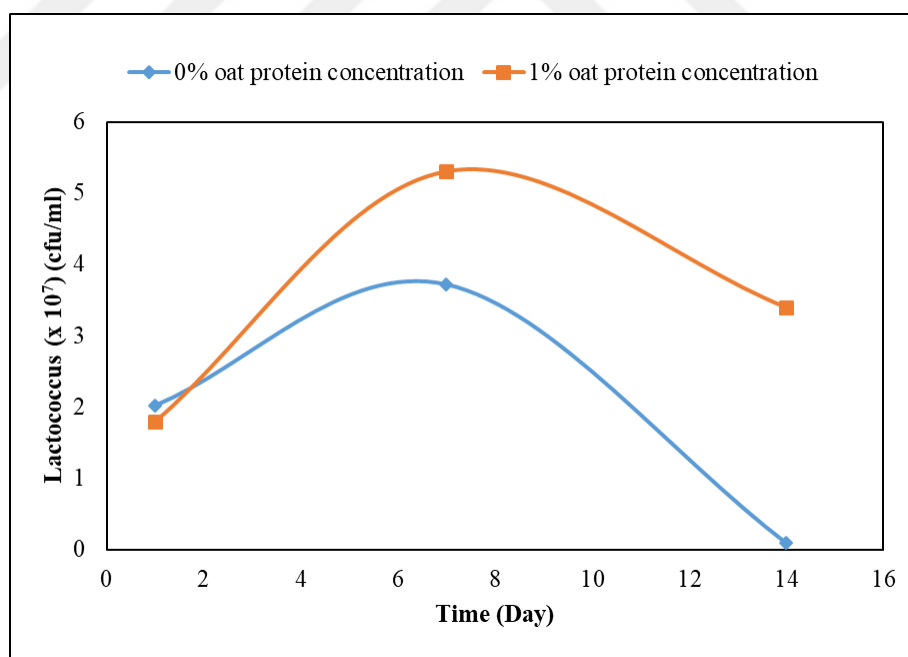


Figure 4.9 Amount of *Lactococcus* of kefir with oat protein

The amount of *Lactococcus* of the samples varied between 0.09×10^7 and 5.3×10^7 cfu/ml. In the Turkish Food Codex Communiqué on Fermented Milk, it is stated that

the number of lactic acid bacteria of kefir should be at least 10^7 cfu/ml. The results found were in accordance with the standard.

As seen in the graphic, microorganisms first multiplied, after a while they started to decrease due to depletion of nutrients and accumulation of toxic substances in the environment.

It has been observed that the addition of oat protein increases the amount of lactococcus in kefir. Oat protein concentration has a significant effect at $p < 0.05$ level on the amount of *Lactococcus* in kefir, while storage time has no significant effect (Appendix A11).

4.2.7 Sensory Analysis

The sensory properties of a product are one of the most important factors that determine consumer taste. The biochemical composition and production method of fermented milk products affect the sensory quality. In this context, vegetable protein additives with unique flavor properties can cause differences in the flavors of the products they are included in the composition (Delikanlı, 2012).

Evaluation was made by giving points from 1 to 5. 1 point means unacceptable, 5 points means excellent. Sensory analysis results in terms of sourness, sweetness, bitterness, pungency, astringency and aroma intensity determined for taste were given in Table 4.16. Average appearance, odor, taste, consistency and general acceptability values obtained as a result of sensory analysis of kefir samples enriched with oat protein were given in Table 4.17. No similar study has been found on this subject.

Table 4.16 The taste assesment of kefir samples

Taste during storage								
Time	Oat Conc. (%)	Sour	Sweet	Bitter	Sharp	Astringency	Aroma intens.	avg
Day 1	0%	3.2	1.5	1.75	3.8	2.8	2.8	2.6
	0.50%	2.8	3.2	2.8	2.9	3.3	2.6	2.9
	1%	2.5	3.5	3.4	3.5	3.6	2.8	3.2
	2%	2.2	2.5	3.2	3.5	3.2	3.4	3
Day 7	0%	4.8	3.4	1.5	2.2	1.4	1.2	2.4
	0.50%	4.2	4.6	3.4	3.1	3.5	3.6	3.7
	1%	3.5	4.5	4.3	4.4	4	4.6	4.2
	2%	3.2	3.8	4.2	4.7	4.7	4.8	4.2
Day 14	0%	3.6	2.8	2.2	2.3	2.3	2.5	2.6
	0.50%	3.6	3.7	3.4	3.2	3.5	3.8	3.5
	1%	3.8	4.7	4.8	3.4	2.2	4.1	3.8
	2%	3.5	4.3	3.9	3.5	4.2	4.6	4

Table 4.17 The sensory assesment of kefir samples with oat protein

Time	Oat Protein Concentration (%)	Appearance	Odor	Taste	Consistency	Overall acceptability
Day 1	0%	3.8	2.8	2.6	3.8	3.3
	0.50%	3.7	2.6	2.9	2.3	2.9
	1%	3.6	3.3	3.2	2.8	3.3
	2%	3.9	3.2	3	2	3
Day 7	0%	3.8	3.1	2.4	3.6	3.2
	0.50%	3.6	3	3.7	2.5	3.2
	1%	3.5	3.5	4.2	2.7	3.5
	2%	3.4	3.7	4.2	2.3	3.4
Day 14	0%	4	3.5	2.6	2.5	3.2
	0.50%	4.2	3.5	3.5	3	3.6
	1%	3.9	3.2	3.8	2.8	3.4
	2%	4.2	3.8	4	2.5	3.6

4.2.7.1. Appearance

The lowest score for appearance was kefir with 1% oat protein on day 7, while the highest score was kefir with 0.5% and 2% oat protein on day 14. The opaque appearance of kefir with 2% oat protein was appreciated by the panelists. It was reported to have a uniform structure and serum separation was less than the others.

Considering the variance analysis results for appearance, no significant difference was observed for concentration while a significant effect was observed for storage time at the $p < 0.05$ level (Appendix A13).

4.2.7.2 Odor

The lowest score for odor was kefir with 0.5% oat protein on first day, while the highest score was kefir with 2% oat protein on day 14.

Considering the variance analysis results for odor, a significant effect was observed for concentration and storage time at the $p < 0.05$ level (Appendix A14).

4.2.7.3 Taste

The lowest score for taste was kefir with 0% oat protein on the 7th day, while the highest score was kefir with 1% and 2% oat protein on the 7th day. Panelists noted that the aroma intensity was most pronounced in kefir with 2% oat protein. They liked the astringency taste caused by the oat protein.

Considering the variance analysis results for taste, a significant effect was observed for concentration and storage time at the $p < 0.05$ level (Appendix A15).

4.2.7.4 Consistency

The lowest score for consistency was kefir with 2% oat protein on the first day, while the highest score was kefir with 0% oat protein on the first day.

Considering the variance analysis results for consistency, a significant effect was observed for concentration and storage time at the $p < 0.05$ level (Appendix A15).

4.2.7.5 Overall Acceptability

According to the results of sensory analysis, kefir with 0.5% and 2% oat protein got the highest scores from the panelists. According to the evaluations of the panelists, kefir samples have the desired properties in terms of odor and consistency. Panelists liked the slightly brown color caused by the oat protein and found the taste and aroma preferable.

Considering the variance analysis results for overall acceptability, a significant effect was observed for concentration while no significant effect for storage time at the $p < 0.05$ level (Appendix A17).

CHAPTER V

CONCLUSION

The outcomes of the study may be resumed as follows:

- Water holding capacity, oil holding capacity, foaming properties, solubility and emulsion properties of oat protein were determined.
- Oat protein solubility was lowest at pH 5 and increased as pH increased.
- It was observed that the emulsion properties changed with the oat protein concentration. The emulsion activity index decreased as the concentration increased. As the concentration increased, the emulsion stability index increased, and accordingly the creaming index decreased.
- Different amounts of oat protein were added to kefir, and the pH, titratable acidity, serum separation, viscosity, total phenolic substance, *Lactococcus* amount and sensory properties of kefirs were determined.
- Oat protein concentration was statistically significant for pH, titratable acidity, serum separation, viscosity, total phenolic substance and *Lactococcus* amount ($p < 0.05$).
- Titratable acidity values increased during the storage period. As the oat protein concentration increased, the acidity value increased. And due to this, pH values decreased.
- It was observed that serum separation decreased as the oat protein concentration increased.
- The viscosity value and total phenolic substances increased as the oat protein concentration increased and during the storage period.
- It has been observed that the addition of oat protein increases the amount of *Lactococcus*.
- Sensory analyzes were performed on the parameters of appearance, taste, odor, structure and general acceptability. According to sensory analysis, kefir with 0.5% and 2% oat protein was the highest score overall among the panelists.

It can be said that functional dairy products will contribute to science in terms of creating a different research area as well as it concerns consumer taste and health. Factors such as durability, sensory acceptance, ingredient absorption and its effect on human health are involved in the implementation of functional dairy products in industry. In addition to product development, there is a need for marketing of products with the production and analysis of functional milk ingredients. Depending on the developments in dairy technology in the future, production of new functional dairy products will be possible. Plant protein sources, especially oats, can be used in the development of functional dairy products. Due to the low variety of products in terms of kefir in our country, studies should be conducted.

In this study, it was aimed to determine the properties of oat protein, to obtain a functional product by adding oat protein to kefir, and to determine the physicochemical and sensory properties of kefir with oat protein. In addition, this study is important in terms of expanding the consumption of kefir by increasing the variety. Considering its functional properties, positive effects on health, we can conclude that more studies should be conducted on the use of oat protein in enrichment of kefir.

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APPENDIX

Table A.1 Variance analysis of water holding capacity values of oat protein.

Descriptive Statistics of Water Holding Capacity of Oat Protein						
	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
WHC	3	1.91	2	1.9507	0.02724	0.04717
Valid N (listwise)	3					

Table A.2 Variance analysis of oil holding capacity values of oat protein.

Descriptive Statistics of Oil Holding Capacity of Oat Protein						
	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
OHC	3	2.39	2.75	2.63	0.12	0.20785
Valid N (listwise)	3					

Table A.3 Variance analysis of foaming capacity values and foaming stability values of oat.

Table A.3.1 Descriptive statistics of foaming capacity of oat protein

Descriptive Statistics of Foaming Capacity of Oat Protein						
	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
Foaming capacity	3	86.22	88.35	87.4	0.62554	1.08347
Valid N (listwise)	3					

Table A.3.2 Descriptive statistics of foam stability of oat protein

Descriptive Statistics of Foam Stability of Oat Protein						
	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
Foam stability	3	66.45	68.28	67.2	0.55344	0.95859
Valid N (listwise)	3					

Table A.4 Variance analysis of protein solubility of oat protein.

ANOVA					
oat protein solubility					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6931.734	2	3465.867	19199.116	0
Within Groups	1.083	6	0.181		
Total	6932.817	8			

Table A.5 Variance analysis and LSD analysis of emulsion activity index and emulsion stability index of oat protein.

Table A.5.1 ANOVA results of emulsion activity index and emulsion stability index of oat protein

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Emulsion Activity Index	Between Groups	12591.3	5	2518.25	31685	0
	Within Groups	0.954	12	0.079		
	Total	12592.2	17			
Emulsion Stability Index	Between Groups	14081.5	5	2816.3	33.768	0
	Within Groups	1000.82	12	83.402		
	Total	15082.3	17			

Table A.5.2 Multiple comparisons of emulsion activity index of oat protein

Multiple Comparisons of Emulsion Activity Index of Oat Protein							
LSD							
Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Emulsion Activity Index	0.20%	0.50%	21,19667 [*]	0.23019	0	20.6951	21.6982
		1%	61,73333 [*]	0.23019	0	61.2318	62.2349
		5%	65,28333 [*]	0.23019	0	64.7818	65.7849
		10%	65,79667 [*]	0.23019	0	65.2951	66.2982
		20%	67,43667 [*]	0.23019	0	66.9351	67.9382
	0.50%	0.20%	-21,19667 [*]	0.23019	0	-21.6982	-20.695
		1%	40,53667 [*]	0.23019	0	40.0351	41.0382
		5%	44,08667 [*]	0.23019	0	43.5851	44.5882
		10%	44,60000 [*]	0.23019	0	44.0985	45.1015
		20%	46,24000 [*]	0.23019	0	45.7385	46.7415
	1%	0.20%	-61,73333 [*]	0.23019	0	-62.2349	-61.232
		0.50%	-40,53667 [*]	0.23019	0	-41.0382	-40.035
		5%	3,55000 [*]	0.23019	0	3.0485	4.0515
		10%	4,06333 [*]	0.23019	0	3.5618	4.5649
		20%	5,70333 [*]	0.23019	0	5.2018	6.2049
	5%	0.20%	-65,28333 [*]	0.23019	0	-65.7849	-64.782
		10%	,51333 [*]	0.23019	0.046	0.0118	1.0149
		20%	2,15333 [*]	0.23019	0	1.6518	2.6549
	10%	0.20%	-65,79667 [*]	0.23019	0	-66.2982	-65.295
		0.50%	-44,60000 [*]	0.23019	0	-45.1015	-44.099
		1%	-4,06333 [*]	0.23019	0	-4.5649	-3.5618
		5%	-,51333 [*]	0.23019	0.046	-1.0149	-0.0118
		20%	1,64000 [*]	0.23019	0	1.1385	2.1415
	20%	0.20%	-67,43667 [*]	0.23019	0	-67.9382	-66.935
		0.50%	-46,24000 [*]	0.23019	0	-46.7415	-45.739
		1%	-5,70333 [*]	0.23019	0	-6.2049	-5.2018
		5%	-2,15333 [*]	0.23019	0	-2.6549	-1.6518
		10%	-1,64000 [*]	0.23019	0	-2.1415	-1.1385

*. The mean difference is significant at the 0.05 level.

Table A.5.3 Multiple comparison of emulsion stability index of oat protein

Multiple Comparisons of Emulsion Stability Index of Oat Protein							
LSD							
Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Emulsion Stability Index	0.20%	0.50%	-0.71667	7.45663	0.925	-16.9633	15.5299
		1%	-7.85	7.45663	0.313	-24.0966	8.3966
		5%	-24,19333*	7.45663	0.007	-40.4399	-7.9467
		10%	-31,26333*	7.45663	0.001	-47.5099	-15.017
		20%	-81,11667*	7.45663	0	-97.3633	-64.87
	0.50%	0.20%	0.71667	7.45663	0.925	-15.5299	16.9633
		1%	-7.13333	7.45663	0.358	-23.3799	9.1133
		5%	-23,47667*	7.45663	0.008	-39.7233	-7.2301
		10%	-30,54667*	7.45663	0.001	-46.7933	-14.3
		20%	-80,40000*	7.45663	0	-96.6466	-64.153
	1%	0.00%	7.85	7.45663	0.313	-8.3966	24.0966
		0.50%	7.13333	7.45663	0.358	-9.1133	23.3799
		5%	-16,34333*	7.45663	0.049	-32.5899	-0.0967
		10%	-23,41333*	7.45663	0.009	-39.6599	-7.1667
		20%	-73,26667*	7.45663	0	-89.5133	-57.02
	5%	0.20%	24,19333*	7.45663	0.007	7.9467	40.4399
		0.50%	23,47667*	7.45663	0.008	7.2301	39.7233
		1%	16,34333*	7.45663	0.049	0.0967	32.5899
		10%	-7.07	7.45663	0.362	-23.3166	9.1766
		20%	-56,92333*	7.45663	0	-73.1699	-40.677
	10%	0.20%	31,26333*	7.45663	0.001	15.0167	47.5099
		0.50%	30,54667*	7.45663	0.001	14.3001	46.7933
		1%	23,41333*	7.45663	0.009	7.1667	39.6599
		5%	7.07	7.45663	0.362	-9.1766	23.3166
		20%	-49,85333*	7.45663	0	-66.0999	-33.607
	20%	0.20%	81,11667*	7.45663	0	64.8701	97.3633
		0.50%	80,40000*	7.45663	0	64.1534	96.6466
		1%	73,26667*	7.45663	0	57.0201	89.5133
		5%	56,92333*	7.45663	0	40.6767	73.1699
		10%	49,85333*	7.45663	0	33.6067	66.0999

*. The mean difference is significant at the 0.05 level.

Table A.6 Variance and LSD analysis of Creaming Index of oat protein stabilized emulsions

Table A.6.1 ANOVA results of creaming index of oat protein stabilized emulsions

ANOVA					
Creaming index of oat protein stabilized emulsions					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	8,782 ^a	11	0.798	13.387	0
Intercept	3.537	1	3.537	59.304	0
time	4.109	6	0.685	11.484	0
concentraiton	3.758	5	0.752	12.605	0
time * concentraiton	0	0	.	.	.
Error	1.789	30	0.06		
Total	15.457	42			
Corrected Total	10.571	41			

a. R Squared = 0.831 (Adjusted R Squared = 0.769)

Table A.6.2 Multiple comparison for storage time

Multiple Comparisons for Storage Time						
Dependent Variable: Creaming Index of oat protein stabilized emulsions						
LSD						
(I) storage time(hour)	(J) storage time(hour)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	8	0.0183	0.14099	0.897	-0.2696	0.3063
	16	0.0425	0.14099	0.765	-0.2454	0.3304
	24	0.045	0.14099	0.752	-0.2429	0.3329
	32	-,8433*	0.14099	0	-1.1313	-0.5554
	40	0.19	0.14099	0.188	-0.0979	0.4779
	48	,3067*	0.14099	0.038	0.0187	0.5946
8	0	-0.0183	0.14099	0.897	-0.3063	0.2696
	16	0.0242	0.14099	0.865	-0.2638	0.3121
	24	0.0267	0.14099	0.851	-0.2613	0.3146
	32	-,8617*	0.14099	0	-1.1496	-0.5737
	40	0.1717	0.14099	0.233	-0.1163	0.4596
	48	,2883*	0.14099	0.05	0.0004	0.5763
16	0	-0.0425	0.14099	0.765	-0.3304	0.2454
	8	-0.0242	0.14099	0.865	-0.3121	0.2638
	24	0.0025	0.14099	0.986	-0.2854	0.2904
	32	-,8858*	0.14099	0	-1.1738	-0.5979
	40	0.1475	0.14099	0.304	-0.1404	0.4354
	48	0.2642	0.14099	0.071	-0.0238	0.5521
24	0	-0.045	0.14099	0.752	-0.3329	0.2429
	8	-0.0267	0.14099	0.851	-0.3146	0.2613
	16	-0.0025	0.14099	0.986	-0.2904	0.2854
	32	-,8883*	0.14099	0	-1.1763	-0.6004
	40	0.145	0.14099	0.312	-0.1429	0.4329
	48	0.2617	0.14099	0.073	-0.0263	0.5496
32	0	,8433*	0.14099	0	0.5554	1.1313
	8	,8617*	0.14099	0	0.5737	1.1496
	16	,8858*	0.14099	0	0.5979	1.1738
	24	,8883*	0.14099	0	0.6004	1.1763
	40	1,0333*	0.14099	0	0.7454	1.3213
	48	1,1500*	0.14099	0	0.8621	1.4379
40	0	-0.19	0.14099	0.188	-0.4779	0.0979
	8	-0.1717	0.14099	0.233	-0.4596	0.1163
	16	-0.1475	0.14099	0.304	-0.4354	0.1404
	24	-0.145	0.14099	0.312	-0.4329	0.1429
	32	-1,0333*	0.14099	0	-1.3213	-0.7454
	48	0.1167	0.14099	0.415	-0.1713	0.4046
48	0	-,3067*	0.14099	0.038	-0.5946	-0.0187
	8	-,2883*	0.14099	0.05	-0.5763	-0.0004
	16	-0.2642	0.14099	0.071	-0.5521	0.0238
	24	-0.2617	0.14099	0.073	-0.5496	0.0263
	32	-1,1500*	0.14099	0	-1.4379	-0.8621
	40	-0.1167	0.14099	0.415	-0.4046	0.1713
Based on observed means.						
The error term is Mean Square(Error) = 0.06.						
*, The mean difference is significant at the 0.05 level.						

Table A.6.3 Multiple comparison for oat protein concentration

Multiple Comparisons for Oat Protein Concentration						
Dependent Variable: Creaming Index of oat protein stabilized emulsions						
LSD						
(I) oat concentration	(J) oat concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0.20%	0.50%	0.0243	0.13053	0.854	-0.2423	0.2909
	1%	0.0507	0.13053	0.7	-0.2159	0.3173
	5%	-,7214*	0.13053	0	-0.988	-0.4548
	10%	0.2071	0.13053	0.123	-0.0594	0.4737
	20%	,3214*	0.13053	0.02	0.0548	0.588
0.50%	0.20%	-0.0243	0.13053	0.854	-0.2909	0.2423
	1%	0.0264	0.13053	0.841	-0.2402	0.293
	5%	-,7457*	0.13053	0	-1.0123	-0.4791
	10%	0.1829	0.13053	0.172	-0.0837	0.4494
	20%	,2971*	0.13053	0.03	0.0306	0.5637
1%	0.20%	-0.0507	0.13053	0.7	-0.3173	0.2159
	0.50%	-0.0264	0.13053	0.841	-0.293	0.2402
	5%	-,7721*	0.13053	0	-1.0387	-0.5056
	10%	0.1564	0.13053	0.24	-0.1102	0.423
	20%	,2707*	0.13053	0.047	0.0041	0.5373
5%	0.20%	,7214*	0.13053	0	0.4548	0.988
	0.50%	,7457*	0.13053	0	0.4791	1.0123
	1%	,7721*	0.13053	0	0.5056	1.0387
	10%	,9286*	0.13053	0	0.662	1.1952
	20%	1,0429*	0.13053	0	0.7763	1.3094
10%	0.20%	-0.2071	0.13053	0.123	-0.4737	0.0594
	0.50%	-0.1829	0.13053	0.172	-0.4494	0.0837
	1%	-0.1564	0.13053	0.24	-0.423	0.1102
	5%	-,9286*	0.13053	0	-1.1952	-0.662
	20%	0.1143	0.13053	0.388	-0.1523	0.3809
20%	0.20%	-,3214*	0.13053	0.02	-0.588	-0.0548
	0.50%	-,2971*	0.13053	0.03	-0.5637	-0.0306
	1%	-,2707*	0.13053	0.047	-0.5373	-0.0041
	5%	-1,0429*	0.13053	0	-1.3094	-0.7763
	10%	-0.1143	0.13053	0.388	-0.3809	0.1523
Based on observed means.						
The error term is Mean Square(Error) = 0.06.						
*. The mean difference is significant at the 0.05 level.						

Table A.7 Variance analysis and LSD test of pH of kefir with oat protein.

Table A.7.1 Variance analysis of pH

Tests of Between-Subjects Effects					
Dependent Variable: pH					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.489 ^a	5	0.098	187.136	0
Intercept	224.814	1	224.814	430494.191	0
Concentration	0.369	3	0.123	235.255	0
Time	0.12	2	0.06	114.957	0
Error	0.003	6	0.001		
Total	225.305	12			
Corrected Total	0.492	11			

a. R Squared = 0.994 (Adjusted R Squared = 0.988)

Table A.7.2 Multiple comparisons of pH of kefir with oat protein for concentration

Multiple Comparisons of Kefir with Oat Protein for Concentration						
Dependent Variable: pH						
LSD						
(I) Oat Protein Concentration of Kefir		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0%	0.50%	,1167*	0.01866	0.001	0.071	0.1623
	1%	,3167*	0.01866	0	0.271	0.3623
	2%	,4533*	0.01866	0	0.4077	0.499
0.50%	0%	-,1167*	0.01866	0.001	-0.1623	-0.071
	1%	,2000*	0.01866	0	0.1543	0.2457
	2%	,3367*	0.01866	0	0.291	0.3823
1%	0%	-,3167*	0.01866	0	-0.3623	-0.271
	0.50%	-,2000*	0.01866	0	-0.2457	-0.1543
	2%	,1367*	0.01866	0	0.091	0.1823
2%	0%	-,4533*	0.01866	0	-0.499	-0.4077
	0.50%	-,3367*	0.01866	0	-0.3823	-0.291
	1%	-,1367*	0.01866	0	-0.1823	-0.091

Based on observed means.
The error term is Mean Square(Error) = .001.
*, The mean difference is significant at the .05 level.

Table A.7.3 Multiple comparisons of pH of kefir with oat protein for storage time

Multiple Comparisons of Kefir with Oat Protein for Storage Time							
Dependent Variable: pH							
LSD							
(I) Storage Time		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval		
					Lower Bound	Upper Bound	
Day 1	Day 7	,1200*	0.01616	0	0.0805	0.1595	
	Day 14	,2450*	0.01616	0	0.2055	0.2845	
Day 7	Day 1	-,1200*	0.01616	0	-0.1595	-0.0805	
	Day 14	,1250*	0.01616	0	0.0855	0.1645	
Day 14	Day 1	-,2450*	0.01616	0	-0.2845	-0.2055	
	Day 7	-,1250*	0.01616	0	-0.1645	-0.0855	
Based on observed means.							
The error term is Mean Square(Error) = .001.							
*. The mean difference is significant at the .05 level.							

Table A.8 The results of variance analysis and LSD test of titratable acidity of kefir with oat protein.**Table A.8.1** Variance analysis of titratable acidity

Tests of Between-Subjects Effects of Titratable Acidity					
Dependent Variable: Titratable Acidity%					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Model	11,440 ^a	6	1.907	1766.17	0
concentration	0.075	3	0.025	23.09	0.001
time	0.036	2	0.018	16.651	0.004
Error	0.006	6	0.001		
Total	11.447	12			
a. R Squared = 0.999 (Adjusted R Squared = 0.999)					

Table A.8.2 Multiple comparisons of titratable acidity of kefir with oat protein for concentration

Multiple Comparisons of Titratable Acidity of Kefir with Oat Protein for Oat Protein Concentration						
Dependent Variable: Titratable Acidity %						
LSD						
(I) oat conc	(J) oat conc	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0%	0.50%	-,1050 [*]	0.02683	0.008	-0.1706	-0.0394
	1%	-,1607 [*]	0.02683	0.001	-0.2263	-0.095
	2%	-,2130 [*]	0.02683	0	-0.2786	-0.1474
0.50%	0%	,1050 [*]	0.02683	0.008	0.0394	0.1706
	1%	-0.0557	0.02683	0.083	-0.1213	0.01
	2%	-,1080 [*]	0.02683	0.007	-0.1736	-0.0424
1%	0%	,1607 [*]	0.02683	0.001	0.095	0.2263
	0.50%	0.0557	0.02683	0.083	-0.01	0.1213
	2%	-0.0523	0.02683	0.099	-0.118	0.0133
2%	0%	,2130 [*]	0.02683	0	0.1474	0.2786
	0.50%	,1080 [*]	0.02683	0.007	0.0424	0.1736
	1%	0.0523	0.02683	0.099	-0.0133	0.118
Based on observed means.						
The error term is Mean Square(Error) = 0.001.						
*. The mean difference is significant at the 0.05 level.						

Table A.8.3 Multiple comparisons of titratable acidity of kefir with oat protein for storage time

Multiple Comparisons of Titratable Acidity of Kefir with Oat Protein for Storage Time						
Dependent Variable: Titratable Acidity %						
LSD						
(I) storage day	(J) storage day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
day1	day7	-,0955*	0.02323	0.006	-0.1524	-0.0386
	day14	-,1293*	0.02323	0.001	-0.1861	-0.0724
day7	day1	,0955*	0.02323	0.006	0.0386	0.1524
	day14	-0.0338	0.02323	0.197	-0.0906	0.0231
day14	day1	,1293*	0.02323	0.001	0.0724	0.1861
	day7	0.0338	0.02323	0.197	-0.0231	0.0906
Based on observed means.						
The error term is Mean Square(Error) = 0.001.						
*. The mean difference is significant at the 0.05 level.						

Table A.9 The results of variance analysis and LSD test of serum separation of kefir with oat protein.

Table A.9.1 Variance analysis of serum separation

Tests of Between-Subjects Effects of Serum Separation					
Dependent Variable: Serum Separation					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Model	,022 ^a	6	0.004	9.676	0.007
concentration	0.004	3	0.001	3.471	0.091
time	0.007	2	0.004	9.529	0.014
Error	0.002	6	0		
Total	0.024	12			
a. R Squared = 0.906 (Adjusted R Squared = 0.813)					

Table A.9.2 Multiple comparisons of serum separation of kefir with oat protein for concentration

Multiple Comparisons of Serum Separations of Kefir with Oat Protein for Oat Protein Concentration						
Dependent Variable: Serum Separation						
LSD						
(I) oat protein concentration	(J) oat protein concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0%	0.50%	0.0233	0.01587	0.192	-0.0155	0.0622
	1%	0.0333	0.01587	0.08	-0.0055	0.0722
	2%	,0500*	0.01587	0.02	0.0112	0.0888
0.50%	0%	-0.0233	0.01587	0.192	-0.0622	0.0155
	1%	0.01	0.01587	0.552	-0.0288	0.0488
	2%	0.0267	0.01587	0.144	-0.0122	0.0655
1%	0%	-0.0333	0.01587	0.08	-0.0722	0.0055
	0.50%	-0.01	0.01587	0.552	-0.0488	0.0288
	2%	0.0167	0.01587	0.334	-0.0222	0.0555
2%	0%	-,0500*	0.01587	0.02	-0.0888	-0.0112
	0.50%	-0.0267	0.01587	0.144	-0.0655	0.0122
	1%	-0.0167	0.01587	0.334	-0.0555	0.0222
Based on observed means.						
The error term is Mean Square(Error) = 0.000.						
*. The mean difference is significant at the 0.05 level.						

Table A.9.3 Multiple comparisons of serum separation of kefir with oat protein for storage time

Multiple Comparisons of Serum Separations of Kefir with Oat Protein for Storage Time						
Dependent Variable: Serum Separation						
LSD						
(I) storage day	(J) storage day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
day1	day7	-0.03	0.01374	0.072	-0.0636	0.0036
	day14	-,0600*	0.01374	0.005	-0.0936	-0.0264
day7	day1	0.03	0.01374	0.072	-0.0036	0.0636
	day14	-0.03	0.01374	0.072	-0.0636	0.0036
day14	day1	,0600*	0.01374	0.005	0.0264	0.0936
	day7	0.03	0.01374	0.072	-0.0036	0.0636
Based on observed means.						
The error term is Mean Square(Error) = 0.000.						
*. The mean difference is significant at the 0.05 level.						

Table A.10 Variance analysis and LSD test of viscosity of kefir with oat protein.

Table A.10.1 Variance analysis of viscosity

Tests of Between-Subjects Effects of Viscosity						
Dependent Variable: Viscosity						
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	
Model	393221,446 ^a	6	65536.9	910.411	0	
concentration	2591.226	3	863.742	11.999	0.006	
time	190.495	2	95.248	1.323	0.334	
Error	431.916	6	71.986			
Total	393653.363	12				
a. R Squared = 0.999 (Adjusted R Squared = 0.998)						

Table A.10.2 Multiple comparisons of viscosity of kefir with oat protein for concentration

Multiple Comparisons of Viscosity of Kefir with Oat Protein for Concentration							
Dependent Variable: Viscosity							
LSD							
(I) oat concentration of kefir		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval		
					Lower Bound	Upper Bound	
0% conc	0.5% conc	-22,8500*	6.92753	0.016	-39.8011	-5.8989	
	1% conc	-30,7500*	6.92753	0.004	-47.7011	-13.7989	
	2% conc	-39,5833*	6.92753	0.001	-56.5344	-22.6323	
0.5% conc	0% conc	22,8500*	6.92753	0.016	5.8989	39.8011	
	1% conc	-7.9	6.92753	0.298	-24.8511	9.0511	
	2% conc	-16.7333	6.92753	0.052	-33.6844	0.2177	
1% conc	0% conc	30,7500*	6.92753	0.004	13.7989	47.7011	
	0.5% conc	7.9	6.92753	0.298	-9.0511	24.8511	
	2% conc	-8.8333	6.92753	0.249	-25.7844	8.1177	
2% conc	0% conc	39,5833*	6.92753	0.001	22.6323	56.5344	
	0.5% conc	16.7333	6.92753	0.052	-0.2177	33.6844	
	1% conc	8.8333	6.92753	0.249	-8.1177	25.7844	
Based on observed means.							
The error term is Mean Square(Error) = 71.986.							
*. The mean difference is significant at the 0.05 level.							

Table A.10.3 Multiple comparisons of viscosity of kefir with oat protein for storage time

Multiple Comparisons of Kefir with Oat Protein for Storage Time							
Dependent Variable: Viscosity							
LSD							
(I) storage day		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval		
					Lower Bound	Upper Bound	
day1	day7	-3.5625	5.99942	0.574	-18.2425	11.1175	
	day14	-9.65	5.99942	0.159	-24.33	5.03	
day7	day1	3.5625	5.99942	0.574	-11.1175	18.2425	
	day14	-6.0875	5.99942	0.349	-20.7675	8.5925	
day14	day1	9.65	5.99942	0.159	-5.03	24.33	
	day7	6.0875	5.99942	0.349	-8.5925	20.7675	
Based on observed means.							
The error term is Mean Square(Error) = 71.986.							
*. The mean difference is significant at the 0.05 level.							

Table A.11 The results of variance analysis and LSD test of total phenolic substance of kefir with oat protein.

Table A.11.1 Variance analysis of total phenolic substance

Tests of Between-Subjects Effects of Total Phenolic Substance of Kefir with Oat Protein					
Dependent Variable: Total Phenolic Substance					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	16603785.417 ^a	5	3320757.083	34.348	0
Intercept	52563602.08	1	52563602.08	543.687	0
concentration	14802730.25	3	4934243.417	51.037	0
time	1801055.167	2	900527.583	9.315	0.014
Error	580079.5	6	96679.917		
Total	69747467	12			
Corrected Total	17183864.92	11			
a. R Squared = .966 (Adjusted R Squared = .938)					

Table A.11.2 Multiple comparisons of total phenolic substance of kefir with oat protein for concentration

Multiple Comparisons of Total Phenolic Substance of Kefir with Oat Protein for Concentration						
LSD						
(I) oat protein conc		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0%	0.50%	-446.6667	253.8765	0.129	-1067.8801	174.5468
	1%	-1928,0000*	253.8765	0	-2549.2134	-1306.7866
	2%	-2757,0000*	253.8765	0	-3378.2134	-2135.7866
0.50%	0%	446.6667	253.8765	0.129	-174.5468	1067.8801
	1%	-1481,3333*	253.8765	0.001	-2102.5468	-860.1199
	2%	-2310,3333*	253.8765	0	-2931.5468	-1689.1199
1%	0%	1928,0000*	253.8765	0	1306.7866	2549.2134
	0.50%	1481,3333*	253.8765	0.001	860.1199	2102.5468
	2%	-829,0000*	253.8765	0.017	-1450.2134	-207.7866
2%	0%	2757,0000*	253.8765	0	2135.7866	3378.2134
	0.50%	2310,3333*	253.8765	0	1689.1199	2931.5468
	1%	829,0000*	253.8765	0.017	207.7866	1450.2134
Based on observed means.						
The error term is Mean Square(Error) = 96679.917.						
*. The mean difference is significant at the 0.05 level.						

Table A.11.3 Multiple comparisons of total phenolic substance of kefir with oat protein for storage time

Multiple Comparisons Total Phenolic Substance of Kefir with Oat Protein for Storage Time						
LSD						
(I) storage day		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
day1	day7	-229.75	219.8635	0.336	-767.7366	308.2366
	day14	-912,2500*	219.8635	0.006	-1450.2366	-374.2634
day7	day1	229.75	219.8635	0.336	-308.2366	767.7366
	day14	-682,5000*	219.8635	0.021	-1220.4866	-144.5134
day14	day1	912,2500*	219.8635	0.006	374.2634	1450.2366
	day7	682,5000*	219.8635	0.021	144.5134	1220.4866
Based on observed means.						
The error term is Mean Square(Error) = 96679.917.						
*. The mean difference is significant at the 0.05 level.						

Table A.12 The results of variance analysis of the amount of *Lactococcus* of kefir with oat protein.

Tests of Between-Subjects Effects of the Amount of Lactococcus of Kefir with Oat Protein					
Dependent Variable: Lactococcus					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Model	48,296 ^a	3	16.099	3.795	0.151
concentration	3.339	1	3.339	0.787	0.44
time	0.022	1	0.022	0.005	0.948
Error	12.725	3	4.242		
Total	61.021	6			

a. R Squared = 0.791 (Adjusted R Squared = 0.583)

Table A.13 Variance analysis and LSD test results of appearance of kefir samples with oat protein

Table A.13.1 Variance analysis of appearance

Tests of Between-Subjects Effects of Appearance of Kefir with Oat Protein					
Dependent Variable: Appearance					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Model	173,868 ^a	6	28.978	1320.52	0
Concentration	0.073	3	0.024	1.114	0.414
Time	0.515	2	0.257	11.734	0.008
Error	0.132	6	0.022		
Total	174	12			

a. R Squared = 0.999 (Adjusted R Squared = 0.998)

Table A.13.2 Multiple comparisons of appearance substance of kefir with oat protein for storage time

Multiple Comparisons of Appearance of Kefir with Oat Protein for Storage Time						
Dependent Variable: Appearance						
LSD						
(I) Storage Time	(J) Storage Time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Day 1	Day 7	0.175	0.10475	0.146	-0.0813	0.4313
	Day 14	-,3250*	0.10475	0.021	-0.5813	-0.0687
Day 7	Day 1	-0.175	0.10475	0.146	-0.4313	0.0813
	Day 14	-,5000*	0.10475	0.003	-0.7563	-0.2437
Day 14	Day 1	,3250*	0.10475	0.021	0.0687	0.5813
	Day 7	,5000*	0.10475	0.003	0.2437	0.7563
Based on observed means.						
The error term is Mean Square(Error) = 0.022.						
*. The mean difference is significant at the 0.05 level.						

Table A.14 Variance analysis and LSD test results of odor of kefir samples with oat protein

Table A.14.1 Variance analysis of odor

Tests of Between-Subjects Effects of Odor of Kefir with Oat Protein					
Dependent Variable: Odor					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Model	129,125 ^a	6	21.521	385.448	0
Concentration	0.5	3	0.167	2.985	0.118
Time	0.572	2	0.286	5.119	0.05
Error	0.335	6	0.056		
Total	129.46	12			
a. R Squared = 0.997 (Adjusted R Squared = 0.995)					

Table A.14.2 Multiple comparisons of odor of kefir with oat protein for concentration

Multiple Comparisons of Odor of Kefir with Oat Protein for Oat Protein Concentration						
Dependent Variable: Odor						
LSD						
(I) Oat Concentration of Kefir	(J) Oat Concentration of Kefir	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0%	0.50%	0.1	0.19293	0.623	-0.3721	0.5721
	1%	-0.2	0.19293	0.34	-0.6721	0.2721
	2%	-0.4333	0.19293	0.066	-0.9054	0.0388
0.50%	0%	-0.1	0.19293	0.623	-0.5721	0.3721
	1%	-0.3	0.19293	0.171	-0.7721	0.1721
	2%	-,5333*	0.19293	0.033	-1.0054	-0.0612
1%	0%	0.2	0.19293	0.34	-0.2721	0.6721
	0.50%	0.3	0.19293	0.171	-0.1721	0.7721
	2%	-0.2333	0.19293	0.272	-0.7054	0.2388
2%	0%	0.4333	0.19293	0.066	-0.0388	0.9054
	0.50%	,5333*	0.19293	0.033	0.0612	1.0054
	1%	0.2333	0.19293	0.272	-0.2388	0.7054
Based on observed means.						
The error term is Mean Square(Error) = 0.056.						
*, The mean difference is significant at the 0.05 level.						

Table A.14.3 Multiple comparisons of odor of kefir with oat protein for storage time

Multiple Comparisons of Odor of Kefir with Oat Protein for Storage Time						
Dependent Variable: odor						
LSD						
(I) Storage Time	(J) Storage Time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Day 1	Day 7	-0.35	0.16708	0.081	-0.7588	0.0588
	Day 14	-,5250*	0.16708	0.02	-0.9338	-0.1162
Day 7	Day 1	0.35	0.16708	0.081	-0.0588	0.7588
	Day 14	-0.175	0.16708	0.335	-0.5838	0.2338
Day 14	Day 1	,5250*	0.16708	0.02	0.1162	0.9338
	Day 7	0.175	0.16708	0.335	-0.2338	0.5838
Based on observed means.						
The error term is Mean Square(Error) = 0.056.						
*. The mean difference is significant at the 0.05 level.						

Table A.15 The results of variance analysis and LSD test results of taste of kefir samples with oat protein**Table A.15.1** Variance analysis of taste

Tests of Between-Subjects Effects of Taste of Kefir with Oat Protein					
Dependent Variable: Taste					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Model	137,970 ^a	6	22.995	222.532	0
Concentration	2.882	3	0.961	9.298	0.011
Time	1.087	2	0.543	5.258	0.048
Error	0.62	6	0.103		
Total	138.59	12			
a. R Squared = 0.996 (Adjusted R Squared = 0.991)					

Table A.15.2 Multiple comparisons of odor of kefir with oat protein for concentration

Multiple Comparisons of Taste of Kefir with Oat Protein for Oat Protein Concentration						
Dependent Variable: Taste						
LSD						
(I) Oat Concentration of Kefir	(J) Oat Concentration of Kefir	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0%	0.50%	-,8333 [*]	0.26247	0.019	-1.4756	-0.1911
	1%	-1,2000 [*]	0.26247	0.004	-1.8422	-0.5578
	2%	-1,2000 [*]	0.26247	0.004	-1.8422	-0.5578
0.50%	0%	,8333 [*]	0.26247	0.019	0.1911	1.4756
	1%	-0.3667	0.26247	0.212	-1.0089	0.2756
	2%	-0.3667	0.26247	0.212	-1.0089	0.2756
1%	0%	1,2000 [*]	0.26247	0.004	0.5578	1.8422
	0.50%	0.3667	0.26247	0.212	-0.2756	1.0089
	2%	0	0.26247	1	-0.6422	0.6422
2%	0%	1,2000 [*]	0.26247	0.004	0.5578	1.8422
	0.50%	0.3667	0.26247	0.212	-0.2756	1.0089
	1%	0	0.26247	1	-0.6422	0.6422
Based on observed means.						
The error term is Mean Square(Error) = 0.103.						
*. The mean difference is significant at the 0.05 level.						

Table A.15.3 Multiple comparisons of taste of kefir with oat protein for storage time

Multiple Comparisons of Taste of Kefir with Oat Protein for Storage Time						
Dependent Variable: Taste						
LSD						
(I) Storage Time	(J) Storage Time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Day 1	Day 7	-,7000*	0.2273	0.022	-1.2562	-0.1438
	Day 14	-0.55	0.2273	0.052	-1.1062	0.0062
Day 7	Day 1	,7000*	0.2273	0.022	0.1438	1.2562
	Day 14	0.15	0.2273	0.534	-0.4062	0.7062
Day 14	Day 1	0.55	0.2273	0.052	-0.0062	1.1062
	Day 7	-0.15	0.2273	0.534	-0.7062	0.4062
Based on observed means.						
The error term is Mean Square(Error) = 0.103.						
*. The mean difference is significant at the 0.05 level.						

Table A.16 The results of variance analysis and LSD test results of consistency of kefir samples with oat protein**Table A.16.1** Variance analysis of consistency

Tests of Between-Subjects Effects of Consistency of Kefir with Oat Protein					
Dependent Variable: Consistency					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Model	130,947 ^a	6	21.824	677.31	0
Concentration	1.057	3	0.352	10.931	0.008
Time	0.527	2	0.263	8.172	0.019
Error	0.193	6	0.032		
Total	131.14	12			
a. R Squared = 0.999 (Adjusted R Squared = 0.997)					

Table A.16.2 Multiple comparisons of consistency of kefir with oat protein for concentration

Multiple Comparisons of Consistency of Kefir with Oat Protein for Oat Protein Concentration						
Dependent Variable: Consistency						
LSD						
(I) Oat Concentration of Kefir	(J) Oat Concentration of Kefir	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0%	0.50%	-0.0333	0.14657	0.828	-0.392	0.3253
	1%	0.3333	0.14657	0.063	-0.0253	0.692
	2%	,7000*	0.14657	0.003	0.3414	1.0586
0.50%	0%	0.0333	0.14657	0.828	-0.3253	0.392
	1%	,3667*	0.14657	0.046	0.008	0.7253
	2%	,7333*	0.14657	0.002	0.3747	1.092
1%	0%	-0.3333	0.14657	0.063	-0.692	0.0253
	0.50%	-,3667*	0.14657	0.046	-0.7253	-0.008
	2%	,3667*	0.14657	0.046	0.008	0.7253
2%	0%	-,7000*	0.14657	0.003	-1.0586	-0.3414
	0.50%	-,7333*	0.14657	0.002	-1.092	-0.3747
	1%	-,3667*	0.14657	0.046	-0.7253	-0.008
Based on observed means.						
The error term is Mean Square(Error) = 0.032.						
*. The mean difference is significant at the 0.05 level.						

Table A.16.3 Multiple comparisons of consistency of kefir with oat protein for storage time

Multiple Comparisons of Consistency of Kefir with Oat Protein for Storage Time						
Dependent Variable: Consistency						
LSD						
(I) Storage Time	(J) Storage Time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Day 1	Day 7	-0.15	0.12693	0.282	-0.4606	0.1606
	Day 14	,3500*	0.12693	0.033	0.0394	0.6606
Day 7	Day 1	0.15	0.12693	0.282	-0.1606	0.4606
	Day 14	,5000*	0.12693	0.008	0.1894	0.8106
Day 14	Day 1	-,3500*	0.12693	0.033	-0.6606	-0.0394
	Day 7	-,5000*	0.12693	0.008	-0.8106	-0.1894
Based on observed means.						
The error term is Mean Square(Error) = 0.032.						
*. The mean difference is significant at the 0.05 level.						

Table A.17 Variance analysis and LSD test results of overall acceptability of kefir samples with oat protein

Table A.17.1 Variance analysis of overall acceptability

Tests of Between-Subjects Effects of Overall Acceptability of Kefir with Oat Protein						
Dependent Variable: Overall Acceptability						
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	
Model	88,172 ^a	6	14.695	90.124	0	
Concentration	1.169	3	0.39	2.39	0.167	
Time	0.062	2	0.031	0.189	0.832	
Error	0.978	6	0.163			
Total	89.15	12				
a. R Squared = 0.989 (Adjusted R Squared = 0.978)						

Table A.17.2 Multiple comparisons of overall acceptability of kefir with oat protein for concentration

Multiple Comparisons of Overall Acceptability of Kefir with Oat Protein for Oat Protein Concentration						
Dependent Variable: overall acceptability						
LSD						
(I) Oat Concentration of Kefir	(J) Oat Concentration of Kefir	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0%	0.50%	0.5333	0.3297	0.157	-0.2734	1.3401
	1%	0.3667	0.3297	0.309	-0.4401	1.1734
	2%	,8667*	0.3297	0.039	0.0599	1.6734
0.50%	0%	-0.5333	0.3297	0.157	-1.3401	0.2734
	1%	-0.1667	0.3297	0.631	-0.9734	0.6401
	2%	0.3333	0.3297	0.351	-0.4734	1.1401
1%	0%	-0.3667	0.3297	0.309	-1.1734	0.4401
	0.50%	0.1667	0.3297	0.631	-0.6401	0.9734
	2%	0.5	0.3297	0.18	-0.3068	1.3068
2%	0%	-,8667*	0.3297	0.039	-1.6734	-0.0599
	0.50%	-0.3333	0.3297	0.351	-1.1401	0.4734
	1%	-0.5	0.3297	0.18	-1.3068	0.3068
Based on observed means.						
The error term is Mean Square(Error) = 0.163.						
*. The mean difference is significant at the 0.05 level.						

Table A.17.3 Multiple comparisons of overall acceptability of kefir with oat protein for storage time

Multiple Comparisons of Overall Acceptability of Kefir with Oat Protein for Storage Time						
Dependent Variable: overall acceptability						
LSD						
(I) Storage Time	(J) Storage Time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Day 1	Day 7	-0.175	0.28553	0.562	-0.8737	0.5237
	Day 14	-0.1	0.28553	0.738	-0.7987	0.5987
Day 7	Day 1	0.175	0.28553	0.562	-0.5237	0.8737
	Day 14	0.075	0.28553	0.802	-0.6237	0.7737
Day 14	Day 1	0.1	0.28553	0.738	-0.5987	0.7987
	Day 7	-0.075	0.28553	0.802	-0.7737	0.6237
Based on observed means.						
The error term is Mean Square(Error) = 0.163.						

Table A.18 Calibration curve of gallic acid

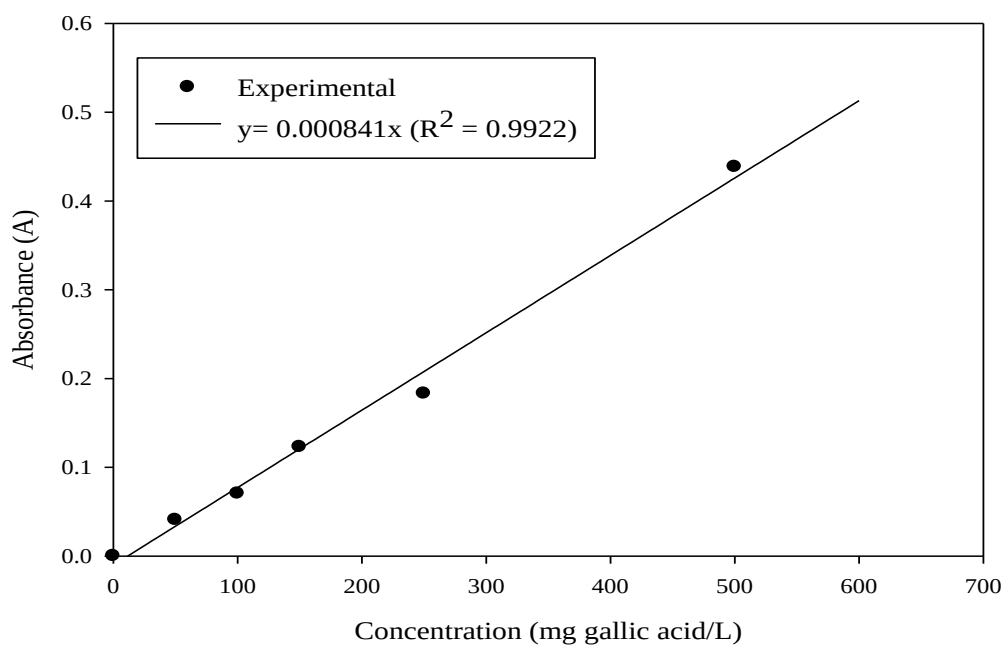
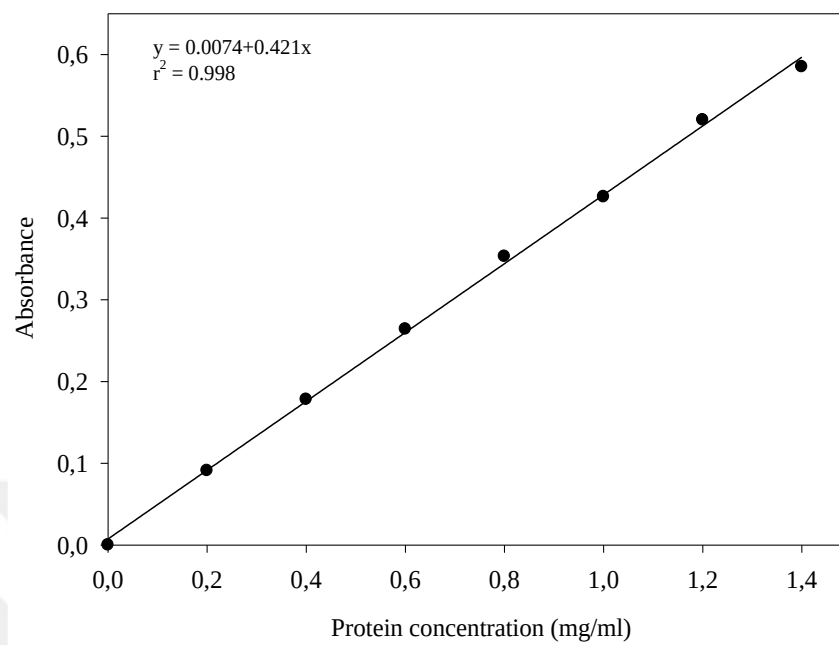


Table A.19 Standard curve for protein determination by Biuret method



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NOTIFICATIONS

1. Karakaş Merve, Erçelebi Emine, Yalçınöz Şelale (2020). Enrichment of kefir with oat protein. GAP International Food, Agriculture and Veterinary Science Congress, 3-5 April Gaziantep, Turkey (Video Conferance), Page 3, ISBN- 978-625-7914-86- 4.
2. Karakaş Merve, Erçelebi Emine, Yalçınöz Şelale (2020). Nutritional and health promoting properties of oat proteins. GAP International Food, Agriculture and Veterinary Science Congress, 3-5 April Gaziantep, Turkey (Video Conferance), Page 4, ISBN- 978-625-7914-86-4.