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DISSERTATION

DEVELOPMENT OF TECHNOLOGY OF FERMENTED MILK DRINKS ENRICHED WITH DIETARY FIBERS

Specialty 181 - Food Technology

Field of study -18 "Production and Technology"

Submitted for a scientific degree of Doctor of Philosophy. The dissertation contains the results of own research. The use of ideas, results and texts of other author have references to the relevant source

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ANNOTATION

Qin Xuanxuan. Development of technology of fermented milk drinks enriched with dietary fibers.

Dissertation for the degree of Doctor of Philosophy in the specialty 181 «Food technology». Sumy National Agrarian University, Sumy, 2023.

The dissertation substantiates the principles and methods that form the scientific basis for the enriching of fermented milk drinks with dietary fibers. Fermented milk drinks are products having certain functional properties according to their chemical composition. They contain proteins, fats, carbohydrates, vitamins A, D, C, E, groups B, H, PP, minerals (K, Ca, F, Cu, Sn, Sr, Mg, Al, Co, Mo), amino acids and beneficial lactic acid microflora. The recommended amount of their daily consumption is 500-750 ml. Among all dairy products produced in the world, the share of kefir production is almost 65%. This product contains all the nutrients the body needs, except for dietary fibers.

According to the theory of rational nutrition the value of food is represented in providing the body with necessary nutrients and its ability to self digest in the human stomach and at the same time be food for those microorganisms that inhabit the intestines and supply our body with the necessary substances. This property is inherent in dietary fiber (ballast substances) that are not part of kefir. Since there are no enzymes in the gastrointestinal tract that break down the fibers, the latter reach the large intestine unchanged. Intestinal bacteria have enzymes that can metabolize fibers. Due to fermentation, they obtain energy for the reproduction and construction of new cells.

Thus, by enriching fermented milk drinks with dietary fiber, in an amount consistent with the recommended daily need for their consumption, it is possible to create a fermented milk drink that will be as balanced as possible in the content of all vital nutrients, including ballast substances.

The purpose of the study is to increase the nutritional value of fermented milk drinks by introducing recycled raw materials containing dietary fiber into their composition.

The object of the research is the technology for the production of fermented milk drinks with plant supplements.

The subject of the study: whole milk; defatted sesame flour; rice bran; model samples of fermented milk drinks enriched with dietary fibers.

The research methodology is based on the chemical composition analysis of raw materials. According to the results of the analysis of the information available in modern literature, a scientific hypothesis is formulated, which is substantiated in the theoretical part of the study and confirmed in the course of experimental research.

During the experimental research, traditional methods of analysis of the subject of research such as physicochemical, microbiological, organoleptic, antioxidant were used. The microbiological composition of products and their storage capacity were analyzed. The influence of dietary fibers on the main properties of products has been established.

According to the materials of the research and the results obtained, the technology for production of fermented milk drinks with the dietary fibers content based on recycled raw material (sesame flour, rice bran) is developed and scientifically grounded.

The first section of the dissertation analyzed the types of raw materials and additives used in the production of fermented milk drinks, modern methods of production of fermented drinks. The functional properties of the components of fermented milk drinks were studied. The functional and therapeutic and preventive properties of fermented milk drinks have been proven by numerous scientifically based facts.

The second section presents the methodology and research methodology. The content of methods of research of organoleptic, physicochemical, microbiological, antioxidant properties of products, the content of vitamins and dietary fibers in them is disclosed. The experimental part of the work was carried out in the laboratory conditions of the School of Food Sciences of the Henan Institute of Science and

Technology (China). The developed product was approved industrially on the basis of the craft enterprise for processing milk of the individual entrepreneur D.V. Opryshko.

The third section describes the results of the study of the influence of the proposed additives on the product properties. Fermented milk drinks with the addition of defatted sesame flour in the amount of 0, 2%, 4%, 6%, 8% were studied during 28 days of storage. Organoleptic properties, microbiological viability, physicochemical properties (total titrated acidity, water-holding capacity, apparent viscosity and pH), antioxidant activity (DPPH radical activity and ·OH radical activity). It was shown that the addition of defatted sesame flour in the amount of 2% had a positive effect on the following indicators: the content of proteins, fats, dietary fibers, pH, total titrated acidity, apparent viscosity, antioxidant activity, water-holding capacity. Microbiological analysis showed that dietary fiber of sesame flour is a good environment for the development of lactic acid microflora. The introduction of defatted sesame flour increases the therapeutic and preventive properties according to the results of DPPH radical activity and ·OH radical activity.

Fermented milk drinks with the addition of rice bran in amounts of 0, 0.1%, 0.3%, 0.5%, 0.7% were studied during 28 days of storage. Organoleptic, physicochemical (total titrated acidity, water-holding capacity, apparent viscosity and pH), microbiological properties, antioxidant activity (activity of DPPH radicals and activity of ·OH radicals) were analyzed. The introduction of rice bran showed a positive effect on these parameters. The fermented milk drink with the addition of rice bran in the amount of 0.1% showed higher sensory evaluation. The recommended shelf life of fortified fermented milk drinks is 14 days. Such results give reason to claim that fermented milk drinks with sesame flour and rice bran can be used for functional nutrition.

The fourth section contains the results of optimization of consumption properties and developed recipes of fermented milk drinks with the addition of defatted sesame flour, rice bran by building a mathematical model, namely: choosing the objective

function, setting the boundary conditions and determining the rational parameters of this mathematical model.

The fifth section of the dissertation discovers the recommendations as regards to the implementation of the developed technologies under industrial conditions. The technological scheme was developed and optimum modes for performing of all technological processes were established. The technological equipment for the production of fermented milk drinks is offered.

Keywords: milk, sour milk products, raw materials of plant origin, sesame flour, rice bran, powder, technology of enrichment, probiotics, relaxation time, viscosity, antioxidant activity, physico-chemical indicators, microbiological indicators, organoleptic indicators, functional nutrition.

АНОТАЦІЯ

Цзінь Сюаньсюань. Розробка технології кисломолочних напоїв збагачених харчовими волокнами. - Кваліфікаційна наукова праця на правах рукопису. Дисертація на здобуття наукового ступеня доктора філософії за спеціальністю 181 «Харчові технології». – Сумський національний аграрний університет, Суми, 2023.

В дисертаційній роботі обґрунтовано принципи і методи, що формують наукові основи збагачення кисломолочних напоїв харчовими волокнами. Кисломолочні напої за хімічним складом є продуктами з певними функціональними властивостями. Вони містять білки, жири, вуглеводи, вітаміни A, D, C, E, групи B, H, PP, мінеральні речовини (K, Ca, F, Cu, Sn, Sr, Mg, Al, Co, Mo), амінокислоти та корисну молочнокислу мікрофлору. Рекомендована кількість їх щоденного споживання становить 500-750 мл. Серед всіх молочних продуктів, що виробляються у світі, частка виробництва кефіру становить майже 65%. Цей продукт містить всі потрібні організму нутрієнти, крім харчових волокон.

Згідно з теорією раціонального харчування цінність їжі полягає у забезпеченні організму необхідними нутрієнтами та її здатності самоперетравлюватися в шлунку людини і одночасно бути поживним середовищем для тих мікроорганізмів, для яких кишечник є середовищем існування. Така властивість притаманна харчовим волокнам (баластним речовинам), які не містяться в кефірі. Оскільки в шлунково-кишковому тракті відсутні ферменти, що розщеплюють волокна, останні потрапляють до товстого кишечника незмінними. Кишкові бактерії мають ферменти, які можуть метаболізувати волокна. Завдяки бродінню вони отримують енергію для розмноження та побудови нових клітин.

Таким чином, збагачуючи кисломолочні напої харчовими волокнами в кількості, що відповідає рекомендованій потребі для їх щоденного споживання,

можливо створити напій, який буде максимально збалансованим за вмістом життєво важливих поживних речовин, в тому числі баластних речовин.

Метою дослідження є підвищення біологічної цінності кисломолочних напоїв за рахунок вторинної сировини, що містить харчові волокна.

Об’єктом дослідження є технологія виготовлення кисломолочних напоїв із рослинними добавками.

Предмет дослідження: незбиране молоко; знежирене кунжутне борошно; рисові висівки; модельні зразки кисломолочних напоїв, збагачені харчовими волокнами.

Методологія дослідження основана на аналізі хімічного складу рослинної сировини. Відповідно до результатів аналізу інформації, доступної в сучасній літературі, сформована наукова гіпотеза, яка обґрунтована в теоретичній частині дослідження та підтверджена в процесі експериментальних досліджень.

В ході експериментальних досліджень використовувалися традиційні методи аналізу фізико-хімічних, мікробіологічних, органолептичних, антиоксидантних властивостей продукту. Проаналізовано мікробіологічний склад продуктів та їх здатність до зберігання. Встановлено вплив харчових волокон на основні властивості продуктів.

Відповідно до матеріалів дослідження та отриманих результатів розроблена та науково обґрунтована технологія виготовлення кисломолочних напоїв з вмістом харчових волокон на основі вторинної сировини (кунжутного борошна, рисових висівок та водяного горіха).

В першому розділі дисертаційної роботи проаналізовано види сировини та добавок, які використовуються при виробництві кисломолочних напоїв, сучасні способи виробництва ферментованих напоїв. Досліджено функціональні властивості компонентів кисломолочних напоїв. Чисельними науково-обґрунтованими фактами доведено функціональні та лікувально-профілактичні властивості кисломолочних напоїв.

У другому розділі представлено методику та методологію проведення досліджень. Розкрито зміст методів дослідження органолептичних, фізико-хімічних, мікробіологічних, антиоксидантних властивостей продуктів, вмісту вітамінів та харчових волокон в них. Експериментальна частина роботи проведена в лабораторних умовах Школи харчових наук Хєнанського Інституту Науки та Технології (Китай). Промислова апробація розробленого продукту здійснена на базі крафтового підприємства з переробки молока ФОП «Опришко Денис Володимирович».

У третьому розділі представлено результати дослідження впливу запропонованих добавок на властивості продукту. Досліджені кисломолочні напої з додаванням знежиреного кунжутного борошна в кількості 0, 2%, 4%, 6%, 8% протягом 28 днів зберігання. Органолептичні властивості, мікробіологічна життєздатність, фізико-хімічні властивості (загальна титрована кислотність, водоутримуюча здатність, умовна в'язкість та pH), антиоксидантна активність (активність радикалів DPPH та активність радикалів·OH). Показано, що додавання знежиреного кунжутного борошна у кількості 2% позитивно вплинуло на такі показники: вміст білків, жирів, харчових волокон, pH, загальну титровану кислотність, умовну в'язкість, антиоксидантну активніст, водоутримуючу здатність. Мікробіологічний аналіз показав, що харчові волокна кунжутного борошна є гарним середовищем для розвитку молочнокислої мікрофлори. Введення знежиреного кунжутного борошна підвищує лікувально-профілактичні властивості відповідно до результатів активності радикалів DPPH та радикалів OH.

Досліджені кисломолочні напої з додаванням рисових висівок в кількості 0, 0.1%, 0.3%, 0.5%, 0.7% протягом 28 днів зберігання. Проаналізовано органолептичні, фізико-хімічні (загальна титрована кислотність, водоутримуюча здатність, умовна в'язкість та pH), мікробіологічні властивості, антиоксидантна активність (активність радикалів DPPH та активність радикалів·OH). Введення рисових висівок показало позитивний вплив на ці параметри. Кисломолочний напій з додаванням рисових висівок в кількості 0.1% показав вище сенсорне прийняття.

В четвертому розділі представлено результати оптимізації споживних властивостей та розроблено рецептури кисломолочних напоїв із додаванням знажиреного кунжутного борошна, рисових висівок та водяного горіха шляхом побудови математичної моделі, а саме: вибору цільової функції, встановлення граничних умов та визначення раціональних параметрів даної математичної моделі.

П'ятий розділ дисертаційної роботи містить рекомендації щодо впровадження розроблених технологій у промислових умовах. Розроблено технологічну схему та встановлено оптимальні режими проведення всіх технологічних процесів. Запропоновано технологічне обладнання для виробництва кисломолочних напоїв.

Ключові слова: молоко, кисломолочні продукти, сировина рослинного походження, кунжутне борошно, рисові висівки, порошок, технологія збагачення, пробіотики, час релаксації, в'язкість, антиоксидантна активність, фізико-хімічні показники, мікробіологічні показники, органолептичні показники, функціональне харчування.

LIST OF THE APPLICANT'S PUBLICATIONS

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9. Qin Xuanxuan. Applications of sesame in fermented drinks. The 2nd International scientific and practical conference "The world of science and innovation" (London, United Kingdom, September 16-18, 2020) p. 216-220. (*PhD participant in carrying out of experimental research, processing of results, and writing the article*).
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11. Qin Xuanxuan, Samilyk M., Luo Yanghe. The influence of dietary fiber in rice bran on kefir. The 1st International scientific and practical conference "Science and technology: problems, prospects and innovations" (Osaka, Japan, October 19-21, 2022) p. 19-27. (*PhD participant in carrying out of experimental research, processing of results, and writing the article*).

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INTRODUCTION

Fermented milk drinks have a number of functional properties according to their chemical composition. They contain proteins, fats, carbohydrates, vitamins A, D, C, E, groups B, H, PP, minerals (K, Ca, F, Cu, Sn, Sr, Mg, Al, Co, Mo), amino acids, fungi, lacto- and bifidobacteria.

According to the theory of rational nutrition the value of food is represented in providing the body with necessary nutrients and its ability to self digest in the human stomach and at the same time be food for those microorganisms that inhabit the intestines and supply our body with the necessary substances. This property is inherent in dietary fiber (ballast substances) that are not part of kefir. Since there are no enzymes in the gastrointestinal tract that break down the fibers, the latter reach the large intestine unchanged. Intestinal bacteria have enzymes that can metabolize fibers. Due to fermentation, they obtain energy for the reproduction and construction of new cells.

Relevance of the topic. By enriching fermented milk drinks with dietary fiber in the amount that corresponds to the recommended need for their daily consumption, it is possible to create a drink that will be as balanced as possible in terms of the content of vital nutrients, including ballast substances.

It is known that the food industry takes the second place after metallurgical industry as regards to the amount of generated production waste. Therefore, the task of scientists and technologists is not just to create products from functional products based on plant raw materials, but to maximize the use of recycled resources (derived products). Derived products of plant processing are valuable raw materials, as they contain not only dietary fibers, but also other biologically active components that can positively affect the nutritional, biological value and physicochemical properties of products.

Connection of work with scientific programs, plans, themes. The dissertation was developed within the research work theme plan of Sumy National Agricultural University of Ukraine, on the subject of research of the Department of Technology and

Food Safety 0119U101237 "Innovative technological solutions in the production of food products" and 0121U111511 "Industrial waste management of food production"

The purpose and the objectives of the research. *The purpose of the dissertation is* to increase the nutritional value of fermented milk drinks by introducing recycled raw materials containing dietary fiber into their composition.

To achieve the goal the following tasks should be solved:

- analyzing the functional properties of the plant raw materials containing dietary fibers;
- investigating the effect of raw materials on organoleptic, physicochemical, microbiological indicators of fermented milk drinks;
- analyzing antioxidant properties of fermented milk drinks enriched with additives and their storage capacity;
- justifying the optimal recipe and rational parameters of the production process of fermented milk drinks enriched with dietary fibers;
- developing the technology of production of fermented milk drinks enriched with food fiber and giving recommendations for their implementation in the production.

The object of the research is the technology for producing fermented milk drinks enriched with plant additives.

The research subject is whole milk; defatted sesame flour; rice bran; model samples of fermented milk drinks enriched with dietary fibers.

The research methods include a complex of traditional and modern methods of sensory assessment, physical, chemical, microbiological properties, simulated in vitro digestion, biomedical experiments, experimental planning, and experimental data processing.

Scientific novelty of the obtained results of the scientific research. The adding of recycled raw materials (defatted sesame flour, rice bran) improves not only properties of the fermented milk drinks (organoleptic, physical and chemical, rheological properties), but gives certain functional properties to them, that has been scientifically substantiated and experimentally proven. The prebiotic properties of the additives have a positive effect on vitality of probiotic microorganisms, the developed products maintain probiotic properties within the whole storage period.

It succeeded in increasing the shelf life of the product to 14 days without the use of preservatives and stabilizers.

For the first time, it is proposed to use recycled raw materials (derivatives of sesame and rice processing) as a functional additive in the production of fermented milk drinks. Thus, the issue of reducing industrial waste in food production and rational integrated use of raw materials is solved.

The practical significance of the results obtained. On the ground of experimental research the optimal recipe of fermented milk drinks with adding of defatted sesame flour, rice bran is offered, the technological modes of all processes for industrial technology of production of new enriched fermented milk drinks are developed.

Personal contribution of the applicant includes: analysis of the state of the problem, development of a research program, organization, execution and generalization of analytical and experimental studies, analysis and generalization of the obtained data in the form of conclusions, preparation of research materials for publications, preparation of the dissertation, conducting industrial testing.

The dissertation work was carried out with the methodological and scientific support of Ph.D., Assistant Professor M.M. Samilyk.

The personal contribution of the dissertation student is documented by scientific works.

Approbation of dissertation results. The dissertation research results are introduced at: All-Ukrainian scientific conference of students and postgraduates dedicated to the International Student's Day (Sumy, November 15-19, 2021); The 2nd International scientific and practical conference "The world of science and innovation" (London, United Kingdom, September 16-18, 2020); The 1st International scientific and practical conference "Priority directions of science and technology development" (Kyiv, Ukraine, September 27-29, 2020.); The 15th International scientific and practical conference “Innovations and prospects of world science” (Vancouver, Canada, October 12-14, 2022); The 1st International scientific and practical conference “Science and technology: problems, prospects and innovations” (Osaka, Japan, October 19-21, 2022).

Publications. The results of the dissertation are reflected in 11 printed works, including: 4 articles in scientific publications according to the specialty included on the date of publication in the list of specialized scientific publications of Ukraine, 1 article in a periodical scientific publication indexed in the Web of Science Core Collection database, 2 an article in a periodical scientific publication indexed in the Scopus database, 1 in a foreign specialist publication and 4 abstracts of reports at scientific, scientific-practical and international conferences. In accordance with the Resolution of the Cabinet of Ministers of Ukraine "On approval of the Procedure for awarding the degree of Doctor of Philosophy and cancellation of the decision of the one-time specialized academic council of the institution of higher education, scientific institution on awarding the degree of Doctor of Philosophy" dated January 12, 2022 No. 44, the number of publications of the recipient is 5.

Structure and scope of the dissertation. The dissertation consists of an introduction, 5 sections, conclusions, a list of used literary sources and appendices. The main content of the work is represented on 160 pages, including 35 tables, 47 figures, appendices (per page). The list of used bibliographic sources contains 253 names (on 34 pages).

SECTION 1
THEORETICAL JUSTIFICATION OF THE FEASIBILITY OF PRODUCTION OF ENRICHED FERMENTED MILK DRINKS

1.1 The characteristics of milk raw materials for production of fermented milk drinks

The raw materials for the production of fermented milk drinks are cow, goat, camel, buffalo milk (Gul et al., 2018), sheep (Yilmaz-Ersan et al., 2018), donkey milk (Annamaria et al., 2018), etc.

One of the ways to produce fermented milk drinks is by mixing several types of milk (Cais-Sokolińska, 2016), or by adding additives to improve their functional properties and final texture (Pawe et al., 2012). An alternative way to produce fermented milk drinks is to use non-dairy substrates such as fruit and molasses to produce sweet kefir, which has unique sensory properties such as a refreshing taste due to the presence of ethanol, a fruity aroma due to the presence of esters, and a body and texture attributed to its glycerin content (Fiorda et al., 2017).

The traditional method of preparing fermented milk drinks is to incubate milk with kefir grains. The kefir grains are inoculated into sterilized milk and fermented at 25°C until a pH of 4.4 is reached. The grain and milk are then separated using a sterilized plastic filter at the end of the fermentation process (Dong-Hyeon et al., 2018).

There is a way of preparing a sweet fermented milk drink. The kefir grains are placed into a solution containing 8% sucrose, dried fruits (typically figs) and some slices of lemon. Fermentation for one or two days at room temperature results in a cloudy, carbonated and straw coloured drink, poor in sugar, slightly alcoholic, and acidic. (Gulitz et al., 2011).

Goat milk is widely known as a main dietary product in many countries. Its nutritional value determines the wide consumption of goat milk products. Goat milk is also known to be beneficial for the health of consumers. Compared with other types of milk, goat milk has unique biologically active properties, such as high digestibility, distinct alkalinity, high buffering capacity, and certain therapeutic properties in human medicine and nutrition (Park et al., 2007).

Observed by size of fat globules, goat milk has smaller size than cow milk Attaie et al., 2000; Hodgkinson et al., 2018) and contains higher concentration of medium chain fatty acids (Park et al., 2007). Other advantages, casein in goat milk is also lower than cow milk (12-15g/L), ranging from 0.9 to 7 g/L (Farrell, 2004). Cinnamon (Cinamomum sp.) is widely used as a food ingredient, mainly as a flavoring agent. Indonesia has been one of the major cinnamon producers in the world for the last ten years, approximately 90000 tons/year (Food and Agricultural Organization, 2015).

A study (Setiyoningrum et al., 2019) examined the addition of ginger and cinnamon extracts to a goat milk fermented beverage.

Several literatures have been published on phenolic content and antioxidant activity of cinnamon such as gallic acid, p-hydroxybenzoic acid, p-hydroxybenzaldehyde, protocatechuic acid, salicylic acid, syringic acid, vanillic acid, vanillin, caffeic acid, quercetin, tannic acid, chlorogenic acid, ferulic acid, p-coumaric acid, cinnamic acid, sinapic acid and eugenol (Helal et al., 2014; Klejdus et al., 2016).

All of the compounds are phenolic compounds. Other literature reported that cinnamon has important biological activities such as antimicrobial and anti-inflammatory (Cheng et al., 2012). Ginger is widely used as a spice and condiment in many countries. Essential oil of ginger consists of sesquiterpenes, carbonyl compounds, alcohols, and monoterpene hydrocarbons (Onyenekwe et al., 1999).

All those compounds contribute to its aroma and flavor. (Si et al., 2018) reported that four main compounds that contributed to the prevention of oxidation are 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol. Ginger extract has different antioxidant capacity depending on the part of the plant. Rhizome has higher antioxidant activity compared to other part which is IC so value 8.29± 1.73 ug/mL (Ali et al., 2018). Results showed that the supplementation of cinnamon or ginger extract has influenced fat content, antioxidant capacity and sensory result of goat kefir. Increasing concentration level of the extract induced an increase of antioxidant capacity and fat content.

Otherwise, adding extract into goat kefir induced a negative sensory result than control.

In the study (PERNA et al., 2018), the phenolic content and antioxidant activity of fermented milk drinks made from donkey milk and fortified with sulla honey and REO during refrigerated storage were studied. Honey is frequently used as a sweetener in fermented milks; it, in fact, can be considered to be a natural syrup, containing primarily fructose and glucose, with flavour derived from flower essences. Sulla honey is a typical product of southern and central Italy. Its smell is faint and floral and it has a sweet and slightly acidic taste. The colour varies from white to straw yellow (Gambacorta et al. 2014).

The addition of essential oils from aromatic herbs is another means of improving the flavour and beneficial properties of dairy products Cutrim and Cortez 2018; Khorshidiana et al. 2018). Essential oils are synthesized in various plant organs, constituted from a complex mixture of polyphenols, which are useful in food preservation, the fragrance industry and aromatherapy (Teixeira et al. 2013). One of the most important sources of natural antioxidants is rosemary (Rosmarinus officinalis L.) of the Labiatae family (Tavassoli and Djomeh 2011).

Today, rosemary is widely used in dairy foods to improve several qualities, including antioxidant properties (Marinho et al., 2015; Cutrim and Cortez, 2018. Rosemary essential oil (REO) is an almost colourless or pale yellow liquid that possesses the characteristic odour of the plant. Due to its major constituents, 1,8-cineole, trans-caryophyllene, camphor, and a-pinene and to the synergy among these, REO can be considered a natural compound with strong antioxidant capacity (Tavassoli and Djomeh 2011).

The results of the study showed that donkey fermented milk drink has a high antioxidant potential that interacts with a wide range of species directly responsible for oxidative damage. This study highlighted the fact that the antioxidant activity of donkey fermented milk drink was strongly influenced by both the type of added polyphenols and the formation of polyphenol–protein complexes, which influence the availability of bioactive components. Sensory analysis demonstrated that the donkey fermented milk drink was well accepted by consumers; also, that fermented milk drink fortified with sulla honey showed the highest acceptability, whereas the one fortified with REO was less acceptable to consumers.

According to the difference of substrate, kefir can be divided as animal milk based kefir and water kefir, such as soy milk (Botelho et al., 2014), peanut milk (Bensmira & Jiang, 2015), honey (Fiorda et al., 2016), vegetables (Marina et al., 2018), juices (Ozcelik et al., 2021) and so on.

1.2 The effect of the main components of fermented milk drinks on the human body

Fermented milk drinks are recommended for use in the treatment of diseases of the gastrointestinal tract, hypertension, allergies, coronary heart disease. Scientific studies have shown that the health benefits of fermented milk drinks consist of anti-pathogenic activity, anti-tumor and anti-cancer activity, lactose dyspepsia, increasing synthesis of vitamin B, anti-inflammatory, immunomodulatory effects and cholesterol-lowering effects (Fuquay, 2011).

It is known that fermented milk drinks act against bacteria such as *Salmonella*, *Shigella*, *Helicobacter pylori*, *Clostridium difficile*, *Escherichia coli*, *Staphylococcus*, *Enterobacter pneumoniae*, *Bacillus subtilis*, *Proteus vulgaris*, *Micrococcus luteus*, *Streptococcus pyogenes*, *Listeria monocytogenes* and *Candida albicans* (Prado et al., 2015; Silva Fernandes et al., 2017).

Fermented milk drinks contain also vitamins, macronutrients such as K, Ca, Mg, P and micronutrients such as Cu, Zn, Fe, Mn, Co, and Mo (Ozer & Akdemir-Evrendilek, 2014).

1.2.1 The effect of probiotics on the human body

The probiotic properties of fermented milk drinks are derived from kefir grains or cultures containing a variety of strains (Gul et al., 2015). The microflora of kefir and kefir grains varies depending on the origin and production methods (Gul et al., 2015**).**

The fermented milk drinks contain lactic acid bacteria such as *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Lactobacillus delbrueckii ssp. bulgaricus*, *Lactobacillus kefir*, *Lactobacillus acidophilus*, *Enterococcus faecium*, *Enterococcus faecalis*, *Streptococcus thermophilus*, *Lactococcus lactis ssp*., Yeast, *Kluyveromyces marxianus*, *Kluyveromyces lactis*, *Candida kefir*, *Saccharomyces cerevisiae*, *Saccharomyces kefir*, *Saccharomyces unisporus*, *Zygosaccharomyces rouxii*, *Torulaspora delbrus*, *Torulaspora delbrueckii*, and *Debarymyces hansenii* (Shah, 2017).

It is known that weight gain and obesity are related to the changes of gut microbiota. The increase of Lactococcus, Lactobacillus and yeasts induces up-regulation of PPARα and promotes lipid oxidation. These microorganisms are included in the fermented milk drinks and can also decrease inflammation and serum cholesterol. These improvements can ameliorate obesity and fatty liver disease, thereby reducing weight and liver damage (Dong-Hyeon et al., 2017).

Colon cancer is the most common type of cancer with the highest mortality and morbidity, and is considered the fourth most common type of cancer in men and the third most common type of cancer in women. Some bacteria can attach to the surface of the epithelium because of a specific protein structure. In this case, there is pressure in the cells at the attachment point, resulting in the cells' mutation and generation, producing cancer tissue and ulcerative colitis in the intestinal tissue. The attachment allows other pathogens to attach to the surface, leading to aggregation (Guzel-Seydim et al., 2016). There are some mechanisms hypothesized to explain the positive results of reducing colon cancer risk after fermented milk drink consumption. The microorganisms present in kefir can attach to the surface of epithelial cells, eliminating the attachment of pathogenic bacteria. Also, lactic acid microflora is able to produce antibacterial compounds that can cause the destruction of pathogenic microorganisms (Guzel-Seydim et al., 2016).

Fermented milk drink probiotics contribute to intestinal flora and immune regulation, reduce TNF-α in the gut and bones, and reduce bone loss.

Food allergy is a worldwide disease, and its prevalence is on the rise (Bonefeld et al., 2014). Eosinophils are the immune system elements that appear in inflammatory areas during anaphylaxis. They play an active role in the pathological process that begins with an allergen. For allergic diseases, such as bronchial asthma and atopic dermatitis, eosinophils are increased in blood and associated tissue. One of the main mechanisms of food allergy is the increasing of immunoglobulin E (IgE) response caused by the imbalance of T helper cell 1/T helper cell 2 (Th1/Th2) (Bourrie et al., 2016). Some studies have shown that lactic acid bacteria can prevent and heal allergic diseases. The mechanism of lactic acid bacteria's antiallergic effect is not fully understood, but it is believed that the cytokines stimulated by lactic acid bacteria play a key role in immune regulation. Activated Th1 reactive probiotics *lactobacillus* are completed by the production of cytokines. IL-12 plays a key role by stimulating Th1-dominated immune response and altering cellular immunity (Hong et al., 2010).

Fermented milk drinks have the potential to improve immune responses in animal and human models (Ebner et al., 2015). There are two main mechanisms by which probiotics exert beneficial effects: direct action on the living microorganisms (probiotics), or indirect action on their metabolites (organisms) (Vinderola et al., 2006). It is established that kefir consumption increased the specific intestinal mucosal immune response against cholera toxin in young adult rats. (Thoreux and Schmucker, 2001). However, the same effect was not observed in senescent rats. Kefir consumption in BALB/c female mice modulated the intestinal mucosal immune response because IgA+ and IgG+ cells were increased after feeding with kefir regardless of the fact that the kefir contained different doses of viable or heat-inactivated bacteria.

In a study by (Vinderola et al., 2005), the importance of kefir dose and cell viability to obtain an intestinal mucosal immune response in the mouse gut was investigated. The fermented milk drink increased the phagocytic activity of peritoneal and pulmonary macrophages. In the second study, these authors investigated the immunomodulating capacity of kefir produced by the kefir microflora L. kefir anofaciens. The oral administration of kefiran (100 mg/kg) for 7 days resulted in improved gut mucosal response with an increased number of IgA+ cells and caused a concurrent increase in small intestine IL-4, IL-6, IL-10+, and IL-6+ cells.

High serum cholesterol levels (>240 mg/dL) increase the risk of cardiovascular diseases (Gürsoy et al., 2017). Results were obtained by Huang et al, who found a significant reduction in serum total cholesterol, triglyceride, and LDL cholesterol levels as well as cholesterol and triglyceride levels in liver of rats fed a high-cholesterol diet supplemented with *Lactobacillus plantarum* Lp09 and Lp45 with bile-salt hydrolase activity isolated from kefir grains. Other *Lactobacillus* stains with cholesterol-reducing effects such as *L. plantarum* Lp27 (Huang, Wu, et al., 2013), *L. plantarum* B23, *Lactobacillus acidophilus* LA15, and *Lactobacillus* kefir D17 were also isolated from Tibetan kefir grains (Liu et al). (H. Liu et al., 2012) also demonstrated that the bile-salt hydrolase activity of *Kluyveromyces marxianus* strains K1 and M3 isolated from Tibetan kefir was the main responsible mechanism for their cholesterol-reducing activity.

Foods containing probiotics can alter the gut flora and provide a beneficial effect on the host, both by ensuring that new strains enter the gastrointestinal system and by promoting the growth of beneficial bacteria (Bourrie et al., 2016). In an animal study, fermented milk drink intake increased the number of beneficial bacteria, such as *Lactobacillus* and bifidobacterial, and reduced harmful bacteria, such as *Clostridium perfringens* (Hamet et al., 2016). Another study showed that kefir can reduce the severity of intestinal Giardia infection in rats (Franco et al., 2013). *Clostridium difficile* is an anaerobic Gram-positive bacterium that can cause problems in the gastrointestinal system, such as diarrhea. With the emergence of antibiotic-resistant strains, the incidence of diarrhea caused by Clostridium difficile has increased. A study in rats has shown that Kefir has a protective effect against enterocolitis caused by *Clostridium difficile* (Bolla et al., 2013).

The research has shown that the presence of d-amino acids in fermented milk drinks is important because they are used to compensate for the complete absence of l-form metabolic requirements. For example, the use of D-amino acids in infants was searched and was determined that both d-alanine and d-valine were used in protein synthesis (Fiorella et al., 2016).

Polysaccharides in kefir particles had a protective effect against lung metastasis in rats, while the non-effervescent polysaccharides could prevent skin metastasis (Fernando Lopitz-Otsoa et al., 2006). One of the mechanisms of its antitumor action is the reduction of TNF-α, TNF-β, and Bc12 secretion. Low levels of TNF-α and TNF-β secretion have an anti-proliferative effect on cancer cells. In addition, the sphingomyelin in kefir increases the secretion of interferon (Sharifi et al., 2017).

The bioactive exopolysaccharides (EPS) produced by kefir microorganisms appear to trigger a cascade that leads to increased insulin release from pancreatic β-cells and increased glucose uptake by cells and peripheral tissues. EPS activates glucagon-hormone (similar to peptide 1 (GLP-1)), gastric inhibitory peptide (GIP), and adenylate cyclase by circulating adenosine monophosphate (c-AMP), sensitizes Ca^{2+} ions, and activates protein kinase A. In this way, the pancreatic β-cells release an increased amount of insulin. Thus, an increase in c-AMP in pancreatic β-cells seems to help the cells produce insulin better.

There are evidences that fermented milk drinks and its polysaccharide extract possess anti-inflammatory activity. In some organisms kefir show anti-inflammatory properties by inhibiting the formation of granuloma tissue (Rodrigues et al., 2005b). Kefir along with other substances exhibits varying degrees of anti-inflammatory activity; in suspensions with molasses, fermented milk and kefiran extract presented an inhibition of 41, 44, and 34%, respectively, for the inflammatory process. For determination of pharmacological application of kefir, (Lee та співавтори, 2007) produced an artificial asthma problem based on ovalbumin sensitization in a mouse model that produced inflammation in the airway system. In such animals when kefir (50 ppm) was administered through intragastric mode, it displayed anti-allergic and anti-inflammatory effects by inhibiting ovalbumin-induced eosinophilia in lung tissue. At the same time, hypersecretion of mucous by goblet cells was also observed in the airway (Elias et al., 2003). In another attempt, a similar result was experienced by administering kefiran (Kwonetal., 2008). Anti-inflammatory effect was also observed when L. plantarum isolated from kefir was administered orally in mouse model (Lee et al., 2007).

1.2.2 The effect of protein and peptides

Bioactive peptides are bioactive ingredients produced during fermentation that have a direct or indirect anti-inflammatory effect on the microbiota (Rosa et al., 2017) . Bioactive peptides in the fermented milk drinks induce macrophage activation, nitric oxide (NO) formation, and phagocytosis. In addition, they increase TNF-α and cytokine secretion and decrease IL-8 secretion. Increased levels of the cytokine IL-5 increased IgA secretion, while decreased levels of IL-8 inhibited neutrophil activity and controlled inflammation (Sharifi et al., 2017).

Metabolic syndrome (MetS) is characterized by anthropometric and physiological abnormalities that lead to high glucose levels, hypertension, obesity, high triglycerides, and low-density lipoprotein cholesterol (HDL-c) levels. MetS is considered to be the leading cause of type 2 diabetes and other diseases such as hyperuricemia, gout, mild kidney disease, oxidative stress, chronic inflammation and endothelial dysfunction (Rosa et al., 2016). Bioactive peptides are produced during fermentation and can reduce the risk of metabolic syndrome and its complications through a variety of mechanisms, such as: regulation of insulin levels; regulation of blood pressure; satiety response; absorption of free radicals; improvement of blood lipid level (Rosa et al., 2016). Thus, the effect of fermented milk drink consumption on metabolic syndrome is associated with effects on obesity, lipid metabolism, hypertension, and diabetes.

Wound healing involves a range of functions, such as cell division, cell migration, chemotaxis, and differentiation of multiple cell types. Although topical antibiotics are commonly used for wound healing, alternative treatments are actively sought due to bacterial resistance and the side effects of overuse of antibiotics. Studies have shown that the antibacterial properties of fermented milk drink polysaccharides produced by probiotics can prevent the proliferation of pathogenic bacteria, reduce the inflammatory response after the aggregation of wound lymphocytes and macrophages, strengthen the immune system, and accelerate wound healing (Huseini et al., 2012). A study of rats reported that fermented milk drinks increased collagen collection in rats with corrosive esophagitis and had a positive effect on wound healing (Yasar et al., 2013). Fermented milk drinks inhibit the growth of bacterial and fungal cells, regulate the immune system, and speed up wound healing Bourrie et al., 2016).

The protein component of kefir promotes an increase in insulin-like growth factor (IGF)-I, which promotes bone formation.

Epidemiological studies have shown that intake of fermented milk products may lessen the risk of onset of breast cancer in women (Reddy et al., 1983; Veeret al., 1989). This reduced risk of breast cancer may be attributed to the presence of certain bioactive components in fermented milk that may include certain proteins and small peptides. Their antitumorigenic activities have been confirmed by feeding trials on animal models both in cancer and in cultured tumor cells (Svensson et al., 1999). These bioactive components have the capacity to prevent the cancer initiation; these also work by suppressing the initiated tumor growth by hindering certain enzymes so that conversion of procarcinogen to carcinogen is eliminated. The other mechanism by which cancer initiation process slows down is the activation of the immune system (Kneating, 1985). Anticarcinogenic effect of kefir and kefir extract has been studied extensively. Several other workers also reported encouraging data about antitumor activities of kefir in animal models, but this time this antitumor activity may be due to presence of some polysaccharides in kefir extracts (Shiomi et al., 1982; Furukawa et al., 1990; Kubo et al., 1992; Cevikbas et al., 1994). In a planned experiment, in which mice were artificially transplanted with solid tumors of E-ascites carcinoma, an oral dose of 100 or 500 mg/kg of kefir resulted in a major decrease in tumor size by activating the immunosuppressive action in spleen (Kubo et. al., 1992). Consumption of 2 g/day kefir product for a period of nine days is more beneficial as compared to yogurt to lower the chance of tumor (Furukawa et al., 1990) but soy-milk kefir has proved the best among all of these (Liu et al., 2002).

Kefiran, which is a water-soluble glucogalactan, either isolated from kefir grain or produced by L. *kefiranofaciens*, a strain isolated from kefir (Wang et al., 2008), also has antitumor activity. However, during comparison study about water soluble and insoluble polysaccharides in kefir, water-soluble polysaccharides appeared more effective for suppression of tumors (Furukawa et al., 2000) and their effectiveness against tumors also improved at higher dosage level (Murofushi et al., 1983).

Apart from nature and dosage of polysaccharides, antitumor activity is also dependent on type of microorganisms during fermentation (Liu et al., 2002) especially Lactobacillus plays an important role (Santos et al., 2003). Another important nutrient that has a role in anticarcinogenic activity is the protein. (Guzel-Seydim et al, 2003) also revealed the idea that milk proteins, especially sulfur containing amino acid, play a major role in providing anticarcinogenic activity in kefir and similar products. Involvement of immune cells in the antitumor effect of kefir in a murine breast cancer model was first time reported by De Moreno. In such a model, immune response in the mammary gland played an important role to avoid tumor growth. Chances of estrogen-dependent human breast cancer cells can also be minimized by the use of kefir extract (Chen et al., 2007).

Several researchers previously claimed the ability of some Lactobacilli for production of antimicrobial compounds. This antimicrobial property may be attributed to the presence of hydrogen peroxide, peptides (bacteriocins), ethanol, carbon dioxide, diacetyl, and organic acids (lactic and acetic acids). These all substances serve towards preservation of food products and reduction in foodborne pathogens, food production, and storage. These also exhibit some nutraceutical effects by preventing gastrointestinal disorders and vaginal infections (Zamfir et al., 1999; Liu et al., 2008; Zhou et al., 2008; Simova et al., 2009).

Fermented milk drinks also contain inherited properties of milk in addition to advantageous factors produced by microflora during fermentation. These beneficial components of kefir inhibit the pathogens by primary and secondary metabolites such as small peptides, diacetyls, and organic acids produced by kefir microflora

(Golowczyc et al., 2008). In general, fermented milk drinks have got bacteriostatic effect on Gram-negative organisms but a better bactericidal effect against Gram-positive organisms was reported (Czamanski et al., 2004). The killing action of kefir against *Listeria monocytogenes*, *Yersinia enterocolitica*, *Escherichia coli* (Gulmez and Guven, 2003), *Listeria innocua* (Morgan et al., 2000), *Salmonella enteritidis* (Czamanski et al., 2004; Golowczyc et al., 2007), and *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella enteritidis*, *Listeria monocytogenes* (Silva et al., 2009). It was noted that kefir grains hydrolyze the nonreducing sugars, which later on converted into organic acids and some inhibitory substances having anti-pathogenic activity. The inhibited species included: *Staphylococcus aureus, Candida albicans, Shigella sonnei, Escherichia coli, Salmonella typhi, Streptococcus pyrogenes and Candida albicans* (Rodrigues et al., 2005a; Silva et al., 2009).

1.2.3 The influence of calcium

Dairy products are a good source of calcium. They promote bone formation, prevent bone loss, and reduce bone absorption (Tu et al., 2015).

The study (Schepper et al., 2017; M.-Y. Tu et al., 2015) showed that direct supplementation of beneficial probiotic bacteria can affect bone health by regulating aspects of the gut such as preventing dysbiosis and /or increases in gut permeability and inflammation.

Dietary components play an important role in protecting the body from oxidative damage. Kefir contains a large number of antioxidants (Ahmed et al., 2013). This effect increased GSH-P x level and decreased malondialdehyde level. Kefir can also bind 1,1-diphenyl-2-picrylhydrazyl (DPPH) and superoxide radical to inhibit the oxidation of linoleic acid (Ahmed et al., 2013). A study on antioxidant capacity of goat milk kefir showed that the content of total phenols decreased significantly with the extension of fermentation time and reached the highest level at the 14th day of storage.

1.2.4 The influence of lactic acid

Cardiovascular disease is one of the most common causes of death. Kefir is rich in lactic acid, which can directly or indirectly reduce cholesterol levels by 33% by binding to hexogen cholesterol and inhibiting intestinal bacteria (Rosa et al., 2017). Lactic acid reduces the pH in the gastrointestinal tract, which is unsuitable for the growth of pathogens. (Marquina et al., 2002) reported that the consumption of kefir increased the population of lactic acid bacteria and decreased the population of *Enterobacteriaceae* and *Clostridia* in the mucosa of mice bowel.

1.2.5 The effect of acetic acid

Acetic acid has an antibacterial effect on pathogens (Shahani & Chandan, 1979). Fermented milk drinks have an antibacterial effect against many pathogenic organisms due to the inherent formation of organic acids, hydrogen peroxide, acetaldehyde, carbon dioxide, and bacteriocins (Powelletal., 2007). The antibacterial effect of kefir produced from a freeze-dried commercial starter culture (PROBAT KC3, Danisco, Denmark) was determined against *Staphylococcus aureus* (ATCC, 29213), *Bacillus cereus* (ATCC 11778), *Salmonella enteritidis* (ATCC 13076), *Listeria monocytogenes* (ATCC 7644), and *Escherichia coli* (ATCC 8739) and compared with ampicillin and gentamicin (Ulusoy et al., 2007).

The antimicrobial effect was determined after 24 h and 48 h fermentations and during 7 days cold storage. Zones of inhibition formed by the antibiotics and the kefir samples were similar for each pathogen. For example, the inhibition zone (in diameter) for E. coli was 19.5 mm, 18.6 mm, 20.2 mm, and 20.8 mm for 24 h fermented kefir, 48 h fermented kefir, ampicillin, and gentamicin, respectively. Antimicrobial activity of kefir was as effective as ampicillin and gentamicin while neither the length of fermentation nor the duration of cold storage significantly affected the antimicrobial activity (Ulusoy et al., 2007).

1.3 The application of functional additives in the production of fermented milk drinks

Fermented milk drinks can be combined with many types of plant ingredients. Many scientists have devoted themselves to healthy nutrition, introducing plant supplements into the fermented milk drink (Zakharova, 2014). Studies have shown that the introduction of plant ingredients will lead to a decrease in viscosity, whey separation and a decrease in sensory properties.

The addition of flavorings and stabilizers leads to a decrease in the population of probiotics, and as a result - a decrease in the biological value of fermented milk drinks (Huang et al., 2015; Liu et al., 2018; Shleikin, 2015). Therefore, it is an urgent task to introduce such plant ingredients that would not have a negative effect on the physico-chemical and rheological properties of fermented milk drinks, especially on the stability of kefir. A large number of different additives are used to create functional fermented milk drinks, for example, anthocyanin-rich juices (Kabakc et al., 2020), coffee (Vimercati et al., 2020), mango peel (Vicenssuto & Castro, 2020), the addition of fava mucilage and chickpeas (Saadi et al., 2020) etc.

There are studies on the use of non-traditional ingredients in the production of milk drinks. Recipes of drinks enriched with wheat germ, medicinal plants, plant extracts, barley grains, and lactulose have been developed (Odarchenko and Karpenko, 2015; Arsenyeva and Baranova, 2017; Lemekhova and Nesterenko, 2011; Romanenko and Didukh, 2011; Hamagayeva and Kachanina, 2005). A study (Saadi et al., 2020) examined the effect of incorporating green bean slime and chickpea slime into kefir. Legumes are a good source of dietary fiber, mucilage is a complex carbohydrate that is part of dietary fiber (Motiwala et al., 2015; Sa´ enz et al., 2004).

Legumes have been found to be used as a source of prebiotics that enhance the growth of lactic acid bacteria in fermented milk drinks (HadiNezhad et al., 2013; Saadi et al., 2017). The effect on the total number of bacteria, physicochemical and rheological properties during four weeks of cold storage was studied. In addition, the acceptability of the fermented milk drink by the coagulant was evaluated. The results showed that the addition of legume mucilage increased the viscosity compared to the control sample. Chickpea and bean mucilage in the product resulted in a slight decrease in sensory acceptability, but no significant differences in odor and texture characteristics. The results showed that mucus is a good source of complex carbohydrates, enhancing the growth of lactic acid bacteria in kefir. Legume mucilage as a food ingredient may also influence probiotic function in the human gut. Clinical studies to determine the prebiotic and probiotic activity of mucus in vivo will be an interesting future study.

A study (Glibowski et al., 2012) examined the effect of replacing skimmed milk powder and milk fat with high-performance and native inulin on the rheological, textural, and sensory properties of kefir. Adding inulin to food products can have a beneficial effect on the health of the consumer. Inulin-polyfructosan, containing fructose polymers of various lengths with β (2–1) glycosidic bonds ending, as a rule, with one glucose unit (Ronkart et al., 2006) is a dietary fiber that is not digested in the gastrointestinal tract (Izzo and Franck, 1998). Consuming 15–20 g of inulin per day helps prevent constipation (Den Hond et al., 2000; Kleessen et al., 1997). Increasing the frequency of defecation reduces the risk of colon cancer (Schneeman, 1999). In addition, inulin has a positive effect on the body by selectively stimulating the growth and activity of one or more beneficial colonic bacteria, such as *Bifidobacterium longum* or *Lactobacillus acidophilus* (Gomes and Malcata, 1999; Roberfroid et al., 1998).

Some studies show that inulin lowers the level of cholesterol in the blood plasma (Roberfroid, 2005). Inulin consumption has been shown to increase the absorption of calcium, magnesium, and iron (Bosscher et al., 2003). Increased absorption of calcium improves bone mineralization, which is especially important for the prevention of osteoporosis (Bosscher et al., 2006).

The results showed that both native and highly effective inulin can be successfully used as a substitute for skimmed milk powder. Fermented milk drinks with different inulins showed similar rheological and sensory properties. Small differences were observed in the texture analysis, where kefir with native inulin had a lower hardness, probably due to a lower degree of inulin polymerization. Fermented milk drinks with inulin showed higher strength as less adhesive and more cohesive properties. Viscoelastic properties were typical for fermented milk drinks, they were more elastic. Rotational rheometry showed that the studied samples exhibited thixotropic and thinning properties. Replacing skimmed milk powder with inulin made it possible to reduce the calorie content by 12% and 35% compared to fermented milk drinks made from skimmed milk powder and whole milk powder, respectively.

Studies have shown (Božanić et al., 2003) that the addition of 2% natural inulin to cow's and goat's milk before fermentation of beverages has a positive effect on their rheological properties. The fortified fermented milk drinks had higher shear stress values and better sensory evaluation scores than the control.

The addition of 2% inulin to reconstituted milk resulted in an increase in viscosity compared to a product made from skimmed milk (Ertekin & Guzel-Seydim, 2010). At the same time, the level of ethanol and acetaldehyde did not differ significantly in the analyzed samples, which was confirmed by sensory analysis. Fermented milk drinks were analyzed in which one-third of the skimmed or whole milk powder (WMP) was replaced with long-chain (LC) and natural inulin (Pawe et al., 2012). It has been established that both native and LC inulin can be successfully used as a substitute for SMP.

As a result of many studies, new types of milk drinks have been created: fermented kefir grains based on apple juice and whey (Sabokbar et al., 2015); a drink based on pumpkin juice (Koh et al., 2015); products made from extracts of yam (Colocasia esculenta L.), seed (Sesamum indicum L.), and legume (Phaseolus vulgaris L.) (Kosta et al., 2018). These drinks have a number of useful properties and are suitable for consumption by vegetarians and people who are allergic to dairy products.

A technology for the production of fermented milk drinks based on whey has been developed (Gilberto V. de Melo Pereira, et al, 2011). Whey, the yellow-green liquid that remains after the precipitation and removal of milk casein during cheese production, is considered one of the main problems in the dairy industry. It represents an important environmental pollutant, showing a biochemical oxygen demand (BOD) equal to the maximum permissible limits of 50,000 mg/l and a chemical oxygen demand (COD) equal to the maximum permissible limits of 80,000 mg/l (Siso, 1996). In addition, deproteinized cheese whey or whey permeate, the liquid fraction obtained by ultrafiltration or diafiltration of cheese whey, accounts for more than 70% of the total whey solids and is mainly responsible for the pollution load. Thus, this liquid presents disposal problems in terms of volumes produced and pollutant load, almost equal to the disposal of raw whey (Guimarães, Teixeira, & Domingues, 2010).

The results showed that although less lactose utilization was observed during the production of whey-based beverages compared to that obtained during the traditional cultivation of kefir grains in milk, no significant differences were found between the samples at the end of fermentation. In a study (Nayereh Sabokbar, 2015), whey was used as a substrate, pomegranate juice was introduced as an additive to obtain a new beverage fermented with kefir grains. Pomegranate (Punica granatum L.) is one of the oldest edible fruits and contains many types of polyphenolic components such as punicalagin isomers, ellagic acid derivatives and anthocyanins (delphinidin, cyanidin, pelargonidin 3- and 3,5-diglucosides) (Pokorny and Schmidt) . 2003). The antioxidant activity of pomegranate juice has been studied previously (Gil et al. 2000; Zaouay et al. 2012; Fawole et al. 2011). The results showed that TPC, DPPH radical scavenging, reducing power, linoleic acid autoxidation inhibitory effect, and ascorbate autoxidation inhibitory effect increased after fermentation, but no significant changes in metal chelating activity were observed. Based on this study, it was found that fermentation of kefir grains has a beneficial effect on the drink, as it can increase antioxidant activity.

The properties of fermented milk drinks made from yam, sesame, and bean extracts after fermentation of the extracts with water kefir grains were evaluated (Kosta et al., 2018). Yams can be considered as an alternative in the preparation of vegetable-based beverages due to their high carbohydrate content in addition to their neutral taste and odor (Miranda 2008). However, yams are a poor source of protein. Beans are legumes that range in protein content from 18.0 to 25.0% and are therefore an excellent source of protein to add to vegetable drinks. Yams and beans have a low lipid content, and the reduced concentration of this biomolecule in vegetable drinks can prevent a more homogeneous mixture from being achieved.

A healthy alternative is to use sesame, which has a high lipid content and can therefore act as an emulsifier in the mixture. The results showed that the fermentation of extracts containing yams, sesame and beans with water kefir grains proved to be suitable for the production of fermented vegetable drinks. The addition of beans was important to speed up the fermentation process. Although cooking affects the nutritional value of yams, reducing the nutrient content of cooked yams did not affect the decrease in pH and increase in titratable acidity during the fermentation process. Thus, the recipe developed with yams and sesame, enriched with 50% beans, was not only the best substrate for obtaining a kefir drink, but also a protein-rich drink. The development of these vegetable-infused fermented milk drinks is directed to solving the problem of non-dairy probiotic deficiencies for the vegan or dairy-allergic population. It's also a great source of plant-based protein.

Almost all types of dairy products can be combined with various plant components. Many scientists are trying to create healthy food by considering different natural components (Zaharova, 2014). However, the introduction of some plant components will lead to a decrease in viscosity, separation of whey, and deterioration of the taste of dairy products. Increasing the amount of flavorings and stabilizers causes these negative reactions.

The activity of probiotics is an important functional characteristic of fermented milk products (Wu, 2012), the introduction of flavors and stabilizers will negatively affect the population of "living microflora" and reduce the biological value of the product. (Huang et al., 2015; Liu et al., 2018; Shleikin, 2015). For this reason, an urgent task of plant-based kefir research is the development of recipes for dairy products that have natural fillers, but have a positive effect on kefir.

Recently, a noticeable trend is the introduction of dietary fibers into fermented milk drinks. Dietary fiber is beneficial for health and prevents colon cancer, constipation, diabetes and weight loss (A. M. a et al., 2017; Carvalho et al., 2019; Fernández et al., 2019; Li et al., 2019). , 2019). Some studies have shown a positive effect of dietary fiber on the probiotic content and rheological properties of fermented milk drinks (Aguiar et al., 2013; Goncu, 2017). Some studies have shown that the introduction of dietary fiber can reduce the acidity of kefir to increase the acceptability of kefir (Hekmat & Irvine, 2011). The introduction of fiber can stimulate the proliferation of probiotics (Desai et al., 2004).

Several studies related to the addition of dietary fiber into dairy products have shown positive effects both on the growth of probiotic bacteria and on sensory, rheological and physicochemical properties (Aguiar et al., 2013; Goncu, 2017). There is a great economic interest in finding new food matrices enriched with dietary fibers.

These dietary fibers masked the sour taste of fermented milk drinks, thus, increasing acceptability (Hekmat & Irvine, 2011). A viscous product that is neither too sour nor too sweet has been found to be generally preferred by consumers, and this combination is only possible if probiotics and dietary fiber are included in the drink (Drunkler et al., 2009). In synbiotic skimmed milk, where Lactobacillus was incorporated into dietary fiber, the doubling time was significantly reduced. Hence, it was concluded that the addition of dietary fiber had a positive effect on both the fermentation time and the viability of the probiotic strain during storage periods (Desai et al., 2004).

1.4 Analysis of functional properties of plant raw materials containing dietary fibers

1.4.1 Defatted sesame flour

Sesame is a drought tolerant crop cultivated and a fast cycle in the region of the Brazilian Northeast, where families produce their food in the family farming system. In spite of the fact that sesame consumption in Asian countries such as India and China are high, Brazilian consumption is reasonably limited due to cultural habits.

Sesame (*Sesamum indicum L.*) is an oleaginous plant of high nutritious value with a high number of proteins composed mainly of sulfur-amino acids (methionine), essential fatty acids, vitamins and minerals, amongst them, high concentration of calcium is found. Sesame flour is a kind of by-product commonly discarded or utilized as an animal food. The process of sesame oil extraction leads to the production of defatted sesame cake, which contains 50% of protein, high calcium content (1,5g/100

g) and crude fiber (10.8 g/100 g) (A. A. A. Mohdaly et al., 2013).

Defatted sesame flour also contains phenolic compounds which possess antioxidant, antimutagenic and antimicrobial activities (Reham et al., 2019; Yashaswini et al., 2017). Other nutraceutical compounds which are present in the sesame flour are lignans (Yang et al., 2019) and several minerals such as: potassium (4.6–5.3 g/kg), phosphorus (1.7–2.3 g/kg) and magnesium (0.018–0.052 g/kg). In India sesame flour is often used as an animal feed when the oil is extracted at village level. The free fatty acid content of Indian sesame flour is high and its keeping quality is poor. Therefore, it must be fed to livestock as soon as possible or it would rapidly become rancid and unpalatable.

It is gaining commercial importance due to the beneficial nutritional and biological effects. A number of studies have been carried out to evaluate sesame flour as a functional ingredient in various foods to improve the nutritional quality. Sesame flour has a high content of dietary fibers, in view of its therapeutic potential, its addition can contribute to the development of value-added foods or functional foods that currently are in great demand. Sesame flour has become increasingly important as a human food due to the following unique properties: the presence of a high level of sulphur-containing amino acids, methionine and cystine (Smith, 1971), its lack of trypsin-inhibiting factors and its pleasant flavor. Sesame flour has a high protein content and is used to fortify foods (Rooney et al., 1972). Its use in the diet of children suffering from kwashiokor has been found to be beneficial. It has been recommended as a protein supplement for soy and legume proteins (Brito & Nunez, 1982).

(Hashempour-Baltork et al., 2018) have prepared a puffed corn snack food using 90 % corn powder and 10 % sesame flour, the results indicate that oleic acid content increased and palmitic acid decreased significantly ($p < 0.05$) in all the samples at 10% and 15% inclusion levels. The content of phenolic compounds, γ-tocopherols sesamolin had significant increases in all the formulated samples. Peroxide value results indicated that the formulated samples had a higher stability when the ratio of sesame powder was increased, while the acidity values showed a significant increase during storage. Incorporation of 10% defatted in the snack formulation had a positive effect on the stability, sensory, and nutritional quality of products. Bread had shown improved nutritional qualities when supplemented with defatted sesame flour. Significant differences ($P < 0.05$) in moisture, ash, protein, fat, and carbohydrate content occurred. The addition of defatted sesame flour leads to significant ($P < 0.05$) increases in protein, fat and fiber of bread compared to control samples. There are significant ($P < 0.05$) differences in the content of Fe, Mg, Zn, Ca, P and K among defatted sesame flour bread and control samples. The introduction of DSF made no significant ($P < 0.05$) difference on sensory characteristics including color, odor, taste, crumb texture, crumb grain and general acceptability.

Defatted sesame flour is sometimes fermented for food in India and Java. In some European countries, it is also used as an ingredient in comminuted meat products. The use of defatted sesame flour for cooking high-protein beverages has been reported (Taskar et al., 1967), (Bandyopadhyay & Ghosh, 2002), prepared a protein liquid from DSF.

1.4.2 Rice bran

Rice is a major cereal crop in the developing world in terms of total world production (672×106 tones), equivalent to that of wheat (FAO/UN, 2012). In Asia and the Pacific (seventeen countries), North and South America (nine countries), and eight countries in Africa, rice is an annual plant. It will provide a great yield under environmental abundance, especially river basin area due to rice being considered to be a semi-aquatic grass plant (Yoshida, 1981).

The major rice production from statistical data was found in Asia (Major producers: China, India, Indonesia, Bangladesh, Vietnam and Myanmar) which produces more than 75 percent (506×106 tones) of rice world production (FAO/UN, 2012).

Besides that, the regular rice consumption can be processed into various products as well, for example: rice flour, rice noodle, used as an ingredient in baked products and producing the alternative healthy beverage etc. During the milling process, rough rice is milled to produce polished edible grain by removing the brownish layer. This process generates agricultural waste as rice bran (RB) (Friedman, 1996).

RB is a by-product of the rice milling industry and constitutes around 10% of the total weight of rough rice (Hu et al., 1996). It is primarily composed of aleurone, pericarp, sub aleurone layer and germ. Each year 90% of the RB produced in the world is utilized cheaply as a feedstock for cattle and poultry, and the remainder is used for extraction of RB oil (Zullaikah et al., 2009).

Protein in RB is a kind of high quality protein, it has appropriate amino acid composition and high biological potency, the lysine content reaches as high as 5.8g/100g. Oil in RB can help to lower blood pressure and improve the lipid profile in mild-to-moderate hypertensive patients (Devarajan et al., 2016; Perez-Ternero et al., 2017), prevent colon cancer (Li et al., 2011). Fiber in RB can help to keep the health of the gut.

Generally, RB still have more important nutrients, though the milling process is applied, previous studies reported about chemical composition of full-fat rice bran and defatted rice bran (% dry basis), RB was composed of carbohydrate (43.5-54.3%), protein (14.1-18.2%), fat (1.6-20.9%), ash (12.8-15.3%), and fiber (8.4-10.5%). (Silva et al., 2006) summarized that the different content of RB composition was possibly caused by different sources and cultivars.

Moreover, it contains high in soluble and non-soluble fibers, vitamins as well as minerals. RB has an abundance of protein which is known as essential amino acid (Sereewatthanawut та ін., 2008). Amino acids (AA) are essential to the body, especially in part of muscle building and maintenance (Han et al., 2015).

In 2004, Patel and Naik noted that Japan contributes 2% from the total production of RB in the world, it is a promising producer of nutraceuticals and other high value products from the derivatives of RB. Bioactive compounds such as polyphenols, vitamin E (Tocopherols, Tocotrienol), and squalene were considered in terms of beneficial health effects, but they provide different functions (Mn et al., 2011).

Consequently, the interesting values as regards to nutritional values and bioactive phytochemicals in RB, today they are partially used during production of several food products due to its health benefits. Moreover, several studies attempted to evaluate RB as potential main food ingredients, designed or substituted to improve the quality and nutrition of the final product. The application of RB in various products was summarized in Table 1.1.

Table 1.1 – Application of RB in various products

Products	Rice bran usage	References
Infant food	Smooth RB as a food ingredient mixing with normal infant food for increasing the nutritional value	(Khan et al., 2011)
Animal feed	Non-smooth rice bran is applied	(Selim et al., 2021)
Bread ingredient	Smooth rice bran is used	(Doan et al., 2021)
Ingredient in meat product (meatball and sausage)	Smooth rice bran is used	(Choi et al., 2008)
Organic rice bran beverage with flavor	Smooth rice bran is used to produce the soft beverage	(Rana et al., 2021)
Dairy product (yogurt and yogurt drink)	Some bioactive constituents are used in rice bran	(Khongla et al., 2021)
Cereal breakfast	RB is added into food and beverage	(Charunuch et al., 2014)

The antioxidant activities of each component in RB are important as regards to the beneficial properties. The antioxidants are known to deactivate free radicals (Gulcin, 2020).

The minor components in RB (γ-oryzanols, phytosterols and other phytosterol) are examined to free radicals scavenging (Pokkanta et al., 2019). (Xu et al., 2001) evinced that γ-oryzanols showed high antioxidant capacities, as indicated that the γ-oryzanols could generate four times antioxidant capacities than vitamin E components (α-tocopherol, β-tocopherol, α-tocotrienol and β-tocotrienol). The health potential of RB depends on type of RB and also depends on bound phenolic acids of each grain.

Several studies (Mirowski, 2021; Shuvo et al., 2021) were related with cholesterol reduction, which mentioned that the unsaponifiable present in RB were shown to reduce liver cholesterol levels significantly. The cholesterol lowering impacted on coronary heart disease (CHD).

Some studies reported that the dietary fiber consumption of cereals grain can reduce the risk of CHD partially, reduce blood pressure, lower blood cholesterol levels and improve insulin sensitivity (Bintanah, 2021; Cara et al., 1992; El-Katcha et al., 2021) Phytochemical compounds in RB and its extracts act as cholesterol properties in the human body.

In recent days, the applications of RB were focused on: the extraction of protein (Phongthai et al., 2016), fiber (Jia et al., 2019), oil (Soares et al., 2016; Trevisani Juchen et al., 2019), vitamin B (Chen et al., 2011) and other biological components (Tabaraki & Nateghi, 2011). The stabilization of RB (Patil et al., 2016); The supplement of RB into bread (Tuncel et al., 2014), sausage (Choi та ін., 2010), drinks (Prestes et al., 2019).

Few research studies were related to the supplement of RB in fermented milk. A few reports were about the usage of RB in beverage production. (Faccin et al., 2009) utilized whole RB for organic rice bran beverage production (with chocolate and strawberry flavors) and studied the chemical, rheological and sensorial properties of the product. They found that this beverage promoted good nutritive values, partially fatty acid and amino acid contents. Furthermore, this product is acceptable according to the expert's evaluation. One more factor that should be concerned for in pasteurization rice bran beverage is viscosity during storing under refrigeration.

Pasteurized rice bran beverage showed the Newtonian behavior and relation to the effect of thermal processing on its rheological properties. Meanwhile, (Issara &

Rawdkuen, 2014) reported the same way for nutritive values except sensorial properties. RB was used in this study: water (1:5, 1:10 and 1:15) to produce the rice bran milk and characterize its properties compared with commercial soymilk. The results found out that the ratio at 1:15 was close to soymilk in terms of physico-chemical properties, total polyphenol content as well as the DPPH radical scavenging activity.

High amount of water added to produce rice bran milk lowered the phenolic content and biological activity. Moreover, sensory profile evaluation found that only color feature was close to the commercial soymilk, while great differences were observed in the attributes of appearance, taste, flavor, sweetness and overall liking. Besides, the authors concluded that RB can be used as a new alternative cereal plant-based beverage production for health concern consumers. These findings could be used as a preliminary result for further development of organic RBM to meet consumer quality requirements.

RB is not as popular for consumption or process into beverages. Therefore, this product has not become commercially available yet and has not been introduced to the market. So, it requires more studies on further development storage stability and others to meet the consumer desirability.

1.5 Methods of production of fermented milk drinks

Several methods are used for the production of kefir, the basis of which is fermentation. According to the traditional method fermented milk drinks are produced mostly from pasteurized whole milk by the inoculation of kefir grains followed by a fermentation period of 20–24 hours at 20–22°C (Rattray & O'Connell, 2011). After the fermentation period, usually kefir is ripened for 7–8 hours at 10–12°C to stimulate the yeast and bacteria growth.

Another production method for traditional fermentation which is more common is fermenting the heat-treated skimmed milk at 20–22°C using kefir grains, then sieving out the grains and using the milk (mother culture, without grains) as an inoculum at rate of 3%–5% for the heat-treated milk.

After the incubation at 20–22°C for 20–24 hours, the milk is cooled to 10–12°C and ripened for another 7–8 hours (Litopoulou-Tzanetaki & Tzanetakis, 2014). The traditional process of kefir production is shown in Fig. 1.2. After straining the kefir grains from milk, they should be rinsed with pasteurized milk or sterile water and then stored at 4°C until the next inoculation for up to 48 hours. If kefir grains are not transferred daily to freshly prepared milk and stored for a long time, they should be frozen or lyophilized (Wszolek et al., 2006).

Only a few manufacturers still use kefir grains as a starter culture in kefir production. The direct-to-vat set (DVS) freeze-dried kefir cultures are currently used by most of the dairy plants (Wszolek et al., 2006). Commercial cultures contain isolates of LAB and yeast species isolated from kefir grains (Samaržija et al., 2013).

During industrial production milk is homogenized, and then heat treatment is carried out at 90–95°C for 5–10 minutes. The milk is then cooled to 20–22°C, and DVS kefir starter culture is added at a rate of 2%–8% in tanks. The fermentation period can be changed between 20 and 24 hours depending on the lactic acid content of the milk (approximately 0.7 mL lactic acid 100 mL^{-1}) at the end of the incubation time.

The milk is ripened at 9–10°C for 15–24 hours after the coagulum is separated by pumping, and the milk is poured into the bottles, and then the kefir is stored at 4°C. Another method involves distributing the inoculated milk in bottles before the incubation period and fermenting the milk in the bottles (Wszolek et al., 2006).

It is possible to produce a product that has an acceptable kefir flavor and longer shelf life (up to 28 days) with industrial production, while kefir produced with grains may have a shelf life of only 3–12 days. But the kefir produced with starter culture may not have the same therapeutic and probiotic characteristics because of the small number of various microbial species contained in it (Samaržija et al., 2013).

The technical process of kefir production is shown in figure 1.2.

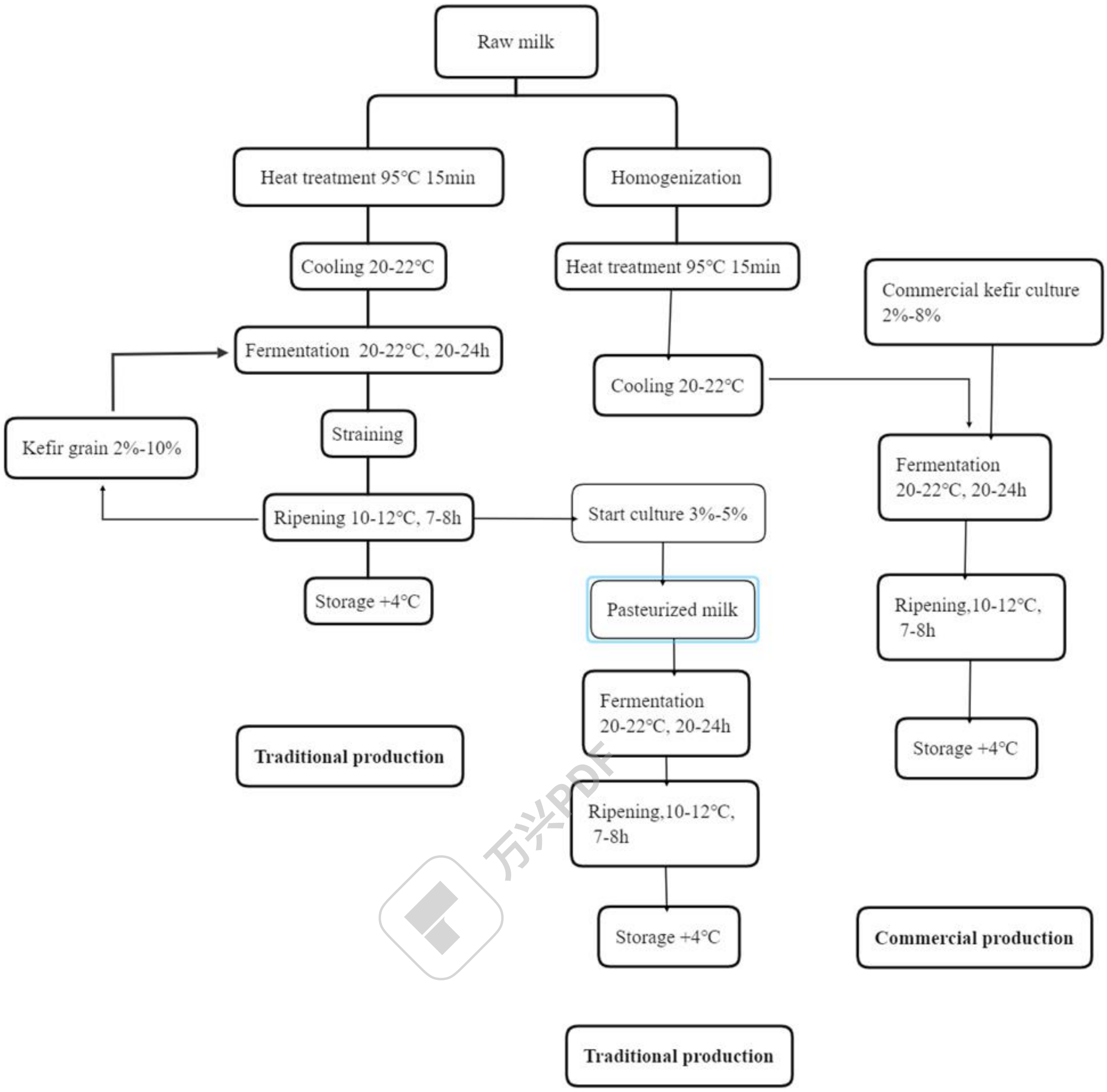


Figure 1.2 - Flow diagram for kefir production

The analysis showed that fermented milk drinks have a number of functional properties. The therapeutic and preventive properties of fermented milk drinks have been confirmed, including with the use of various functional additives. The introduction of dietary fibers into the composition of fermented milk drinks will make them biologically complete food products that contain all the substances needed by the body.

It is worth noting that among the derivatives of plant processing, which are usually considered as production waste, there are those that are suitable for enriching fermented milk drinks according to their organoleptic characteristics. Thus, their introduction into the recipe composition of fermented milk drinks will allow solving two problems at once: effective use of plant raw materials and reduction of production waste, as well as enriching products with dietary fibers and other useful substances.

Considering the prebiotic properties of dietary fibers, it can be assumed that the shelf life of products developed on the basis of sesame flour, rice bran will increase. At the same time, the activity of lactic acid microorganisms will be preserved during the entire storage period.

Analysis of the assortment of fermented milk drinks on the markets of China and Ukraine showed that recycled raw materials are practically not used for the production of fermented milk products. Therefore, this study is relevant from the point of view of expanding the range of fermented milk products with functional properties based on plant derivatives.

Conclusions to Section 1

The types of raw materials used for the production of milk drinks were analyzed. It has been established that cow milk is the optimal raw material for the production of kefir-type fermented milk drinks.

The analysis showed that the main components of fermented milk drinks, which have a positive effect on the human body, are probiotics, proteins, peptides, calcium, lactic and acetic acids.

A wide variety of plant ingredients are used to increase the biological value of fermented milk drinks. However, some of them lead to a decrease in viscosity, separation of serum and a decrease in sensory properties. The addition of flavorings and stabilizers leads to a decrease in the population of probiotics. The urgent task is to introduce such plant ingredients that would not have a negative effect on the physicochemical and rheological properties of fermented milk drinks, especially on the stability of kefir.

Some plant-derived products (sesame and rice) have been found to be a good source of dietary fiber. Using them as fillers will not only improve the functional properties of the product, but also help reduce the amount of waste in the form of by-products.

Among the industrial methods of production of fermented milk drinks, the tank method is the most economically expedient and convenient as regards to implementation, which is proposed to be the basis of the developed technology.

SECTION 2
RESEARCH METHODOLOGY

2.1 Object, subjects and research materials

The object of the research is the technology for producing fermented milk drinks enriched with plant additives.

The research subject is whole milk; defatted sesame flour; rice bran; model samples of fermented milk drinks enriched with dietary fibers.

The following *research materials* were used during research.

Kefir grains were obtained from private households in Tibet, China. Cow milk was supplied from Mengniu Dairy Group Co, Neimenggu, China. All chemicals were of analytical grade. Other media (agar) and reagents used for microbiological analyses were obtained from Merck (Darmstad, Germany).

In all analyses, the ultrapure water (18.2 MΩ.cm) was used (Millipore Simplicity UV, Molsheim, France).

Sesame seeds (white) were procured from the local market (Hezhou, Guangxi, China). Defatted sesame flour was obtained from sesame seed which was roasted at 120-130°C for 10 min and pressed the fat.

It was finely powdered and homogenized by sieving through a fine screen (100 mesh).

he figure of defatted sesame flour was shown in figure 2.1.

Figure 2.1 - Pretreated defatted sesame flour

Rice bran was procured from the local market (Xingtai, Hebei, China). RB was homogenized by sieving through a fine screen (200 mesh). RB was steam sterilized at 121°C for 15 minutes before used. The figure of RB is shown in figure 2.2.

Figure 2.2 - Pretreated rice bran

Five samples based on defatted sesame flour were studied: without the addition of defatted sesame flour (control sample) and with the addition of sesame flour in the amount of 2% (A), 4% (B), 6% (C), 8% (D).

And five samples with the addition of rice bran: cow milk without the addition of rice bran (control sample, K), with the addition of rice bran in the amount of 0.1% (A), 0.3% (B), 0.5 % (C), 0.7% (D).

All kinds of mixture were fermented at 28°C for about 12h until pH reached to 4.7, control kefir samples and research samples were stored at 4 °C for 28 days.

The content of the main nutrients (proteins, fats, dietary fiber, vitamin E, amino acids, water, ash), microbiological indicators (*Lactobacillus*, *Lactococcus* and *yeast*), physicochemical properties (total titrated acidity, water-holding capacity, apparent viscosity and pH), rheological properties, antioxidant activity (DPPH radical scavenging activity and •OH radical scavenging activity) and sensory properties were investigated for all samples. All indicators were studied every 7 days for 28 days.

Based on the research materials and the obtained results, the technology of fermented milk drinks with the addition of defatted sesame flour and rice bran was developed and scientifically substantiated.

The following reagents were used for the research (Table 2.1).

Table 2.1 – Reagents, used in the process of research

Reagents	Usage	Manufacture factory
1	2	3
concentrated sulfuric acid	Kjeldahl method for the test of protein	Xilong scientific company, Guangdong, China
$CuSO_4$	Kjeldahl method for the test of protein	Xilong scientific company, Guangdong, China
K_2SO_4	Kjeldahl method for the test of protein	Xilong scientific company, Guangdong, China
NaOH	Kjeldahl method for the test of protein; acid-base titration for the test of titrable acid	Xilong scientific company, Guangdong, China
H_3BO_3	Kjeldahl method for the test of protein	Xilong scientific company, Guangdong, China
HCl	Kjeldahl method for the test of protein	Xilong scientific company, Guangdong, China
Ammonium hydroxide	Ross Gottrell Method for the test of fat in kefir	Xilong scientific company, Guangdong, China
α- amylase	Enzyme - gravimetric method for the test of dietary fiber	Xilong scientific company, Guangdong, China
CH_3CH_2OH	Ross Gottrell Method for the test of fat in kefir; Enzyme - gravimetric method for the test of dietary fiber; For the test of DPPH-radical scavenging activity; For the test of Hydroxyl radical scavenging activity	Xilong scientific company, Guangdong, China
diethyl ether	Ross Gottrell Method for the test of fat in kefir/ DSF/ RB; Enzyme - gravimetric method for the test of dietary fiber	Xilong scientific company, Guangdong, China

Continuation of the table 2.1

1	2	3
petroleum ether	Ross Gottrell Method for the test of fat in kefir/ DSF/ RB; Enzyme - gravimetric method for the test of dietary fiber	Xilong scientific company, Guangdong, China
glucosidase	Enzyme - gravimetric method for the test of dietary fiber	Xilong scientific company, Guangdong, China
protease	Enzyme - gravimetric method for the test of dietary fiber	Xilong scientific company, Guangdong, China
acetone	Enzyme - gravimetric method for the test of dietary fiber	Xilong scientific company, Guangdong, China
phenothalin	Acid-base titration for the test of titrable acid	Xilong scientific company, Guangdong, China
potassium acid phthalate	Acid-base titration for the test of titrable acid	Xilong scientific company, Guangdong, China
MRS agar	For the cultivate of S. thermophilus	Difco Company (KS, USA)
M_{17} agar	For the cultivate of L. delbrueckii subsp. bulgaricus	Oxoid Company (ON, Canada)
Potato Dextrose Agar	For the cultivate of yeast	Xilong scientific company, Guangdong, China
Vitamin E assay kit	For the test of VE	Nanjing Jiancheng Bioengineering Institute, Nanjing, China
$FeSO_4$	for the test of Hydroxyl radical scavenging activity	Xilong scientific company, Guangdong, China
H_2O_2	For the test of Hydroxyl radical scavenging activity	Xilong scientific company, Guangdong, China

The end of the table 2.1

1	2	3
salicylic acid	For the test of Hydroxyl radical scavenging activity	Xilong scientific company, Guangdong, China
hydrochloric acid	For the test of amino acid	Xilong scientific company, Guangdong, China
ninhydrin	For the test of amino acid	Xilong scientific company, Guangdong, China
$Sncl_2$	For the test of amino acid	Xilong scientific company, Guangdong, China
phosphate buffer	For the test of amino acid	Xilong scientific, Guangdong

The scheme of theoretical and experimental research on the topic of the dissertation is presented in fig. 2.3.

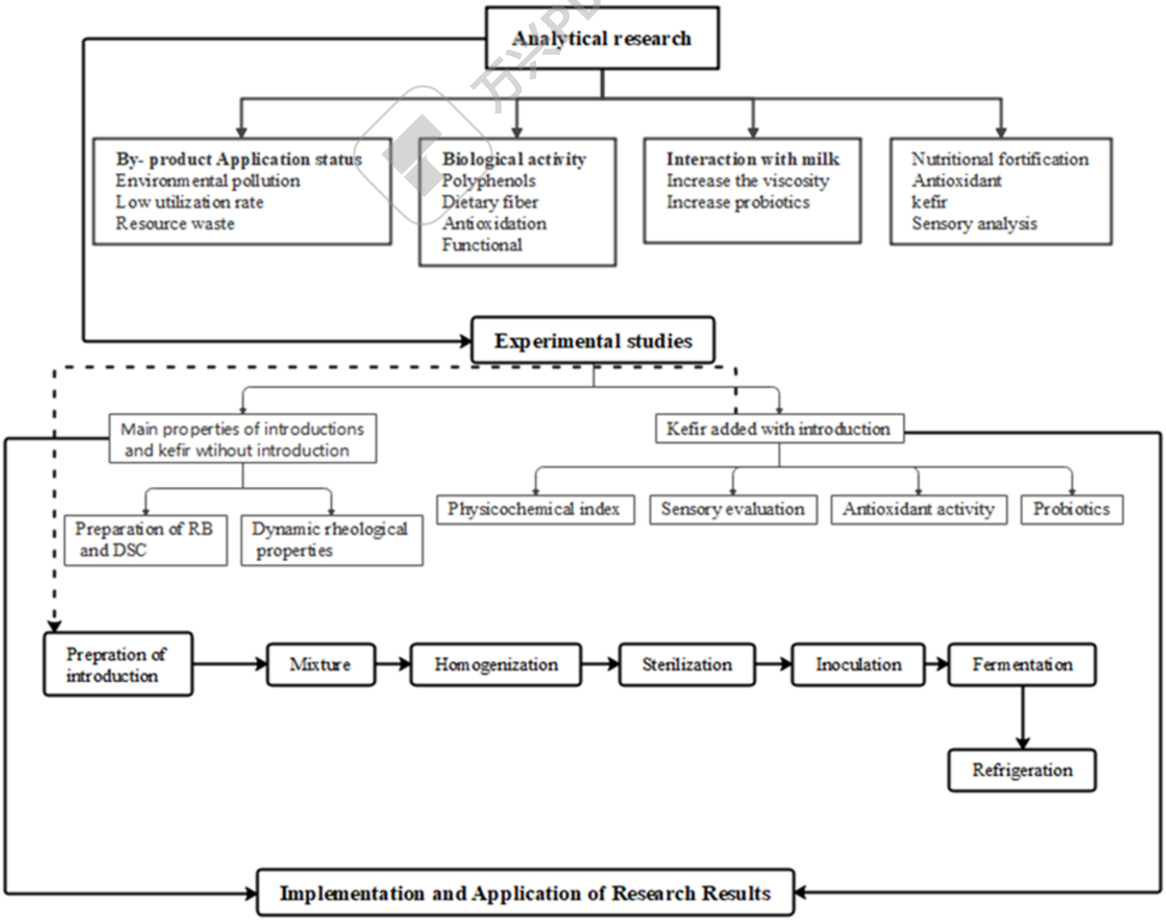


Figure 2.3 - The scheme of theoretical and experimental research

2.2 Research methods

2.2.1 Research of chemical composition of fermented milk drinks The content of protein was determined by the Kjeldahl method (ISO, 2014). In order to convert percent nitrogen to percent protein, a multiplication factor of 6.38 was used.

Vitamin E was measured by an assay kit (Colorimetric method) for analysis of vitamin E (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

The content of amino acids was determined by Hitachi amino acid analyzer.

Mass fraction fat was confirmed by means of Gerber method (ISO/IDF, 2008).

Mass fraction of water was determined by vacuum drying method.

The content of ash was determined by high-temperature burning method.

2.2.2 Study of physical and chemical properties

The pH value was determined by a pH meter (METTLER TOLEDO LE438, Switzerland). The titratable acidity was measured by titrating 10 g of sample with 0.1n NaOH using phenolphthalein indicator (Guler-Akin and Akin 2007). The apparent viscosity of the samples was measured with a digital viscometer (NDJ-8S, Shanghai, China).

WHC (water holding capacity) of kefir was determined using a centrifuge (Mudgil et al., 2016). 10g of kefir (X) samples were weighed into a 50mL test tube and centrifuged at 3000 rpm for 20 min at 4°C. The separated whey (Y) was removed and weighed. The water-holding capacity was calculated as:

$$WHC = \frac{X - Y}{X_s} 100\%, \tag{2.1}$$

To conduct color measurements, a reflectance colorimeter (CHROMA METER CR-400, JAPAN) was used to determine Hunter L, a and b color parameters of the kefir samples. The source of light and the observation angle are D65 and 10°.

2.2.3 Study of rheological properties

To conduct the nuclear magnetic resonance experiment, a sample of about 1.5 g was weighed and placed into a glass tube (caliber: 18 mm), the sample tube was placed in the NMR probe (maintain temperature: 32°C), and the transverse relaxation time T_2 was measured using the CPMG sequence, where the receiver bandwidth was SW=100 kHz.

The control parameters of sampling starting point RFD=0.150ms, repeat sampling interval TW=4 500.000 ms, analog gain RG1= 20.0dB, 90° and 180° pulse width were P90= 10.00μs, P180= 19.52 μs, sampling points Td =800034, digital gain DRG1=1, data radius DR=1, cumulative scanning times NS=2, echo number NECH=8000. The test measured 3 copies.

2.2.4 Microbiological analysis

A 25 ml sample was suspended in 225 ml sterile normal saline (0.85%, v/v) solution in a ratio of 1:9 (w/w) in a sterile bag. The mixture was homogenized in an orbital shaker (Guansen 200JR, Shanghai, China) at 400 rpm for 1.0 min. Then, serial dilutions in normal saline (0.85%, w/v) were prepared, and proper dilution was transferred onto the appropriate media for each microorganism.

The microbial loads of *Lactobacillus* were determined on Man-Rogosa-Sharpe agar, *Lactococcus* on M_{17} agar, yeast and molds on Potato Dextrose Agar. The plates were then incubated at 30 °C for 72 h for the detection of *Lactobacillus*, at 37 °C for 48 h for *Lactococcus*, at 28 °C for 5 days for yeast and molds.

All microbiological incubations were carried out in incubators (BPC-150F, Shanghai, China). Colony counts were converted to log CFU/ml. Each sample for all measurements was replicated twice.

2.2.5 Methodology for research of antioxidant properties

DPPH-radical scavenging activity.

Aliquots of 1g of each sample were diluted by a ratio of 1:9 (sample: anhydrous ethanol), the diluent was centrifuged with the rotating speed set at 4000r/min for 10 min (cence L550, Hunan, China). 3 ml of the supernatant was placed in eppendorf tubes with 3 ml of the radical DPPH (0.1mmol/L in anhydrous ethanol).

The reaction mixtures were homogenized and left at room temperature in the absence of light for 30 min. A standard curve was prepared using a standard solution of vitamin C, and absorbance was read using a spectrophotometer (Thermo Fisher Evolution 300, USA) at 517 nm. A standard curve was prepared with different concentrations of vitamin C (0.015-0.075mg/mL), and the results are expressed as mg VC/100 g.

Hydroxyl radical scavenging activity. Aliquots of 1g of each sample were diluted by a ratio of 1:3 (sample: anhydrous ethanol), the diluent was centrifuged with the rotating speed set at 4000r/min for 10 min (cence L550, Hunan, China). 2 ml of the supernatant was placed in eppendorf tubes with 2 ml of 9.0 mmol/L $FeSO_4$ aqueous solution, 2ml 6mmol/L salicylic acid ethanol solution, and 2ml 1mmol/L H_2O_2 aqueous solution.

The reaction mixtures were homogenized and water-bath heating at 37°C for 30 min. A standard curve was prepared using a standard solution of vitamin C, and absorbance was read using a spectrophotometer (Thermo Fisher Evolution 300, USA) at 510 nm. A standard curve was prepared with different concentrations of vitamin C (0.015-0.075 mg/mL), and the results are expressed as mg VC/100 g.

2.2.6 Sensory assessment methodology

Sensory evaluation was conducted using 40 untrained panelists (25 women and 15 men, age 20–36, all non-smokers). Panelists examined and tasted the samples and recorded their perceptions by making marks on nine-score grades. Different samples were presented to sensory evaluators in a double-blind manner.

Color, consistency, sour, texture and flavor of samples were evaluated in a room designed for sensory analyses, general acceptability was obtained by summing of the five attributes. During sensory analyses, panelists were provided with plain cracker sticks and water, instructed to clear their mouth between tastings. The scoring criteria were shown in table 2.2.

Table 2.2 Criteria for sensory evaluation

Indicators	Evaluation indicators	The magnitude
Color	uniform color, slightly yellowish tint	8-9
	slight delamination, slightly yellowish tint	7-8
	delamination, uneven color	<7
Consistence	moderate consistency and viscosity	8-9
	slightly liquid consistency	7-8
	liquid or thick consistency	5-7
	very liquid or thick consistency	<5
Taste	the taste is moderately sour, without tartness	8-9
	light sour taste, without tartness	7-8
	slight acidity and astringency	6-7
	slight acidity and noticeable astringency	<6
Structure	homogeneous and delicate structure, without gas formation	8-9
	slightly homogeneous and delicate structure, without gas formation	7-8
	slightly disturbed structure, slight gas formation	5-7
	too dense structure, significant gas formation	<5
Aroma	rich aroma of food supplement and fermented milk drink, without acid smell and burnt taste	8-9
	light taste of food supplement and fermented milk drink, without acid smell and burnt taste	7-8
	light taste of food supplement and fermented milk drink, slightly sour smell and taste of burnt taste	5-7
	strong acid smell and burnt taste	<5

2.2.7 Research of dietary fibers content

Dietary fiber was measured according to AOAC Official Method 991.43 about total dietary fiber in Foods (AOAC, 2000). The dried samples were digested with heat-stabilized α-amylase, protease and glucosidase to remove protein and starch. After ethanol precipitation and filtration, the residue was washed with ethanol and acetone, dried and weighed, which was the total dietary fiber residue. The total dietary fiber content in the sample can be calculated by deducting the corresponding protein, ash and reagent blank contents in the dietary fiber residue. The set for the test is dietary fiber determination system (CSF6, Italy).

First, defat and desugar before determining dietary fiber, defatting by the use of Soxhlet extraction, desugar by extracting 2-3 times with 85% ethanol, 10 mL/g, decanting, and then drying overnight at 40°C. Run 2 blanks/assay with samples to measure any contribution from reagents to residue.Weigh duplicate 1.000±0.005 g samples (M1 and M2), accurate to 0.1 mg, into 600 mL tall-form beakers. Add 40 mL MES-TRIS buffer solution, pH 8.2, to each. Stir on magnetic stirrer until sample is completely dispersed (to prevent lump formation, which would make test material inaccessible to enzymes).

Add 50 uL heat-stable o-amylase solution, stirring at low speed. Cover beakers with Al foil, and incubate in 95-100℃ H_2O bath 15 min with continuous agitation. Start timing once the bath temperature reaches 95℃ (total of 35 min is normally sufficient). Remove all beakers from the bath, and cool to 60°C. Remove foil. Scrape any ring from inside of the beaker and disperse any gels in the bottom of the beaker with spatula. Rinse beaker walls and spatula with 10 mL H_2O.

Add 100 uL protease solution to each beaker. Cover with Al foil, and incubate 30 min at 60 ± 1℃ with continuous agitation. Start timing when the bath temperature reaches 60°C. Remove foil. Dispense 5 mL 0.561N HCl into beakers while stirring. Adjust pH to 4.0-4.7 at 60°C, by adding 1N NaOH solution or 1N HCl solution. Add 300 uL amyloglucosidase solution while stirring. Cover with Al foil, and incubate 30 min at 60± 1℃ with constant agitation. Start timing once the bath reaches 60℃.

To each digested sample, add 225 mL (measured after heating) 95% ethanol at 60℃. Ratio of ethanol to sample volume should be 4:1. Remove from the bath, and cover beakers with large sheets of Al foil. Let precipitate form 1 h at room temperature.

Wet and redistribute Celite bed in previously tared crucible B(b), using 15 mL 78% ethanol from wash bottle. Apply suction to the crucible to draw Celite onto fritted glass as an even mat.

Filter alcohol-treated enzymes digestate through the crucible. Using a wash bottle with 78% ethanol and rubber spatula, quantitatively transfer all remaining particles to the crucible. Using vacuum, wash residue 2 times each with 15 mL portions of 78% ethanol, 95% ethanol, and acetone. Dry crucible containing residue overnight in 105℃ oven. Cool crucible in desiccator ca 1 h. Weigh crucible, containing dietary fiber residue and Celite, to nearest 0.1 mg, and calculate residue weight by subtracting weight of dry crucible with Celite, B(b).

Use one duplicate from each sample to determine protein, by method 960.52, using N x 6.25 as conversion factor. For ash analysis, incinerate the second duplicate 5 h at 525℃. Cool in a desiccator, and weigh to the nearest 0.1 mg. Subtract weight of crucible and Celite, B(b), to determine ash weight.

Blank (B, mg) determination:

$$B = [(BR1 + BR2)/2] - PB - AB, \qquad (2.2)$$

where BR1 and BR2= residue weights (mg) for duplicate blank determinations;

PB and AB = weights (mg) of protein and ash, respectively, determined on first and second blank residues.

Dietary fiber (DF, g/100 g) determination:

$$TDF = \{[(R1+R2)/2] - P - A - B\} / [(M1 + M2)/2] \times 100, \qquad (2.3)$$

where R1 and R2 - residue weights (mg) for duplicate samples;

P and A = weights (mg) of protein and ash, respectively, determined on first and second residues;

B = blank weight (mg); and M1 and M2= weights (mg) for samples.

2.3 The calculation method for optimizing of consumers' properties of the product

The variables in the used mathematical model are the mass fractions of the constituents in the studied samples of food additives. The values of the indicators were determined by the content of food substances in the recipe components of the studied samples. Boundary conditions for target functions were established based on organoleptic indicators.

When finding rational mass fractions of recipe components, it is necessary that the amount of food substances in the resulting filling is as close as possible to the recommended norms. To solve this problem, it is necessary that the condition be fulfilled (method of least squares):

$$\sum_{i=1}^{n}(f(x_i)-V\cdot K)^2 \rightarrow \min \quad , \qquad (2.4)$$

where n – the number of indicators for which optimization is carried out;

Ki – normative value of i-th indicator;

V – normalizing indicator, which is determined by the formulas:

$$V_1 = \frac{46x_1 + 46x_2 + 10x_3 + 374x_4}{1000},$$

$$V_2 = \frac{46x_1 + 46x_2 + 10x_3 + 374x_4}{1000}, \qquad (2.5)$$

where V_1, V_2 are the normalizing indicators for DSF, RB fillings, respectively.

Finding the value of variables under condition (3) was carried out using the standard Minimize function of the MathCad software package.

2.3 Statistical analysis

All experimental studies were performed in three flasks, the results are presented as the average of three independent experiments with standard error. The multiple range test was used to determine significant differences between the mean values at the level of P = 0.05. Statistical analysis was performed according to the known method using data processing software (DPS version 7.05, SAS Institute).

SECTION 3
THE RESEARCH OF FERMENTED MILK DRINKS ENRICHED WITH PLANT ADDITIVES

3.1 The results of the study of fermented milk drinks enriched with defatted sesame flour

3.1.1 Chemical composition of fermented milk drinks enriched with defatted sesame flour

The approximate composition of the studied batch of fermented milk drinks is given in the table 3.1. The content of fat, protein, dietary fibers and vitamin E increased when adding defatted sesame flour (p<0,05).

Table 3.1 – Chemical composition of fermented milk drinks (n=3, ±SD)

Samples	Mass fraction of fat (g/100g)	Mass fraction of proteins (g/100g)	Mass fraction of dietary fibers (g/100g)	Content of vitamin E (mg/100g)
K	3.99±0.08d	3.36±0.08d	0.02±0.00c	0.32±0.02d
A	4.10±0.25cd	4.30±0.13c	0.68±0.11c	0.93±0.01cd
B	4.45±0.14bc	4.89±0.13bc	1.14±0.00bc	2.02±0.16c
C	4.85±0.06b	5.74±0.37ab	2.12±0.48b	5.53±0.83b
D	5.29±0.15a	6.31±0.62a	4.52±0.44a	9.07±0.61a

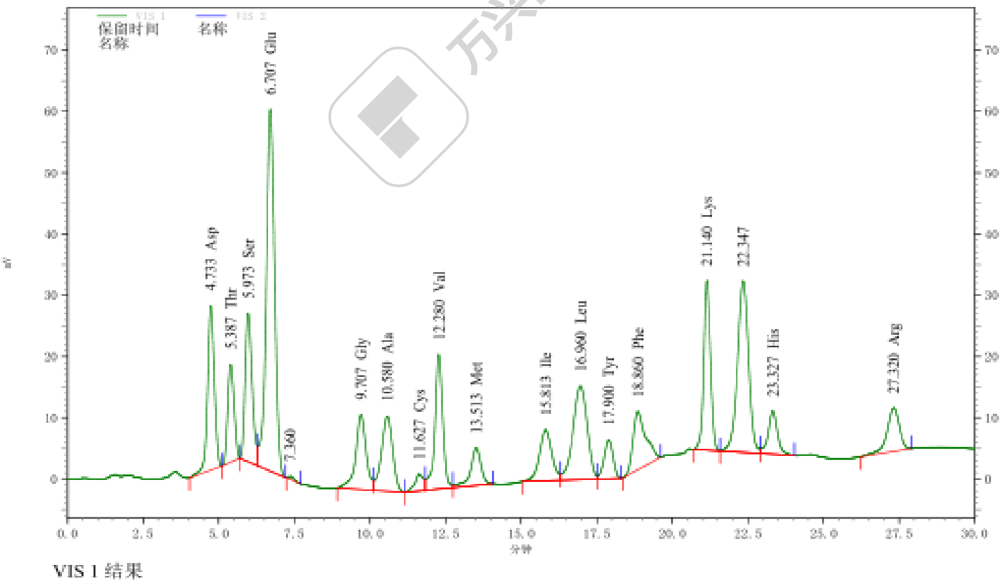
* Values expressed as mean ± standard deviation of duplicate samples.
* K= control sample, A= fermented milk drink with adding 2% of defatted sesame flour, B= fermented milk drink with adding 4% of defatted sesame flour, C= fermented milk drink with adding 6% of defatted sesame flour, D= fermented milk drink with adding 8% of defatted sesame flour.
* Different superscript letters within a column indicate significant difference ($p < 0.05$) according to Tukey test.

The introduction of defatted sesame flour could significantly improve the total content of proteins, dietary fibers, and vitamin E in the fermented milk drink, which are beneficial to the human body. The addition of defatted sesame flour could give the fermented milk drink a more healthy effect.

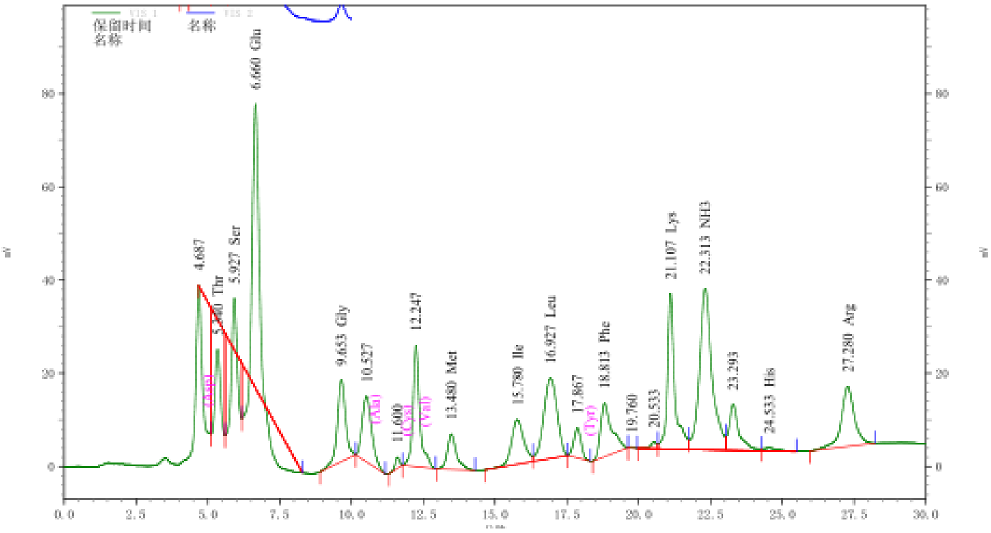
The protein content (3.36 g/100g) in K sample was much lower than that (6.31 g/100g) in D sample. The fat content (3.99 g/100g) in K sample was much lower than that (5.29 g/100g) of D sample. The content of dietary fiber in K sample (0.02 g/100g) was much lower than that of D sample (4.52 g/100g). VE content was found as the lowest in K kefir sample (0.32 mg/100g) while the highest concentration was found in D sample (9.07 mg/100g).

This mainly due to the higher addition of defatted sesame flour, since defatted sesame flour has approximately 38% protein, 10.8 g/100 g crude fiber, 19.4 g/100g fat (Erika et al., 2018; Yu et al., 2016).

The introduction of defatted sesame flour could significantly improve the total content of amino acids in the studied batch. The content of amino acids in the sample were shown in figures 3.1 (*a*, *b*, *c*, *d*, *e*).



a



保留时间
名称
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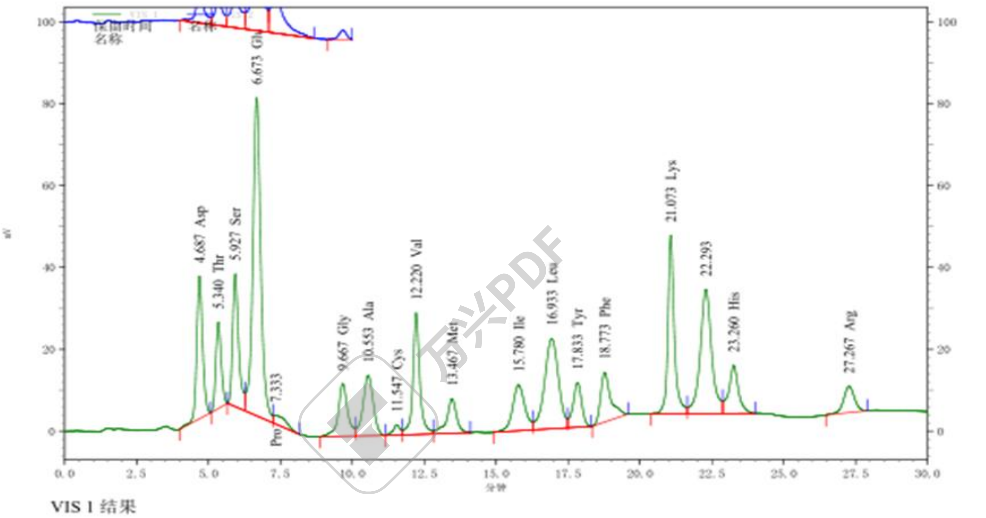
5.340 Thr
5.927 Ser
6.660 Glu
9.633 Gly
10.527
12.247
13.480 Met
15.780 Ile
16.927 Leu
17.867
18.813 Phe
19.760
20.533
21.107 Lys
22.313 NH3
23.293
24.533 His
27.280 Arg

b



保留时间
名称
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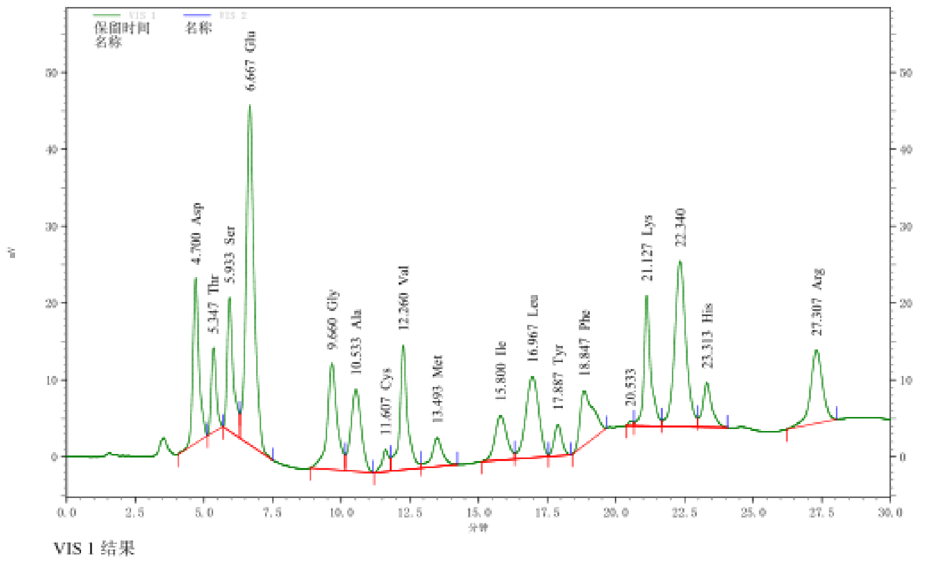
5.340 Thr
5.927 Ser
6.673 Glu
7.333
Pro
9.667 Gly
10.553 Ala
11.547 Cys
12.220 Val
13.467 Met
15.780 Ile
16.933 Leu
17.833 Tyr
18.773 Phe
21.073 Lys
22.293
23.260 His
27.267 Arg
VIS 1 结果

c



保留时间
名称
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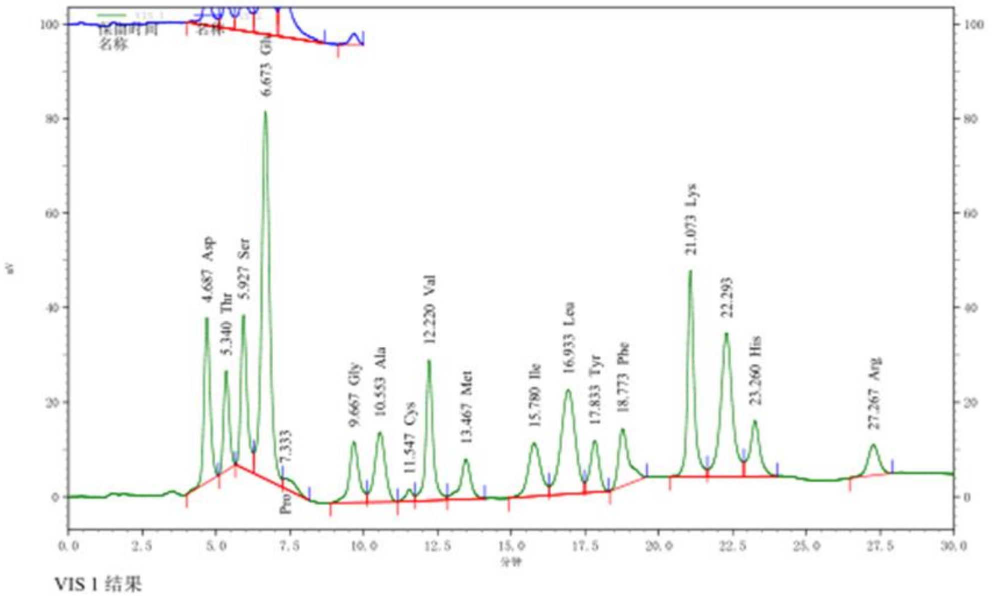
5.933 Ser
6.667 Glu
9.660 Gly
10.533 Ala
11.607 Cys
12.260 Val
13.493 Met
15.800 Ile
16.967 Leu
17.887 Tyr
18.847 Phe
20.533
21.127 Lys
22.340
23.313 His
27.307 Arg
VIS 1 结果

d



e

Figure 3.1 - Composition of amino acids of studied samples: a – sample A; b – sample B; c – sample C; d – sample D; e – control sample

The results of amino acid composition of the studied batch was represented in table 3.2.

Table 3.2 – Composition of amino acid samples

Amino acid	K	A	B	C	D
	g/100g	g/100g	g/100g	g/100g	g/100g
1	2	3	4	5	6
Asp	0.29	0.29	0.33	0.43	0.67
Thr	0.14	0.14	0.13	0.16	0.33
Ser	0.21	0.22	0.24	0.28	0.5
Glu	0.9	0.99	1.27	1.36	2.32
Gly	0.08	0.07	0.15	0.19	0.17
Ala	0.12	0.12	0.18	0.21	0.28
Cys	0.02	0.02	0.02	0.04	0.04
Val	0.17	0.18	0.23	0.27	0.43
Met	0.08	0.09	0.11	0.1	0.22

The end of the table 3.2

1	2	3	4	5	6
Ile	0.14	0.16	0.19	0.19	0.38
Leu	0.3	0.38	0.4	0.41	0.89
Tyr	0.12	0.18	0.15	0.14	0.43
Phe	0.18	0.17	0.27	0.29	0.39
Lys	0.23	0.3	0.36	0.3	0.71
His	0.08	0.12	0.12	0.14	0.29
Arg	0.15	0.11	0.31	0.42	0.25
Pro	0.25	0.36	0.3	0.33	0.84
	3.46	3.9	4.76	5.26	9.14

K= control sample, A= fermented milk drink with adding 2% of defatted sesame flour, B= fermented milk drink with adding 4% of defatted sesame flour, C= fermented milk drink with adding 6% of defatted sesame flour, D= fermented milk drink with adding 8% of defatted sesame flour.

In all the amino acids, the content of Glu and Arg has obtained the largest increase, which was caused by the rich content of Glu and Arg in defatted sesame flour.

3.1.2 Physical and chemical indicators of fermented milk drinks enriched with defatted sesame flour

3.1.2.1 Change of pH and acidity of fermented milk drinks enriched with defatted sesame flour

The results of pH and titratable acidity were shown in table 3.3. The pH of the samples ranged between 3.93 and 5.01. The titratable acidity ranged between 67.78·T and 234.91·T. Addition of defatted sesame flour caused decrease of pH values and increase of the acidity of fermented milk drinks. The results indicate that fermented milk drink samples with adding 8% of defatted sesame flour had the lowest pH and the highest acidity contents.

Table 3.3 – Acidity of fermented milk drinks in 28 days (n=3, ±SD)

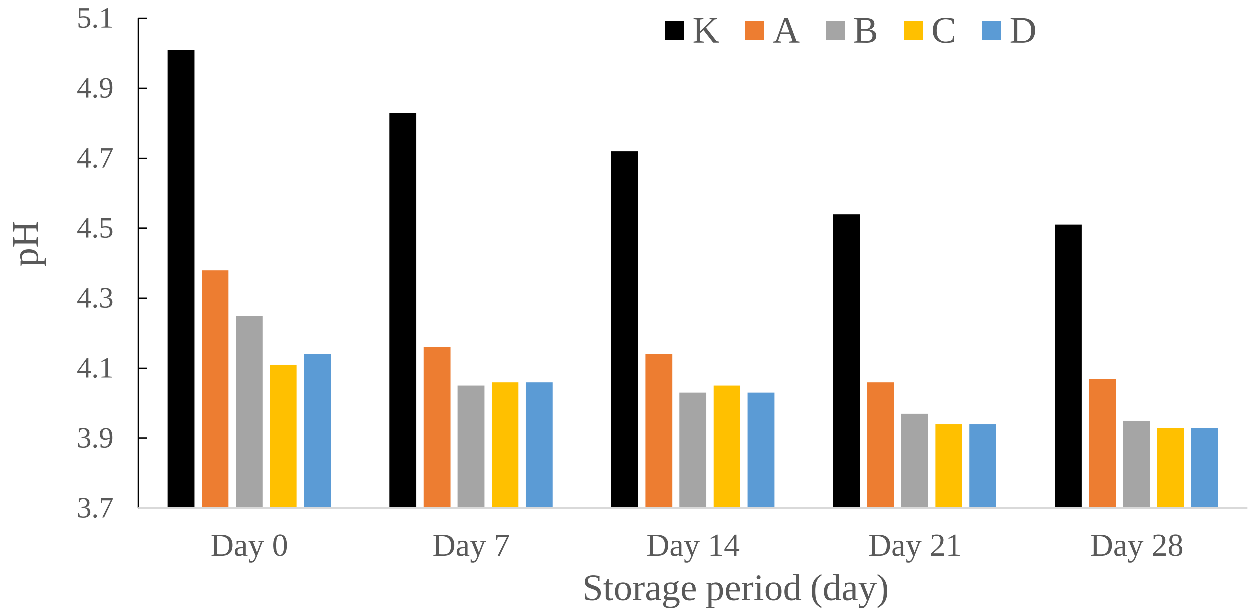
Indicators	Samples	Day 0	Day 7	Day 14	Day 21	Day 28
pH	K	$5.01\pm0.02^{a, X}$	$4.83\pm0.05^{a, Y}$	$4.72\pm0.02^{a, Z}$	$4.54\pm0.00^{a, W}$	$4.51\pm0.00^{a, W}$
	A	$4.38\pm0.03^{b, X}$	$4.16\pm0.02^{b, Y}$	$4.14\pm0.02^{b, Y}$	$4.06\pm0.00^{b, Z}$	$4.07\pm0.02^{b, Z}$
	B	$4.25\pm0.04^{c, X}$	$4.05\pm0.04^{c, Y}$	$4.03\pm0.00^{c, YZ}$	$3.97\pm0.00^{c, ZW}$	$3.95\pm0.00^{c, W}$
	C	$4.11\pm0.03^{d, X}$	$4.06\pm0.02^{c, Y}$	$4.05\pm0.02^{c, Y}$	$3.94\pm0.00^{d, Z}$	$3.93\pm0.00^{d, Z}$
	D	$4.14\pm0.05^{d, X}$	$4.06\pm0.01^{c, Y}$	$4.03\pm0.00^{c, Y}$	$3.94\pm0.00^{d, Z}$	$3.93\pm0.00^{d, Z}$
Titratable acidity, °T	K	$67.78\pm0.38^{d, M}$	$75.44\pm1.10^{d, W}$	$82.92\pm1.75^{e, Z}$	$92.83\pm1.17^{e, Y}$	$99.22\pm2.25^{d, X}$
	A	$112.07\pm0.92^{c, Z}$	$125.88\pm4.75^{c, Y}$	$133.46\pm1.62^{d, Y}$	$157.43\pm4.03^{d, X}$	$163.52\pm6.04^{c, X}$
	B	$117.34\pm2.18^{c, M}$	$155.27\pm1.38^{b, W}$	$167.25\pm2.77^{c, Z}$	$191.12\pm3.53^{c, Y}$	$205.57\pm8.26^{b, x}$
	C	$138.88\pm5.26^{b, M}$	$179.23\pm3.89^{a, W}$	$192.53\pm5.33^{b, Z}$	$208.41\pm1.04^{b, Y}$	$222.74\pm3.26^{a, X}$
	D	$163.13\pm6.02^{a, W}$	$188.61\pm9.84^{a, Z}$	$209.48\pm0.71^{a, Y}$	$222.00\pm2.52^{a, XY}$	$234.91\pm1.76^{a, X}$

$^{a, b, c, d}$ Means in the same column with different superscripts significantly differ (P<0.05)

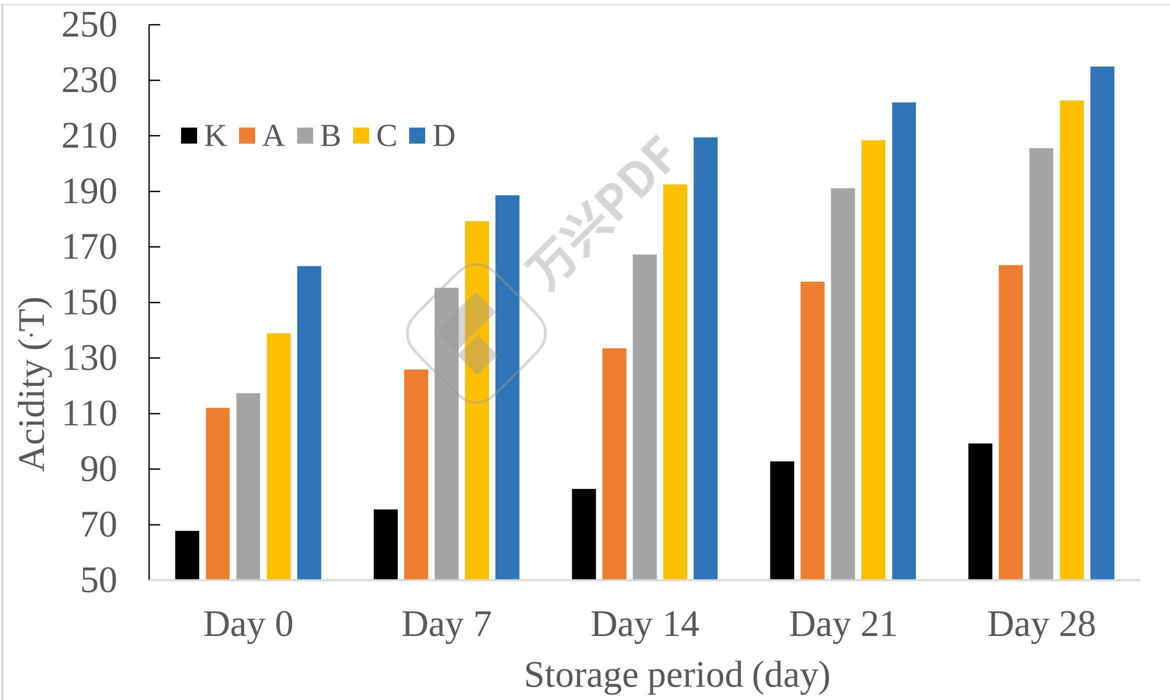
$^{X, Y, Z, W, M}$ Means in the same row with different superscripts among fermented milk drink samples significantly differ (P<0.05)

SD (Standard deviation): K= control sample, A= fermented milk drink with 2 % DSF, B= fermented milk drink with 4 % DSF, C= fermented milk drink with 6 % DSF, D= fermented milk drink with 8 % DSF.

PH values reduced significantly when the content of defatted sesame flour was 4% (P < 0,05) (fig. 3.2, *a*).



a



b

Figure 3.2 - Acidity of the studied batch during storage (n=3, ±SD): *a* – active acidity (pH); *b* – titratable acidity, °T

The research showed that titratable acidity increased as the content of DSF was up to 6% (fig.3.2, *b*). It could be related to stimulation of lactic acid bacteria by fiber. Previous studies have also demonstrated that fermented milk drink as a probiotic Guzel-Seydim et al., 2011; Mariana et al., 2011) and dietary fiber had a potential prebiotic effect (Shah et al., 2020; Wu et al., 2020). However, when the adding amount of DSi was up to 4%, the more DSF content made no difference on the pH of fermented milk drink ($P > 0.05$).

When the adding amount of DSF was up to 6%, more DSF made no difference on the titratable acidity of fermented milk drink ($P > 0.05$). The result of that study could be related to lower water activity (aw) of fermented milk drink samples enriched with 4 % DSF and more. As it was shown in this study, DSF had a good water holding capacity, and lactic acid bacteria in the fermented milk drinks couldn't find enough water for growing or producing lactic acid. 2%-4% of DSF was enough to significantly decrease the pH and titratable acidity, the more amount of DSF had a little effect on them.

As expected, the storage time affected significantly the level of pH and acidity in the samples ($p < 0.05$). Substantial decrease occurred in pH in the first week and the third week for all the samples. Titratable acidity increased sustained in 4 weeks. The reason for this phenomenon might come from the catabolism of lactose during storage.

3.1.2.2 Change of water holding capacity of fermented milk drinks enriched with defatted sesame flour

Water holding capacity (WHC) is the tendency to retain water or resistance towards phase separation of the product. The results of WHC of samples were shown in table 3.4 and figure 3.3.

Table 3.4 – Water holding capacity of the fermented milk drinks in 28 days (n=3, ±SD)

Indicators	Samples	Day 0	Day 7	Day 14	Day 21	Day 28
Water holding capacity,%	K	54.57±4.91$^{b, X}$	56.31±1.30$^{d, X}$	55.49±1.90$^{c, X}$	59.69±1.423^{d,X}	60.97±0.93$^{d, X}$
	A	67.76±0.85$^{a, X}$	64.4±1.28$^{c, Y}$	62.07±0.45$^{b, YZ}$	62.56±1.198$^{c, YZ}$	61.21±1.36$^{cd, Z}$

The end of the table 3.4

Indicators	Samples	Day 0	Day 7	Day 14	Day 21	Day 28
	B	67.49±2.26^{a,XY}	68.44±0.41^{b,X}	66.73±1.23^{a,XY}	64.49±0.28^{bc,YY}	64.30±0.79^{bc,YY}
	C	71.60±1.02^{a,X}	70.44±0.46^{ab,XY}	68.35±1.06^{a,YZ}	65.99±0.52^{ab,ZW}	64.87±1.35^{ab,W}
	D	70.17±0.97^{a,XY}	72.27±0.08^{a,X}	67.94±1.01^{a,YZ}	67.58±0.63^{a,YZ}	67.84±1.33^{a,Z}

$^{a, b, c, d}$ Means in the same column with different superscripts significantly differ (P<0.05)

$^{X, Y, Z, W}$ Means in the same row with different superscripts among fermented milk drink samples significantly differ (P<0.05)

SD (Standard deviation): K= control sample, A= fermented milk drink with 2 % DSF, B= fermented milk drink with 4 % DSF, C= fermented milk drink with 6 % DSF, D= fermented milk drink with 8 % DSF.

The WHC of fermented milk drinks supplemented with DSF was significantly increased compared with blank one (p<0.05), which was due to the high WHC of dietary fiber and a high content of protein in DSF. Fiber may act as a stabilizer due to its capacity for binding water (Mudgil & Barak, 2013). The amino acid composition of the proteins, the structures of the proteins and the ratio of surface polarity/hydrophobicity of the proteins are the major effects that influence water holding capacity of foods (Ilyas & Atalar, 2019).

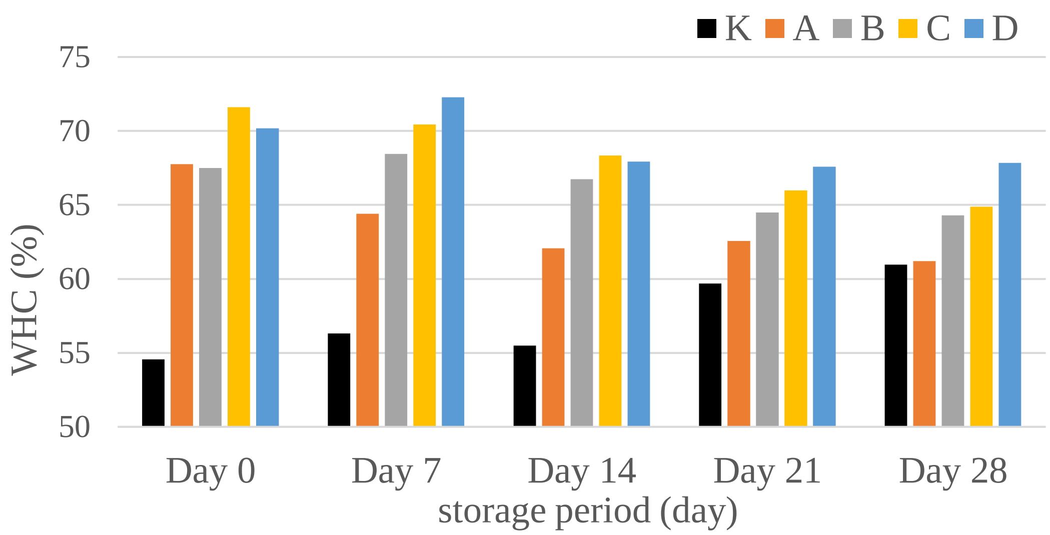


Figure 3.3. - Water holding capacity of the studied batch in the process of storage (n=3, ±SD)

There were no significant changes in WHC among fermented milk drinks added with 2%, 4%, 6%, 8% amount of DSF, which might be caused by the lack of free water, most molecules of water were combined in samples added with 2% DSF, let alone fermented milk drink added with 4%, 6%, 8% amount of DSF. Storage period made no difference on the WHC of control samples, but significant decrease of WHC occurred during storage period, which might be caused by the slow coagulation of DSF.

The research showed that adding of defatted sesame flour contributes to increase of water holding capacity in comparison with the control sample. This is explained by the capacity of the dietary fibers, contained in the defatted sesame flour, to absorb moisture.

3.1.2.3 Apparent viscosity of fermented milk drinks enriched with defatted sesame flour

The results of apparent viscosity were shown in table 3.5 and figure 3.4.

Table 3.5 – Viscosity of fermented milk drinks in 28 days (n=3, ±SD)

Indicator	Samples	Day 0	Day 7	Day 14	Day 21	Day 28
Apparent viscosity, Pa*s	K	$0.19\pm0.02^{c,Z}$	$0.23\pm0.02^{b,YZ}$	$0.27\pm0.05^{d,XY}$	$0.30\pm0.02^{b,XY}$	$0.35\pm0.03^{b,X}$
	A	$4.87\pm0.37^{b,Y}$	$6.01\pm1.47^{a,X}$	$3.26\pm0.34^{c,Z}$	$3.88\pm0.11^{a,Z}$	$5.06\pm0.64^{a,Y}$
	B	$7.53\pm0.92^{ab,X}$	$3.72\pm0.19^{a,Z}$	$6.52\pm0.09^{b,XY}$	$3.52\pm0.94^{a,Z}$	$5.72\pm0.67^{a,Y}$
	C	$7.33\pm1.12^{ab,X}$	$5.52\pm0.63^{a,X}$ Y	$5.88\pm0.29^{b,XY}$	$4.14\pm0.31^{a,Y}$	$5.95\pm1.31^{a,XY}$
	D	$9.33\pm1.69^{a,X}$	$5.52\pm1.11^{a,YZ}$	$8.45\pm1.31^{a,X}$ Y	$4.90\pm0.62^{a,Z}$	$5.68\pm0.60^{a,YZ}$

$^{a, b, c, d}$ Means in the same column with different superscripts significantly differ ($P<0.05$)

$^{X, Y, Z, W}$ Means in the same row with different superscripts among fermented milk drink samples significantly differ ($P<0.05$)

SD (Standard deviation): K= control sample, A= fermented milk drink with 2 % DSF, B= fermented milk drink with 4 % DSF, C= fermented milk drink with 6 % DSF, D= fermented milk drink with 8 % DSF

Compared with blank samples, even 2% amount of DSF increased significantly the apparent viscosity of kefir ($p<0.05$), not to mention samples added with 4%, 6% and 8% amount of DSF, which was caused by the adding amount of dry matter and the hydrophilicity of protein and fiber in DSF (Ilyas & Atalar, 2019; Mudgil & Barak, 2013).

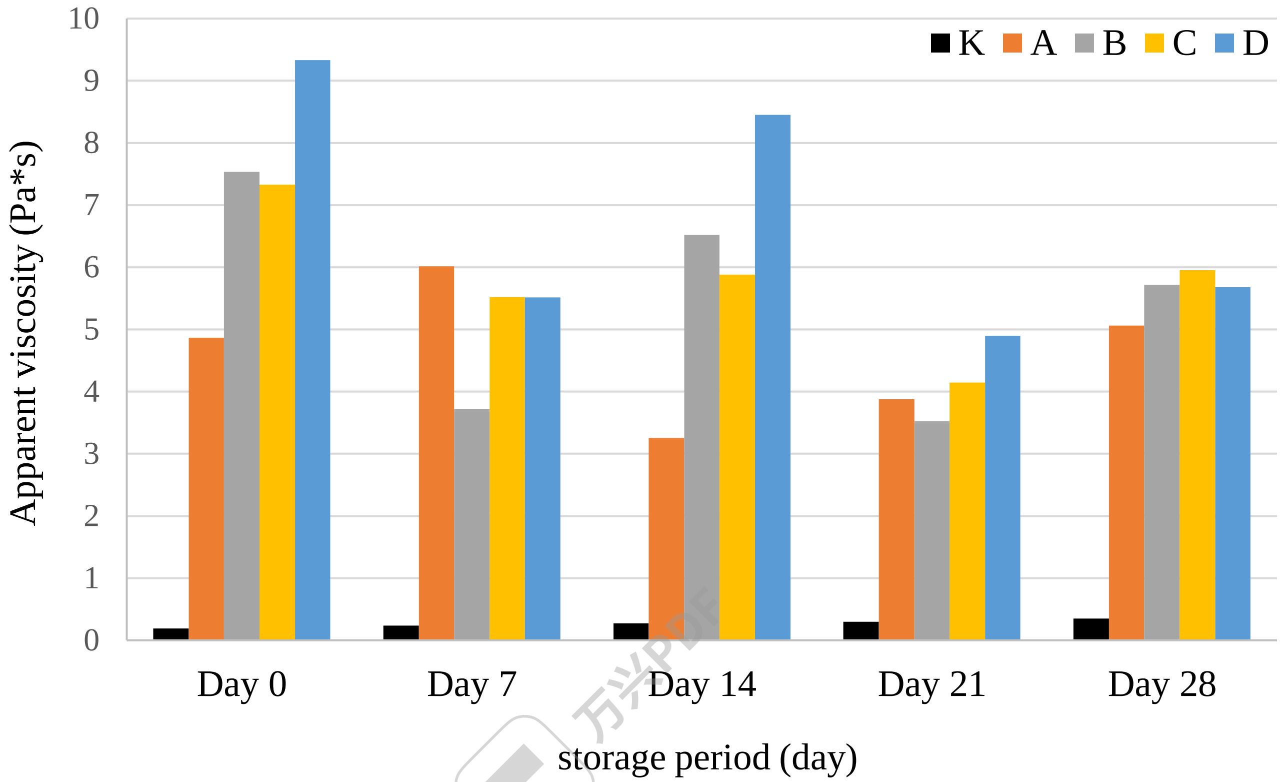


Figure 3.4 - Apparent viscosity of the studied batch in the process of storage (n=3, ±SD)

The apparent viscosity kept steady among samples added with 2%, 4%, 6% and 8% amount of DSF during storage period ($p > 0.05$), this might partly be explained by the balance of sedimentation and hydrophilic interaction of DSF. The storage period significantly influenced the apparent viscosity of the control sample. The apparent viscosity of the control sample improved during the storage period ($p<0.05$).

Sharply contrasted, the apparent viscosity of samples added with DSF were in a fluctuant downward trend during the storage period, which could be explained by the sedimentation of DSF due to presence of the dietary fibers in it.

3.1.2.4 The color of fermented milk drinks enriched with defatted sesame flour

The effect of DSF on the colour parameters of the studied batch were shown in Table 3.6.

Table 3.6 - Color parameter changes during cold storage

Samples	Storage period (day)	L	a	b
K	0	97.08±0.02$^{a, Y}$	1.96±0.09$^{d, Z}$	2.25±0.02$^{d, X}$
	7	97.42±0.33$^{a, XY}$	2.06±0.05$^{d, YZ}$	2.00±0.11$^{d, Y}$
	14	97.71±0.13$^{a, X}$	2.23±0.05$^{e, X}$	2.02±0.03$^{e, Y}$
	21	97.73±0.13$^{a, X}$	2.14±0.05$^{e, XY}$	1.84±0.05$^{e, Z}$
	28	97.38±0.10$^{a, XY}$	2.05±0.04$^{d, YZ}$	1.94±0.01$^{e, YZ}$
A	0	95.6±0.40$^{b, Y}$	3.04±0.04$^{c, Y}$	4.03±0.09$^{c, X}$
	7	96.06±0.30$^{b, XY}$	3.21±0.06$^{c, XY}$	3.92±0.03$^{c, X}$
	14	96.35±0.03$^{b, X}$	3.34±0.08$^{d, X}$	3.91±0.10$^{d, X}$
	21	96.45±0.08$^{b, X}$	3.05±0.05$^{d, Y}$	3.55±0.08$^{d, Y}$
	28	96.41±0.11$^{b, X}$	3.27±0.09$^{c, X}$	3.62±0.11$^{d, Y}$
B	0	94.35±0.32$^{c, X}$	3.43±0.06$^{b, Z}$	5.53±0.11$^{b, X}$
	7	94.94±0.57$^{c, X}$	3.61±0.08$^{b, X}$	5.25±0.03$^{b, Y}$
	14	94.59±0.11$^{c, X}$	3.6±0.04$^{c, XY}$	5.21±0.12$^{b, Y}$
	21	95.15±0.09$^{c, X}$	3.46±0.02$^{c, YZ}$	5.12±0.09$^{c, YZ}$
	28	94.90±0.06$^{c, X}$	3.49±0.04$^{b, XYZ}$	4.89±0.04$^{c, Z}$
C	0	93.53±0.38$^{c, X}$	3.88±0.23$^{a, Y}$	6.1±0.49$^{ab, Y}$
	7	93.47±0.29$^{d, X}$	4.14±0.07$^{a, XY}$	6.62±0.72$^{a, Y}$
	14	93.48±0.25$^{d, X}$	4.28±0.06$^{a, X}$	6.78±0.47$^{a, Y}$
	21	91.47±0.27$^{e, Y}$	3.99±0.12$^{a, XY}$	9.25±0.03$^{a, X}$
	28	91.55±0.09$^{e, Y}$	4.16±0.06$^{a, XY}$	8.79±0.06$^{a, X}$
D	0	93.79±0.40$^{c, X}$	3.72±0.13$^{ab, XY}$	6.45±0.33$^{a, X}$
	7	94.29±0.03$^{cd, X}$	3.79±0.09$^{b, XY}$	5.94±0.10$^{ab, Y}$
	14	94.33±0.17$^{c, X}$	3.81±0.05$^{b, X}$	5.85±0.04$^{b, Y}$
	21	94.16±0.06$^{d, X}$	3.73±0.05$^{b, XY}$	5.99±0.03$^{b, Y}$
	28	93.99±0.21$^{d, X}$	3.56±0.11$^{b, Y}$	5.93±0.03$^{b, Y}$

The parameters “L”, “a” and “b” represent lightness (100) and blackness (0), red (+ve)-green (−ve) and yellow (+ve)-blue (−ve) hues separately (Sanz та ін., 2008). The colorimetric parameters (L, a and b) showed significant differences ($p < 0.05$) among different formulations. The luminosity values (L) ranged from 97.73 to 91.47 in all samples.

The "L" value of samples added with DSF were significantly decreased compared with samples without DSF (K), the more DSF was added, the smaller the "L" value was. It was shown that the introduction of DSF significantly decreased the brightness of fermented milk drink, which made samples look dark. The values of "a" ranged from 1.96 to 4.28. The values of "a" of samples added with DSF were significantly increased compared with control samples (K), the more DSF was added, the bigger the "a" value was, which showed that the introduction of DSF into fermented milk drink significantly deepened the red color of fermented milk drink. The values of "b" ranged from 1.84 to 9.25. The values of "b" of samples introduced with DSF were significantly increased compared with control samples (K), the more DSF was added, the bigger the values of "b" was, which showed that the introduction of DSF significantly deepened the yellow of fermented milk drink. Generally speaking, the introduction of DSF led to the dark color of kefir. DSF had a dark color because of the progress of the high temperature stir-frying.

As the data showed in Table 3.6, storage period made no significant difference on the brightness ("L" value) of all samples ($P > 0.05$). With the prolongation of storage time, the red color ("a" value) of all samples showed a trend of deepening at first and then weakening ($P < 0.05$), and the red was the deepest on the 14th day.

3.1.3 The rheological properties of fermented milk drinks enriched with defatted sesame flour

The spin-spin relaxation time of fermented milk drinks added with sesame flour during storage was measured by NMR, the results were shown in the following figure 3.5. Three peaks were obtained after software fitting. The transverse relaxation time range T_{21} (0.051~ 1.831) and T_{22} (5.722~ 24.771) of fermented milk drinks were small, the signal amplitudes were also small, which showed that the signal of T_{21} and T_{22} might be the water that was less easy to flow in the pores that form the network structure.

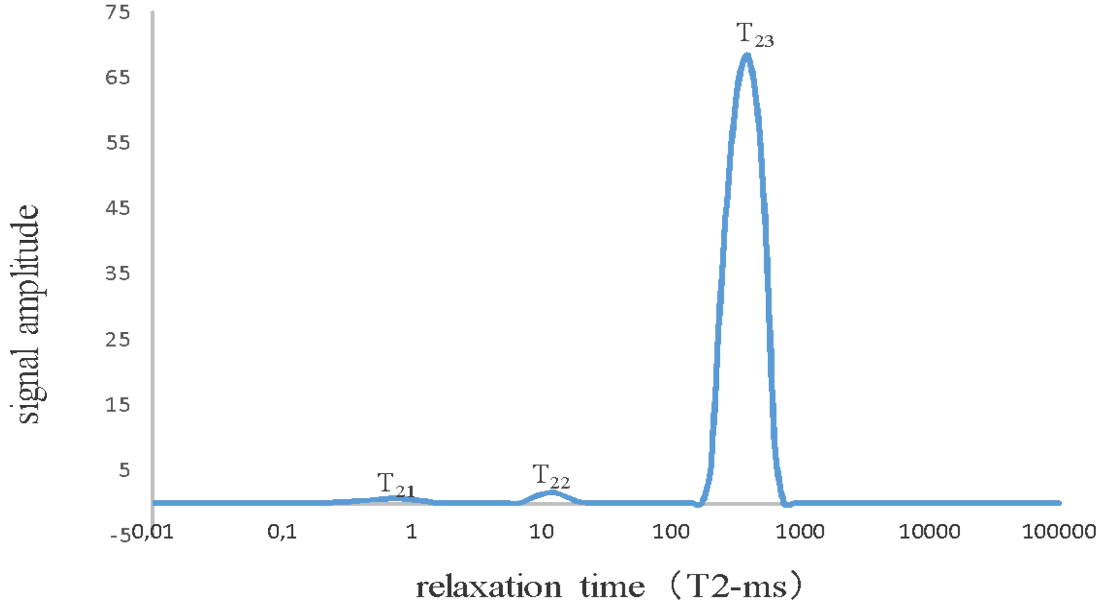
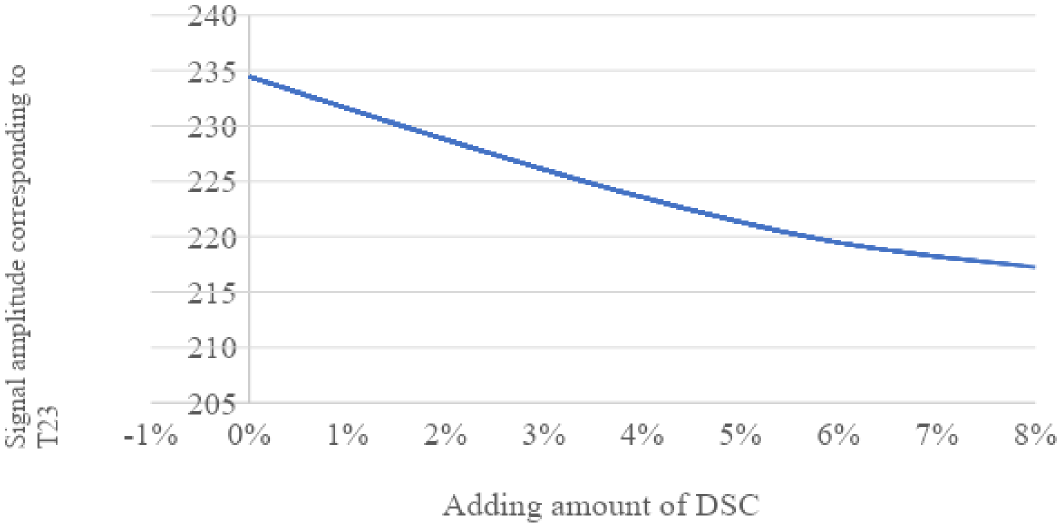


Figure 3.5 - The spin-spin relaxation time of studied samples

The hydrogen bond strength of this kind of water was strong, or it could be the parts of the water that bind tightly to large molecules like protein. The transverse relaxation time range of T_{23} (174.753~ 756.463 ms) was higher than that of T_{21} and T_{22}, The signal amplitude of M_{23} was also high, which indicated that the combination of T_{23} water molecules was relatively loose and had high fluidity (CHEN et al., 2006; Cheng et al., 2013; LIN et al., 2006). Therefore, the peak area changes of T_{23} were mainly studied. T_{23} and the signal amplitude M_{23} were taken as the main indexes, the moisture transfer characteristics of samples were studied.

The signal amplitude corresponding to T_{23} of different kefir samples (K, A, B, C, D) were shown in figures 3.6 *(a, b, c, d)*.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7%
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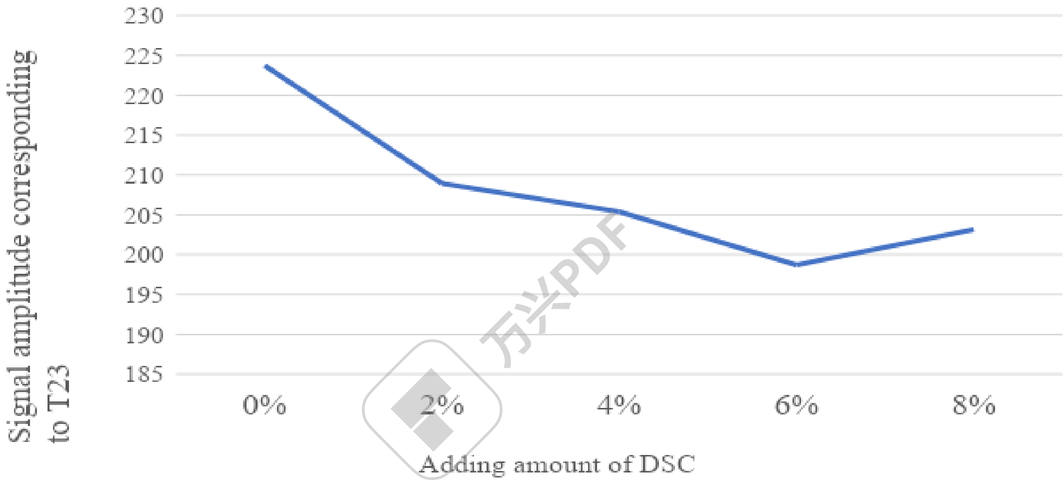
Signal amplitude corresponding to T23
Adding amount of DSC

a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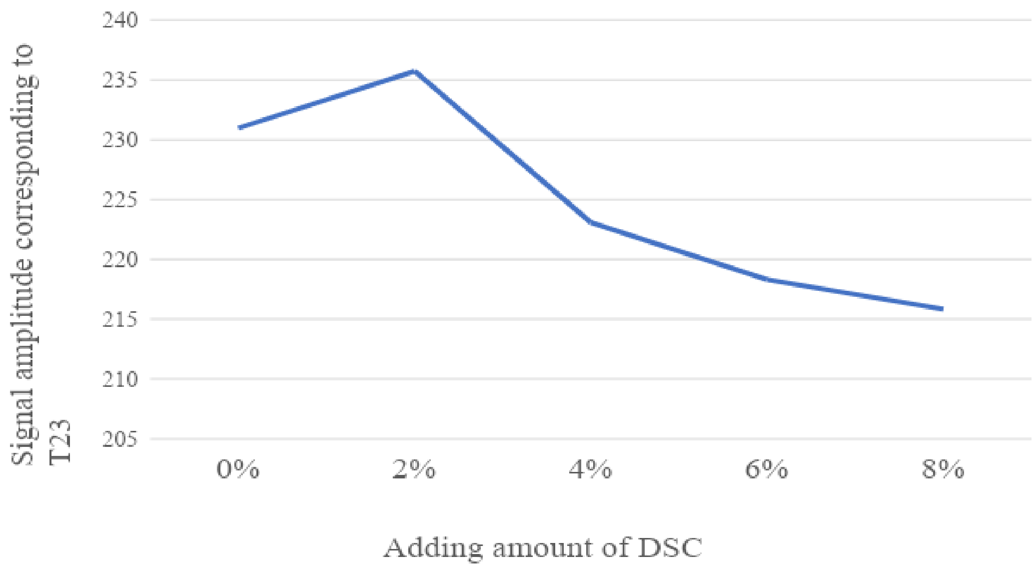
Signal amplitude corresponding to T23
Adding amount of DSC

b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Signal amplitude corresponding to T23
Adding amount of DSC

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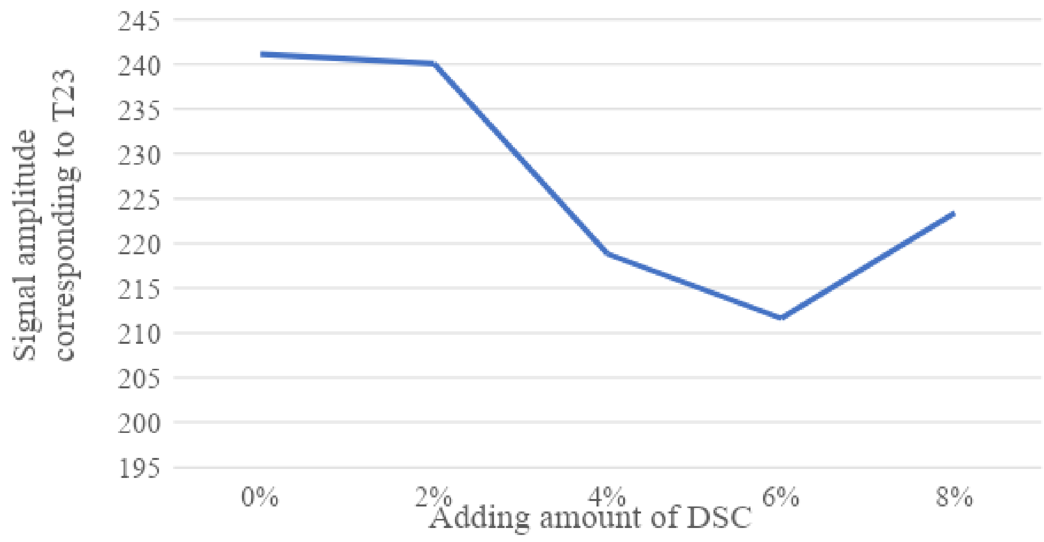


d

Figure 3.6. Signal amplitude corresponding to T_{23} of the studied batch : a - on 0 day; b – on 7th day; c - on 21st day; d - on 28th day.

The summarized results of transverse relaxation time distribution of kefir samples were shown in figure 3.7.

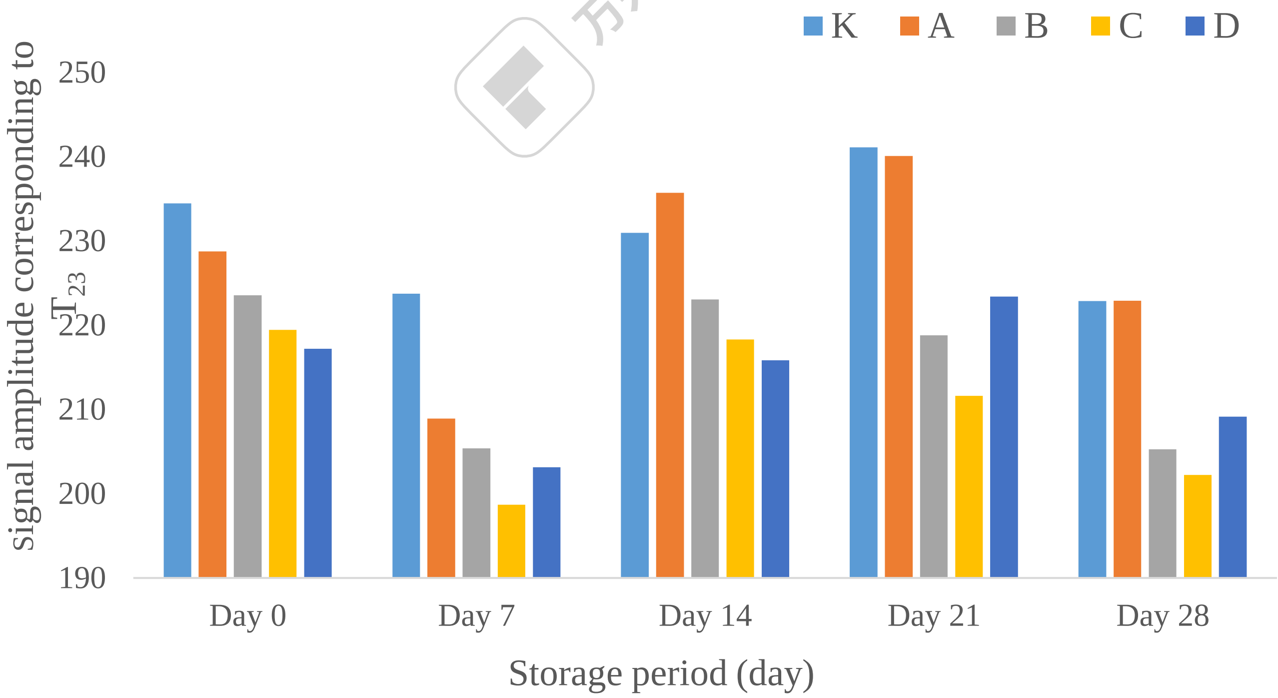


Figure 3.7 - Transverse relaxation time distribution of kefir samples during storage for 28 days (n=3, ±SD)

With the extending of storage time, the signal amplitude corresponding to T_{23} presented a down-up-down fluctuation. During the first week of storage, the signal amplitude decreased significantly, indicating that the content of free water reached the lowest, the retention power of the sample reached the highest, and the solidification state was the best.

Then on the 21st day, the signal amplitude increased significantly, indicating that the content of free water increased, the water holding capacity of the samples decreased, and the solidification structure also decreased. Until the 28th day of storage, the water holding capacity of the samples increased again, and the solidification structure increased too.

Within the 21 days of storage, the signal amplitude T_{23} of the samples significantly decreased as the adding amount of DSF rose, indicating that the content of free water significantly decreased, and the solidification state of the samples enhanced. On the 28th day of storage, the signal amplitude T_{23} of all samples was completely consistent, indicating that the solidification state of all samples tended to be consistent at the later stage of storage. According to these data, the storage period of fermented milk drinks should be not more than 14 days.

When the amount of DSF exceeded 6%, the amount of DSF had a little effect on the signal amplitude T_{23} of samples, this might be caused by the lack of free water. The dietary fibers included in the composition of sesame flour retain moisture, which ensures the stability of the structure.

3.1.4 Microbiological indicators of fermented milk drinks enriched with defatted sesame flour

The changes of the population of *Lactobacillus*, Yeast and *Lactococcus* in 28 days were shown in figures 3.8-figure 3.10.

The *Lactobacillus* counts were between 8.04- 9.62 log CFU mL^{-1} during storage time. The *Lactococcus* counts were between 9.02-9.77 log CFU mL^{-1} during storage time. Yeast counts were between 7.81-10.25 log CFU mL^{-1} during storage time. The introduction of DSF made no difference on the microbiological count, which may be due to the combined effect of the promoting effect of dietary fiber and the bacteriostasis effect of sesame phenolic substances on the growth of microorganisms.

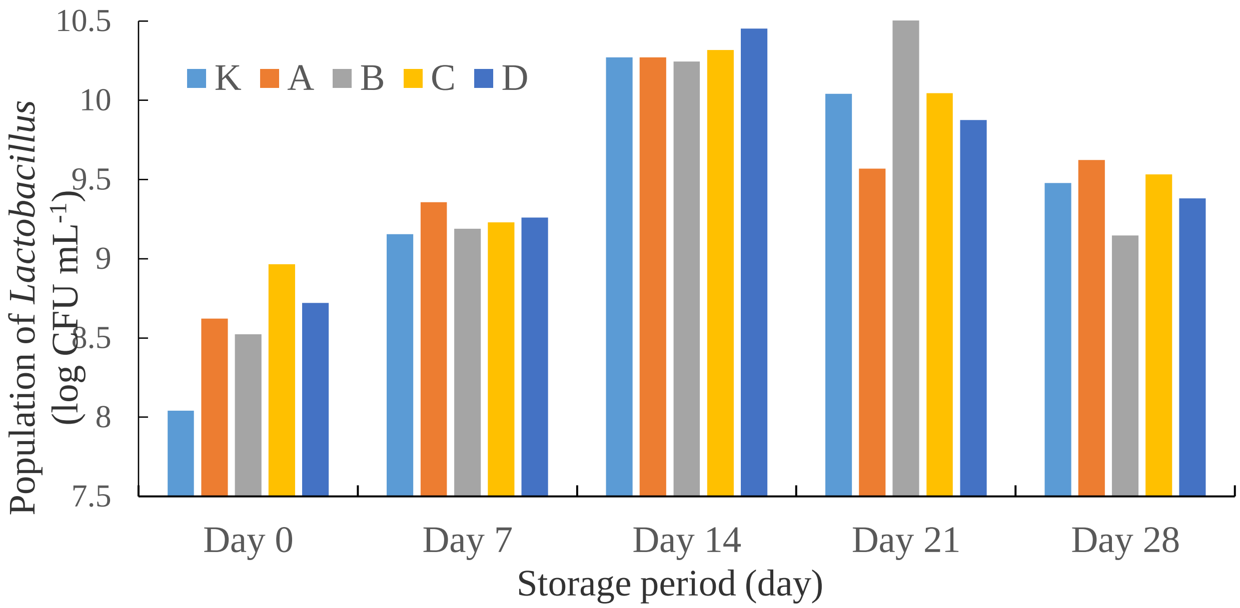


Figure 3.8 - Population of *Lactobacillus* of the studied samples

Compared with other samples, the *Lactobacillus* and *Lactococcus* counts of samples added with 8% DSF slightly decreased due to the inhibitory effect of antimicrobial and phenolic agents present in DSF. Some scientists (Alfuraydi et al., 2019) reported that DSF showed high activity against bacteria, authors also indicated that antimicrobial properties originate from phenolic compounds in DSF. Another reason for this reduction may be resulted in antagonistic effect of higher yeast content against lactic acid bacteria. The co-cultured organism may compete for nutrients or may produce metabolic products that may stimulate or inhibit each other's growth (Santos et al., 2014).

Viable counts of lactobacillus in samples varied from log 8.0 to 8.7 after production and important reductions occurred in some samples after 14 days of storage (figure 3.9).

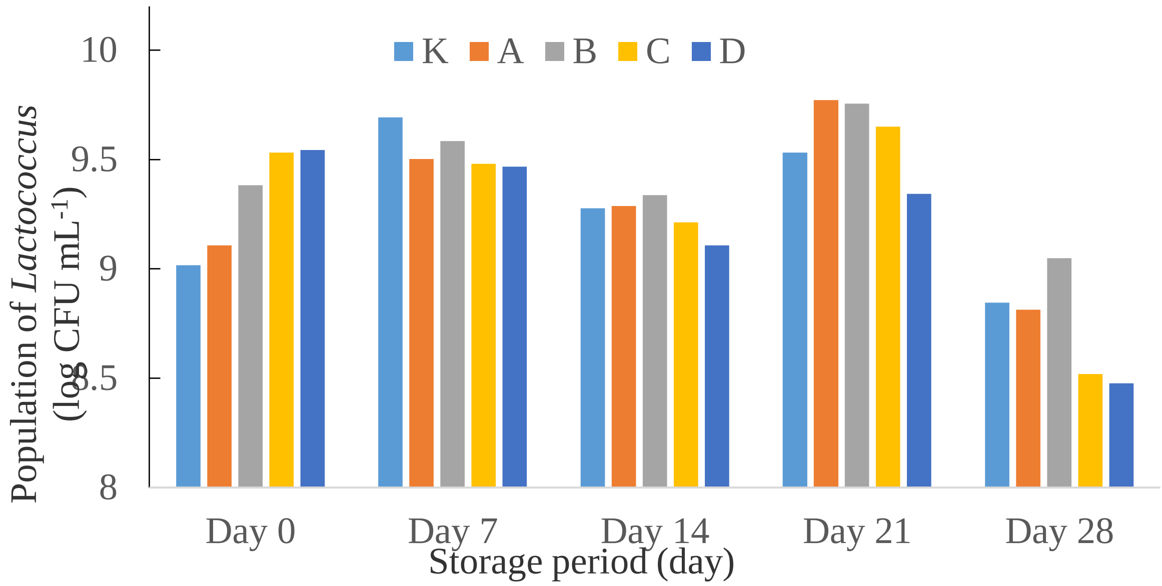


Figure 3.9 - Population of *Lactococcus* of the studied samples

Viable counts of *Lactococcus* in samples varied from log 9.0 to 9.5 after production and important reductions occurred in some samples after 7 days of storage, the number of *Lactococcus* was the highest on the 7th day.

Viable counts of yeast in samples varied from log 5,3 to 7,4 after production and important reductions occurred in some samples after 14 days of storage (figure 3.10).

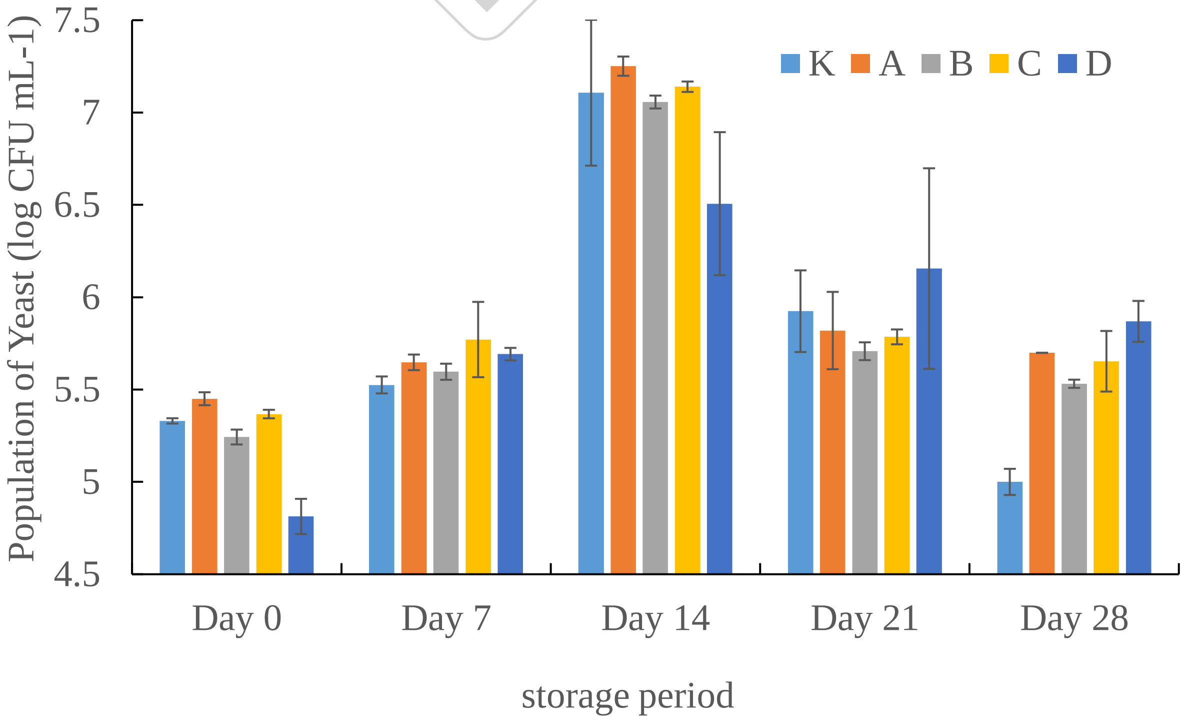


Figure 3.10. Population of Yeasts of the studied samples

The number of yeasts was the highest on the 14th day. The CFU valuesLactococcus > lactobacillus > yeast. Fermented milk drink is considered to be a probiotic product with numerous therapeutic benefits (Otles & Cagindi, 2003).

However, to achieve the desired functionality of the intrinsic microbiota, the product must have minimal cell viability which, according to the Food and Agriculture Organization of the United Nations/World Health Organization - (FAO/WHO, 2003), recommendations, is at least 4 and 7 log CFU/g of yeast and bacteria respectively.

Therefore, the kefir formulations developed in this study were in accordance with these specifications. The concentration, type and characteristics of the ingredients added in the preparation of fermented milks play a fundamental role in the endogenous bacteria activity of the product (Baú et al., 2013).

The dietary fibers of sesame flour are prebiotics that act as an environment for the development of beneficial microflora. In the absence of a sufficient amount of fiber, microorganisms cannot fully synthesize vitamins, amino acids, hormones, micro- and macroelements.

This also allows to ensure the shelf life of the product within 14 days without the use of preservatives and structural stabilizers.

3.1.5 Antioxidant properties of fermented milk drinks enriched with defatted sesame flour

In the test of antioxidant properties, a standard curve expressing the relationship between absorption values and content of VC (mg/mL) should be built. A standard curve was prepared using a standard solution of vitamin C, and absorbance was read using a spectrophotometer (Thermo Fisher Evolution 300, USA) at 517 nm. The data were shown in the following table 3.7.

Table 3.7 The relationship between absorption values and content of VC (mg/mL).

Absorbance	Content of VC (mg/ml)
0.441	0.015
0.393	0.025
0.343	0.035
0.29	0.045
0.251	0.055

The standard curve expressing the relationship between absorption values and content of VC (mg/ml) was shown in figure 3.11.

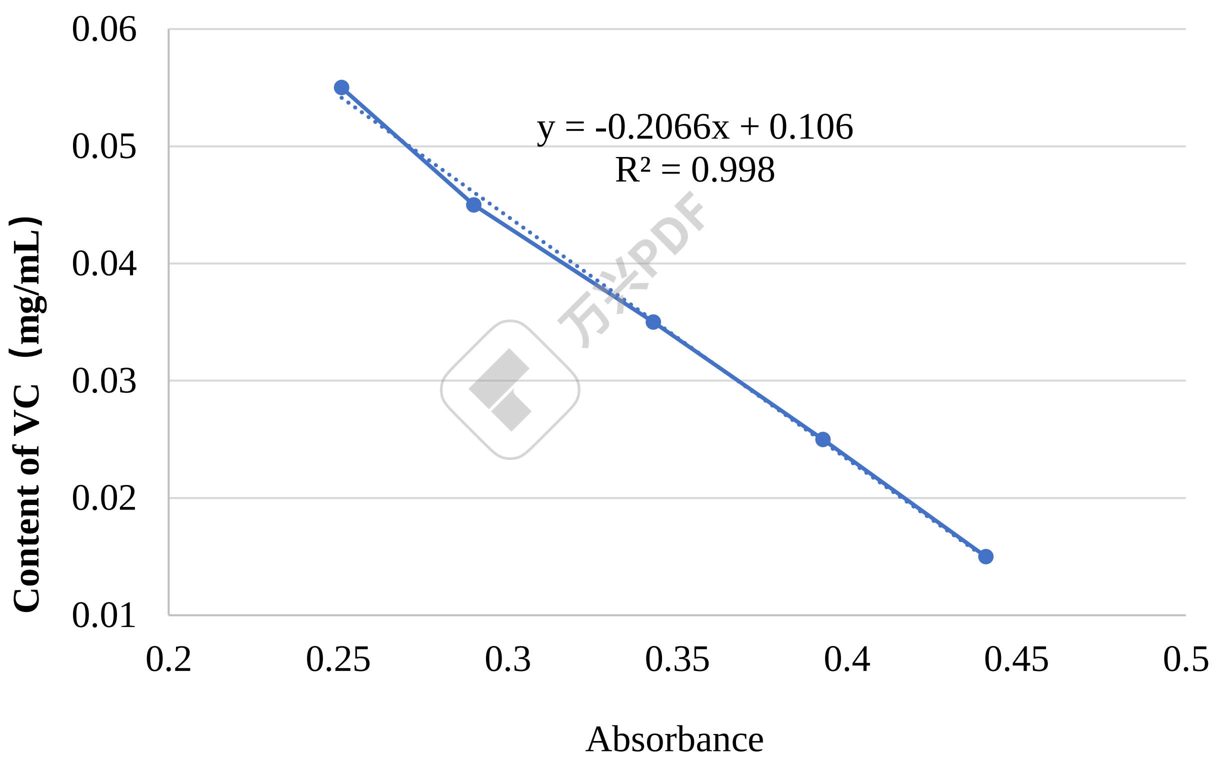


Figure 3.11 - The standard curve that expresses the relationship between absorption values and content of VC

When antioxidant capacity of samples were determined, diluted sample would detect a absorbance value according to the experimental method, the absorbance value reflected the antioxidant capacity of diluted sample, through standard formula:

$$y = -0.2066x + 0.106, \quad (3.1)$$

where y - absorbance value,

x - content of VC (mg/mL), and dilution ratio and volume, the absorbance value would be transformed into mg VC equivalent.

DPPH radical scavenging activity and ·OH-radical scavenging activity are most used methods to measure the antioxidant capacity of natural extracts. The antioxidant properties of fermented milk drinks were shown in figure 3.12 and figure 3.13.

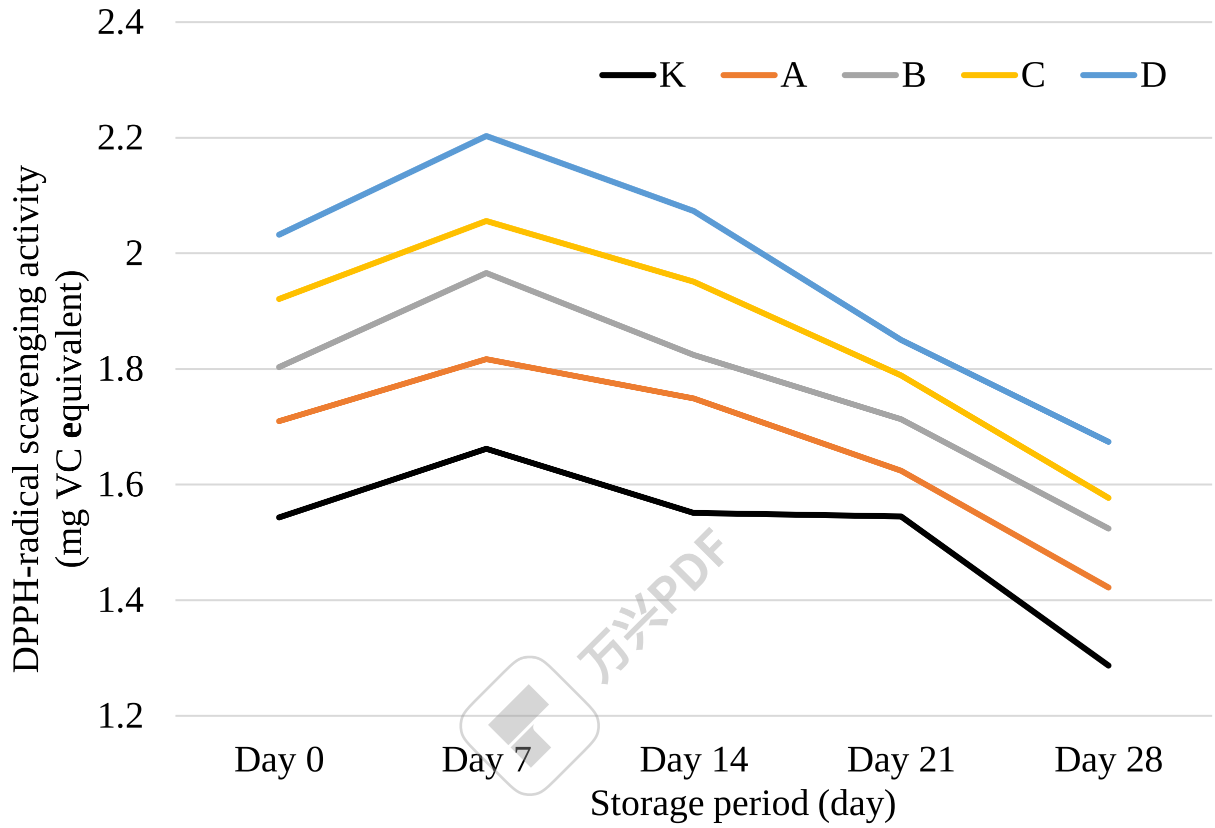


Figure 3.12 - DDPH-radical scavenging activity of the studied samples

It was found that the introduction of DSF increased significantly levels of DDPH-radical scavenging activity (Fig. 2.12) ($P<0.05$) and ·OH-radical scavenging activity (Fig.13) ($P<0.05$). Sample D had the highest antioxidant activity with 2.023 mg VC/ml (DPPH-radical scavenging activity) and 11.803 mg VC/ml (·OH-radical scavenging activity) whereas sample K had lowest value as mg 1.543 mg VC/ml (DPPH-radical scavenging activity) or 9.755 mg VC/ml (·OH-radical scavenging activity).

This indicated that some antioxidants components in DSF improve the antioxidant properties of kefir (Reham et al., 2019; Yashaswini et al., 2017). The antioxidant properties of DSF have been attributed to high levels of sesamol (Erika et al., 2018). (Sallam et al., 2021) evaluated the antioxidant capacity of sesame oil and also found that the addition of sesame oil and sesamol provided high antioxidant capacity.

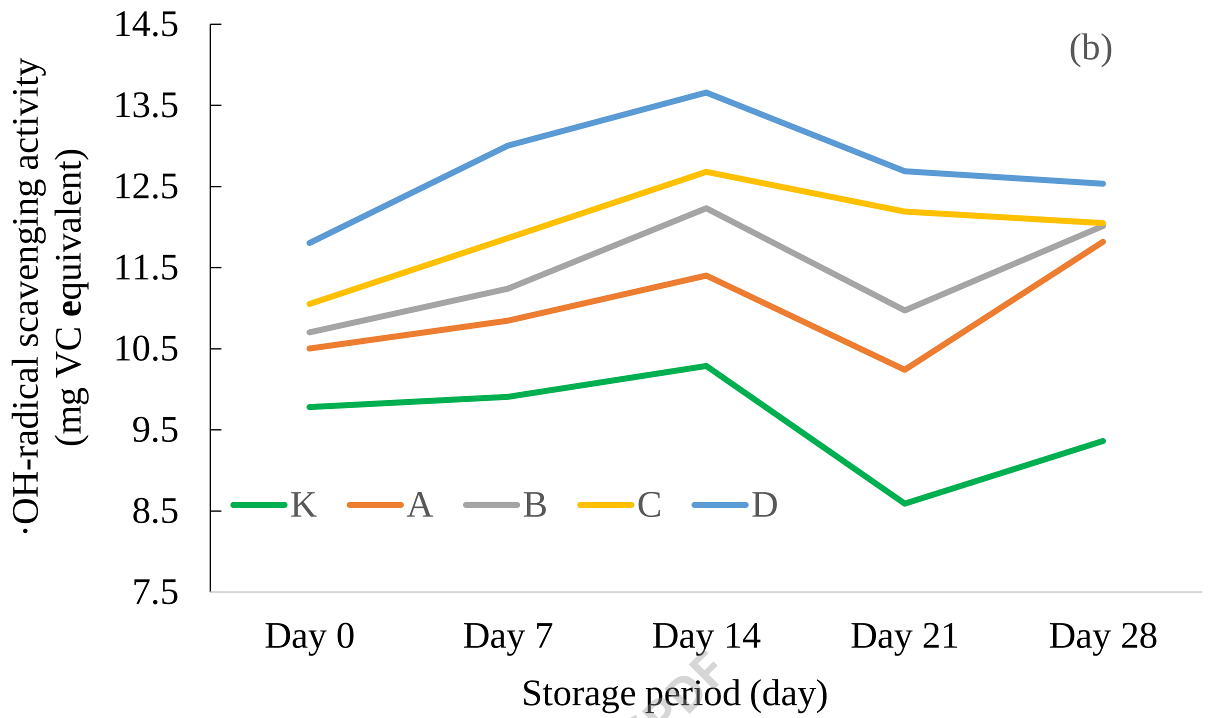


Figure 3.13 - ·OH-radical scavenging activity of the studied samples

The DDPH-radical scavenging activity of all samples decreased after 7 days of storage while ·OH-radical scavenging activity decreased for all samples after 14 days of storage. (Ilyas & Atalar, 2019) stated that the degradation of polyphenols and decrease of yoghurts antioxidant activities followed first order kinetic during storage.

The DPPH-radical scavenging activity was the highest on the 7th day of storage while the ·OH-radical scavenging activity was the highest on the 14th day of storage. Similar to our results, the results were received during investigating sesame oil and sesamol in meatballs during cold storage for 18 days at 3 ± 1°C. Addition of either sesame oil or sesamol significantly delayed lipid oxidation when compared with control. Sesamol exhibited more potent antioxidant activities than sesame oil.

Thus, it can be affirmed that sesame flour has a positive effect on the antioxidant properties of the product, allowing it to be preserved throughout the entire storage period.

3.1.6 Sensory indicators of fermented milk drinks enriched with defatted sesame flour

The concentrations of defatted sesame flour to be added into the plain fermented milk drink were determined by taking into consideration sensory evaluation results. The samples were shown in figure 3.14.

Figure 3.14 -. The studied batch of fermented milk drinks with adding of defatted sesame flour

Addition of defatted sesame flour had a significant effect on the sensory characteristics of fermented milk drinks ($p<0.05$). From top left corner to lower right corner are: fermented milk drink fermented with 0 DSF, fermented milk drink fermented with 2% DSF, fermented milk drink kefir fermented with 4% DSF, fermented milk drink fermented with 6% DSF, fermented milk drink fermented with 8% DSF.

The studied sample of fermented milk drink with adding 2 % of DSF had the highest sensory scores. The studied sample of fermented milk drink with adding 8 % of DSF had the lowest sensory scores.

In this research adding of defatted sesame flour had a positive effect on the taste and consistency due to better neck thickness and pleasant sourness.

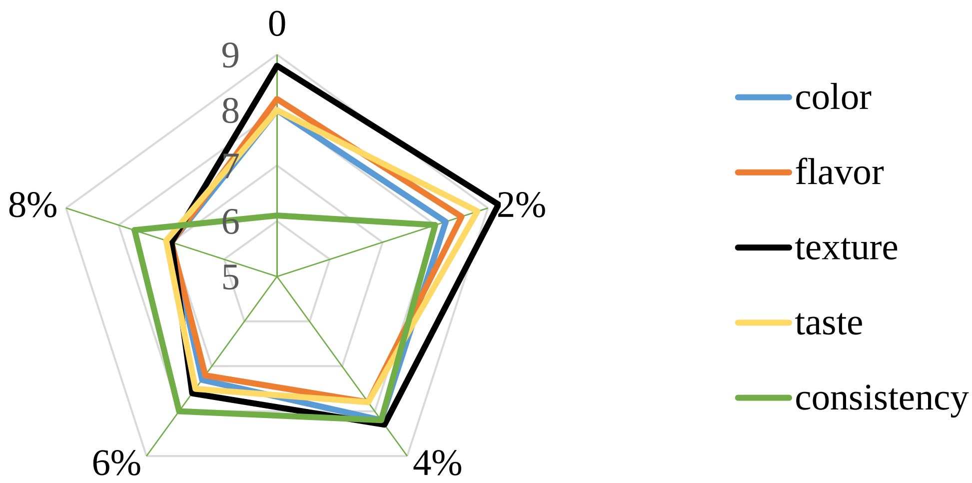


Figure 3.15 – Sensory characteristics of fermented milk drinks with adding of defatted sesame flour

When the adding amount of DSF was higher than 2%, the color of the sample darked seriously, so as to flavor and taste. Higher DSF adding amount caused lower sensory scores. The scorched taste was also increased with the increase of the amount of DSF, because DSF was cooked at high temperature at about 120-130°C.

The fermented milk drink sample with adding 2% of DSF had the highest sensory characteristics. It showed higher viscosity, suitable sour taste, light yellow color, fresh flavor of sesame and aroma of sesame. The main factor leading to the decline of sensory evaluation was the scorched taste. The research showed that the optimal amount of sesame flour additive was 2%.

3.2 The study of fermented milk drinks enriched with rice bran

3.2.1 The study of chemical composition of rice bran

The main components of RB are shown in Table 3.8.

Table 3.8 - Chemical composition of rice bran

Component	Fat	Protein	Dietary fiber	Ash	Water
Content, %	21.56±0.21	11.18±0.33	28.57±1.98	8.57±1.34	9.77±0.07

The results showed that RB was rich in dietary fiber, the content was up to 28.57%. The content of fat and protein were high (21.56% and 11.18% respectively), too. Its high content of dietary fiber and protein could play a good thickening effect on fermented milk drink.

3.2.2 The effect of rice bran on physical and chemical parameters of fermented milk drinks enriched with rice bran

3.2.2.1 Change of pH and acidity of fermented milk drinks enriched with rice bran

The results of pH and titratable acidity were shown in table 3.9 and figures 3.16-3.17.

Table 3.9 – pH and titratable acidity of samples of fermented milk drinks

Indicators	Samples	Day 0	Day 7	Day 14	Day 21	Day 28
pH	0	5.06±0.02 $^{a, A}$	4.94±0.01 $^{a, B}$	4.79±0.00 $^{a, C}$	4.61±0.01 $^{a, D}$	4.54±0.01 $^{a, E}$
	0.1%	4.92±0.00 $^{b, A}$	4.74±0.04 $^{b, B}$	4.65±0.01 $^{b, C}$	4.49±0.02 $^{b, D}$	4.43±0.02 $^{b, E}$
	0.3%	4.74±0.01 $^{c, A}$	4.63±0.01 $^{c, B}$	4.52±0.01 $^{c, C}$	4.38±0.01 $^{c, D}$	4.34±0.00 $^{c, E}$
	0.5%	4.71±0.00 $^{d, A}$	4.58±0.01 $^{c, B}$	4.47±0.01 $^{d, C}$	4.29±0.01 $^{d, D}$	4.31±0.01 $^{d, E}$
	0.7%	4.57±0.00 $^{e, A}$	4.52±0.02 $^{d, B}$	4.41±0.01 $^{e, C}$	4.37±0 $^{c, D}$	4.23±0.01 $^{e, E}$
Titratable acidity, (°T)	0	64.21±0.42 $^{e, D}$	67.14±1.08 $^{d, CD}$	72.00±1.97 $^{d, AB}$	68.93±0.68 $^{e, BC}$	75.14±0.51 $^{d, A}$
	0.1%	70.02±0.89 $^{d, B}$	74.01±3.16 $^{c, B}$	82.05±0.26 $^{c, A}$	80.30±0.72 $^{d, A}$	82.69±1.32 $^{c, A}$
	0.3%	77.58±1.05 $^{c, D}$	86.15±0.41 $^{b, C}$	91.42±0.36 $^{b, AB}$	89.01±2.27 $^{c, BC}$	94.33±0.61 $^{b, A}$
	0.5%	83.39±0.37 $^{b, D}$	85.71±0.37 $^{b, C}$	91.36±1.06 $^{b, B}$	92.32±0.50 $^{b, B}$	96.92±1.03 $^{ab, A}$
	0.7%	93.9±1.98 $^{a, C}$	97.86±0.94 $^{a, BC}$	100.64±3.28 $^{a, AB}$	102.28±0.12 $^{a, A}$	105.22±0.56 $^{a, A}$

* $^{a, b, c, d, e}$ Means in the same column with different superscripts significantly differ ($P<0.05$)

* $^{A, B, C, D, E}$ Means in the same row with different superscripts among kefir samples significantly differ ($P<0.05$)

* SD: Standard deviation

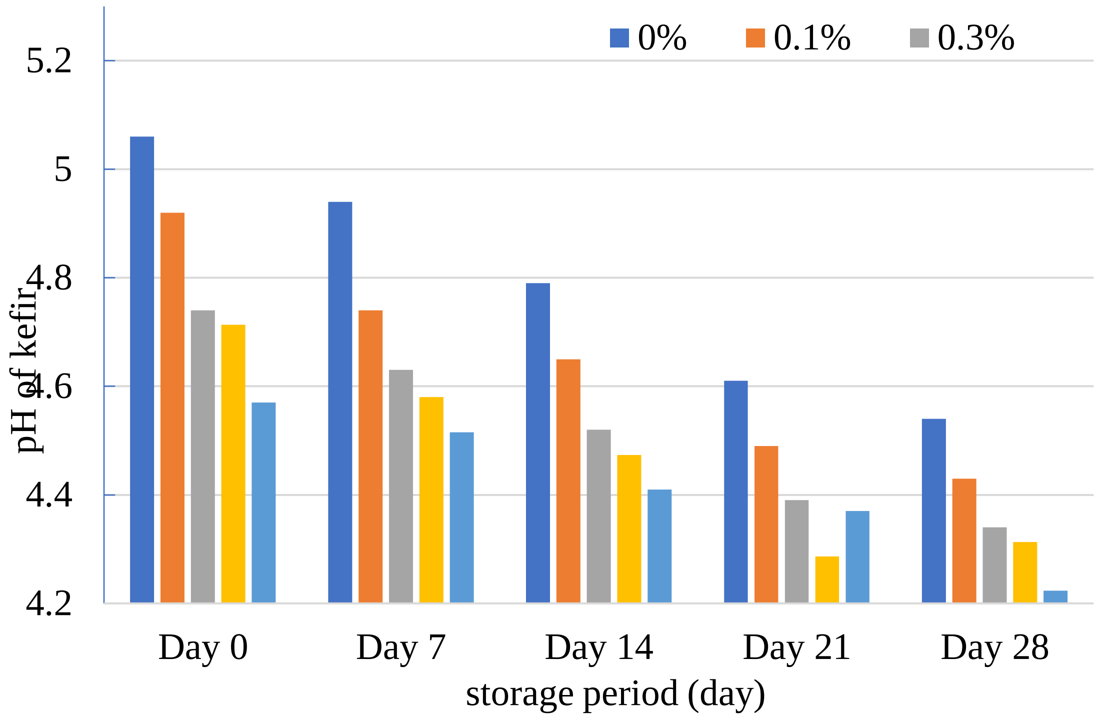


Figure 3.16 – Change of pH of fermented milk drink enriched with rice bran

The pH of the samples ranged between 4.23 and 5.06. The titratable acidity was between 70.02·T and 105.22·T. Addition of RB caused decrease of pH values and increase of the titratable acidity of fermented milk drinks. Results indicated that 0.7% DSF added fermented milk drink samples had the lowest pH and the highest titratable acidity. It could be related to:

1) Stimulation of lactic acid bacteria by fiber. Previous studies have also demonstrated that fermented milk drink as a probiotic Guzel-Seydim et al., 2011; Mariana et al., 2011) and dietary fibers had a potential prebiotic effect (Shah et al., 2020; Wu et al., 2020);

2) The degradation of polysaccharides present in RB also contributes to the reduction of pH, since these polysaccharides are transformed into glucose, which is eventually converted into lactic acid by microorganisms (Jawad et al., 2013). Adding the different amounts of RB made significant differences on pH and titratable acidity of fermented milk drinks.

As expected, storage period significantly affected the level of pH and acidity of samples ($p<0.05$).

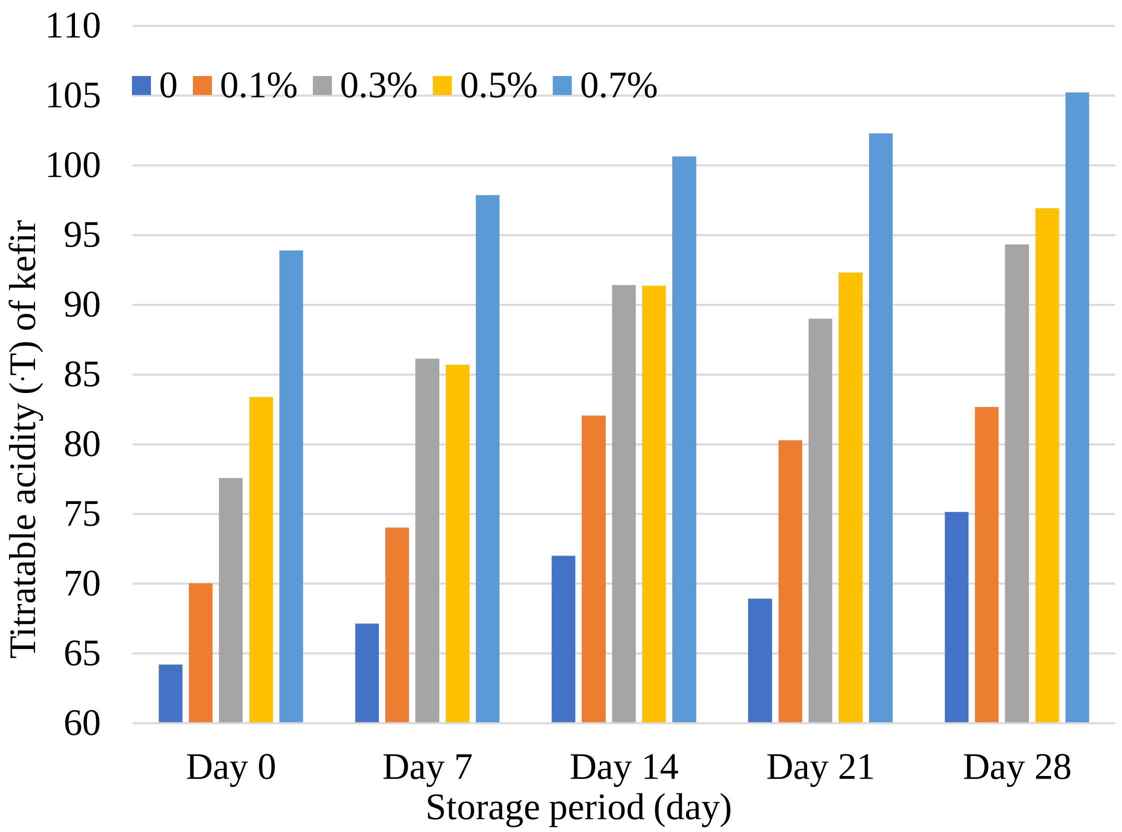


Figure 3.17 – Change of titratable acidity of fermented milk drink, enriched with rice bran

With the prolonging of storage period, the pH value of all samples decreased significantly (P<0.05), this is due to the post -fermentation of fermented milk drinks. The titrated acidity increased significantly; the change of pH value was more significant than that of titration acidity.

The appropriate titrated acidity was between 70 ·T and 95·T, when the storage period was up to 28 days, titrated acidity of fermented milk drinks added with RB exceeded this value, which showed that fermented milk drink added with RB was not suitable for further consumption on 28th day.

3.2.2.2 Change of water-holding capacity of fermented milk drinks enriched with rice bran

Water holding capacity is one of the desirable features as regards to the fermented milk drink quality, which is related to the water keeping ability of proteins within the kefir. Results of WHC were shown in table 3.10 and figure 3.18. WHC ranged from 44.84% to 56.36% during storage.

Table 3.10 – Water holding capacity of samples of fermented milk drinks enriched with rice bran (n=3, ±SD)

Samples	Day 0	Day 7	Day 14	Day 21	Day 28
Water holding capacity, %					
0	50.17±2.34 b, A	44.84±0.69 b, B	49.30±0.30 b, A	48.33±0.44 b, AB	47.47±0.29 c, AB
0.1%	50.67±1.94 b, A	49.82±0.71 a, A	49.49±0.32 b, A	49.33±0.17 ab, A	50.55±0.42 ab, A
0.3%	55.58±0.65 a, A	48.71±0.67 a, B	49.84±0.17 b, B	48.81±0.51 ab, B	49.40±0.01 b, B
0.5%	56.36±0.37 a, A	48.98±0.62 a, B	51.29±0.17 a, B	51.09±1.62 a, B	51.09±0.42 a, B
0.7%	55.91±0.38 a, A	50.04±0.63 a, B	50.89±0.64 a, B	50.86±0.42 a, B	50.64±0.06 a, B

WHC of plain fermented milk drink was lower than fermented milk drink added with RB, regardless of the addition level of RB. As the RB addition ratio increased, WHC of the samples increased, but there were not statistically differences ($p > 0.05$) among fermented milk drinks added with different RB levels. This may be explained that RB contains dietary fibers such as b-glucan, pectin, galactooligosaccharide (GOS), hemicellulose, and arabinogalactan, which could improve the water holding capacity of fermented milk drinks.

Furthermore, (Hu & Yu, 2015) showed that hemicellulose and insoluble dietary fibers from RB had many desirable properties including high water holding capacity and swelling capacity; these specialties provided fermented milk drinks with firmer texture.

(Ozcan & Kurtuldu, 2014) also observed that barley and oat b-glucan dramatically decreased whey separation in yoghurts containing B. bifidum.

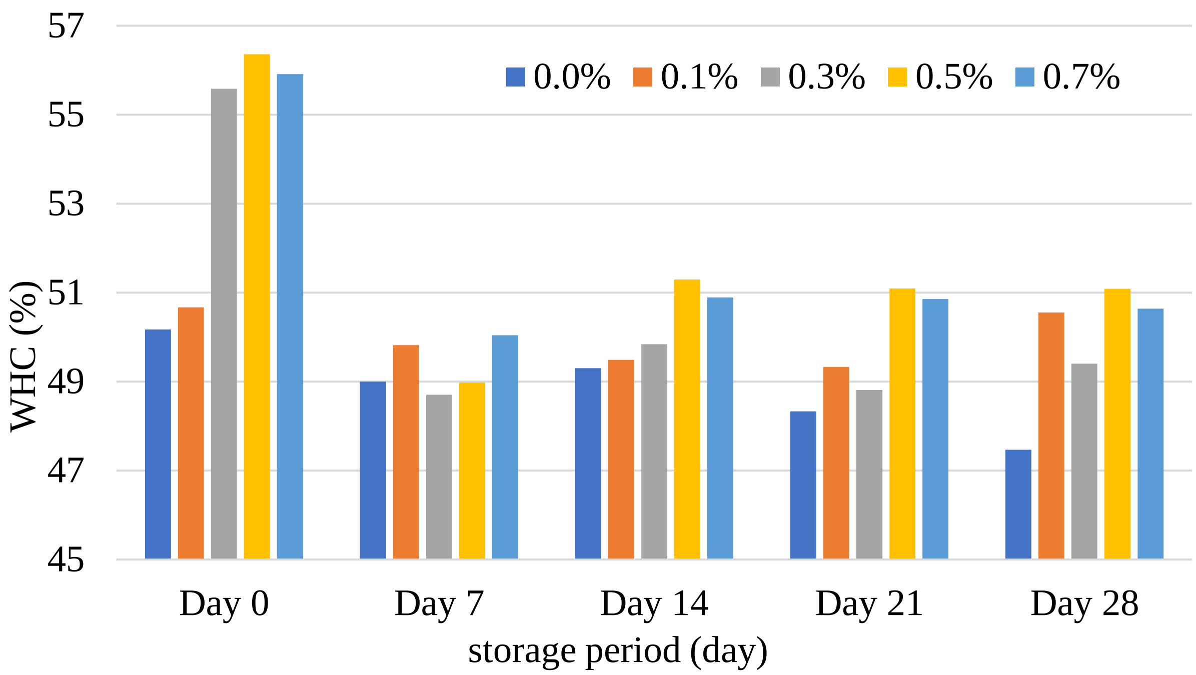


Figure 3.18 – Change of water holding capacity of samples of fermented milk drinks enriched with rice bran for 28 days

Storage period made no significant effect ($P > 0.05$) on WHC of fermented milk drinks except for the first week. However, a small amount of added rice bran had a positive effect on the structure of the product, preventing the separation of moisture. This is explained by the hydrophilic properties of dietary fibers contained in rice bran.

3.2.2.3 Change of apparent viscosity of fermented milk drinks enriched with rice bran

Figure 3.19 and table 3.11 presented the apparent viscosity values of fermented milk drinks in 28 days.

Compared with control sample, RB can significantly improve the apparent viscosity of fermented milk drinks, because the textures of these products were affected by weak physical bonds, electrostatic and hydrophobic interactions, the introduction of RB into fermented milk drinks could improve these interactions (Ertekin & Guzel-Seydim, 2010).

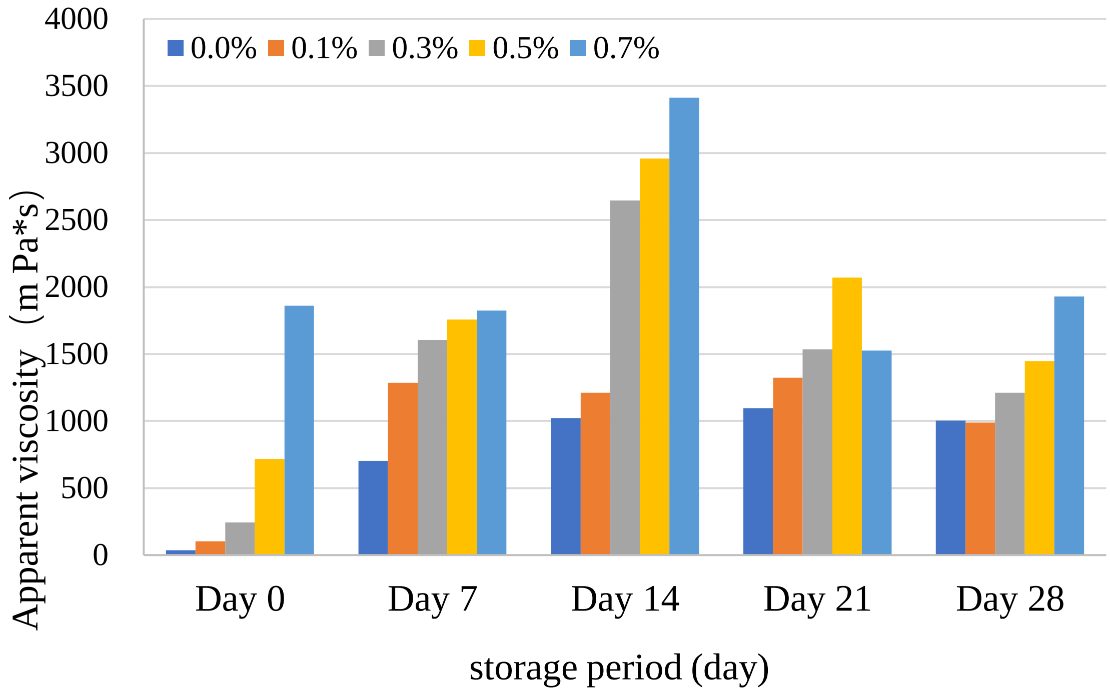


Figure 3.19 – Apparent viscosity of fermented milk drink samples enriched with rice bran for 28 days

(Donkor et al., 2007) reported that the yoghurts added with inulin and peach dietary fiber had significantly higher apparent viscosity than the plain yoghurt. On the other hand, (Tseng & Zhao, 2013) reported that increasing levels of wine grape pomace addition as prebiotic in yoghurts decreased apparent viscosity values. Also, (El-Said et al.,2014) stated that increasing the concentration of pomegranate peel extracts decreased the viscosity values and they associated these results with the effect of pomegranate peel extract on the aggregation of the network in yoghurts via electrostatic interactions.

Table 3.11 – Change of apparent viscosity of fermented milk drink samples enriched with rice bran for 28 days (n=3, ±SD)

Samples	Day 0	Day 7	Day 14	Day 21	Day 28
Apparent viscosity, Pa*s					
1	2	2	4	5	6
K	64.17±3.86$^{e, C}$	702.83±3.01$^{c, B}$	1023.33±17.74$^{c, A}$	1095.83±80.01$^{d, A}$	1003.33±147.95$^{c, BC}$

The end of the table 3.11

1	2	2	4	5	6
A	131.20±1.06 $^{d, C}$	1284.33±73.05 $^{b, A}$	1211.83±32.04 $^{c, A}$	1322.5±49.94 $^{c, A}$	988.75±5.30 $^{c, BC}$
B	243.33±20.23 $^{c, D}$	1605±10.00 $^{a, B}$	2645±45.83 $^{b, A}$	1536.67±46.46 $^{b, B}$	1210.83±45.85 $^{bc, A}$
C	716.00±41.94 $^{b, D}$	1758.17±3.55 $^{a, BC}$	2958.33±315.29 $^{a, A}$	2070.83±5.20 $^{a, B}$	1446.67±52.70 $^{b, C}$
D	1861.25±15.91 $^{a, AB}$	1824.33±239.64 $^{a, B}$	3413.33±65.06 $^{a, A}$	1525.83±9.46 $^{b, B}$	1930±56.57 $^{a, B}$

*a, b, c, d, e Means in the same column with different superscripts significantly differ (P<0.05)

*A, B, C, D, E Means in the same row with different superscripts among kefir samples significantly differ (P<0.05)

*SD: Standard deviation

The storage period could significantly affect the apparent viscosity of samples. For all the samples, the apparent viscosity showed a trend of rising first and falling later, the apparent viscosity value was the highest on the 14th day, which showed that the best storage period of fermented milk should be not more than 14 days. A significant increase of viscosity, compared to the control sample, is explained by the presence of dietary fibers, which are able to increase the content of dry substances in the product without the use of artificial thickeners and structure stabilizers.

3.2.2.4 The color of fermented milk drinks enriched with rice bran

The influence of RB color parameters «L», «a», «b» values of fermented milk drinks was shown in table 3.12. The RB amounts significantly affected «L», «a», «b» values of fermented milk drinks (P<0,05).

Table 3.12 – “L”, “a”, “b” values of fermented milk drink samples enriched with rice bran in 28 days (n=3, ±SD)

Samples	Day	L	a	b
0%	0	97.91±0.18$^{a, A}$	2.57±0.03$^{c, D}$	3.77±0.04$^{b, A}$
	7	97.59±0.20 $^{a, AB}$	3.12±0.02 $^{c, A}$	3.45±0.10$^{b, BC}$
	14	95.31±0.20 $^{a, C}$	2.74±0.10 $^{b, C}$	3.39±0.08$^{ab, C}$

The end of the table 3.12

Samples	Day	L	a	b
	21	97.47±0.21$^{a, AB}$	2.77±0.06$^{c, BC}$	3.61±0.03$^{b, AB}$
	28	97.32±0.18$^{a, B}$	2.92±0.06$^{c, B}$	3.59±0.05$^{bc, B}$
0.1%	0	97.07±0.05$^{b, B}$	2.42±0.03$^{d, D}$	3.28±0.05$^{d, AB}$
	7	97.25±0.05 $^{b, A}$	2.99±0.03$^{d, A}$	3.08±0.05$^{c, B}$
	14	95.42±0.02$^{a, D}$	2.86±0.04$^{b, B}$	3.11±0.05$^{c, B}$
	21	97.20±0.05$^{a, AB}$	2.73±0.03$^{c, C}$	3.40±0.11$^{c, A}$
	28	96.90±0.09$^{a, C}$	2.96±0.08$^{c, AB}$	3.28±0.15$^{cd, AB}$
0.3%	0	96.56±0.17$^{c, A}$	2.59±0.11$^{c, C}$	3.53±0.06$^{c, B}$
	7	96.45±0.02$^{c, AB}$	2.78±0.04$^{e, BC}$	3.09±0.06$^{c, C}$
	14	94.87±0.07$^{b, D}$	2.96±0.2$^{b, B}$	3.27±0.19$^{bc, C}$
	21	96.19±0.08$^{b, C}$	2.78±0.03$^{c, BC}$	3.34±0.06$^{c, BC}$
	28	94.31±0.03$^{b, BC}$	3.82±0.10$^{b, A}$	4.06±0.03$^{b, A}$
0.5%	0	96.44±0.03$^{c, A}$	2.94±0.03$^{b, D}$	3.34±0.13$^{cd, A}$
	7	96.53±0.06$^{c, A}$	3.26±0.05$^{b, C}$	3.28±0.04 $^{bc, AB}$
	14	93.27±0.12$^{d, D}$	3.49±0.05$^{a, B}$	3.90±0.03$^{d, C}$
	21	95.15±0.06$^{c, C}$	3.44±0.08$^{a, B}$	3.08±0.11$^{d, B}$
	28	95.90±0.19$^{b, B}$	3.65±0.06$^{b, A}$	3.03±0.15$^{d, B}$
0.7%	0	95.76±0.03$^{d, A}$	3.38±0.02$^{a, BC}$	4.51±0.08$^{a, B}$
	7	95.50±0.12$^{d, A}$	3.66±0.05$^{a, B}$	4.09±0.15$^{ab, BC}$
	14	93.99±0.06 $^{c, C}$	3.35±0.14$^{a, BC}$	3.62±0.04$^{a, D}$
	21	94.51±0.12$^{d, D}$	3.13±0.14$^{b, C}$	3.88±0.02$^{a, CD}$
	28	93.45±0.33$^{c, B}$	3.50±0.20$^{a, A}$	5.43±0.36$^{a, A}$

As the rise of the amount of RB, the Lightness (L) of samples decreased, “a” value of samples added with 0.5% and 0.7% RB were significantly higher than other samples, showing that 0.5% amount of RB significantly deepened the red color of fermented milk drinks. “b” value of samples added with 0.7% amount of RB significantly increased, showed that samples with 0.7% amount of RB significantly deepen the yellow color of fermented milk drinks, so the adding amount of RB should be no more than 0.3%.The “L”, “a”, “b” values were closer for all samples at different storage periods. As the data showed in Table 3.12, storage period had no significant effect on the brightness of all samples (P> 0.05). The storage period significantly raised the “a” value of all samples, especially on the 7th day, showing that storage period significantly affected the red color of samples, the longer storage period was, the deeper red color of samples was.

Storage period significantly affected “b” value of samples, with the prolonging of storage period, “b” value showed a trend of increase at first and then decrease, showed that the yellow color of samples rose at first and then decreased, the lowest color of yellow of samples were on the 7th day.

3.2.2.5 Antioxidant properties of fermented milk drinks enriched with rice bran

The antioxidant properties of the samples were determined by nuclear magnetic resonance experiment in the storage process. Nuclear magnetic resonance of different samples (K, A, B, C, D) was shown in 28 days in figures 3.20 - 3.24.

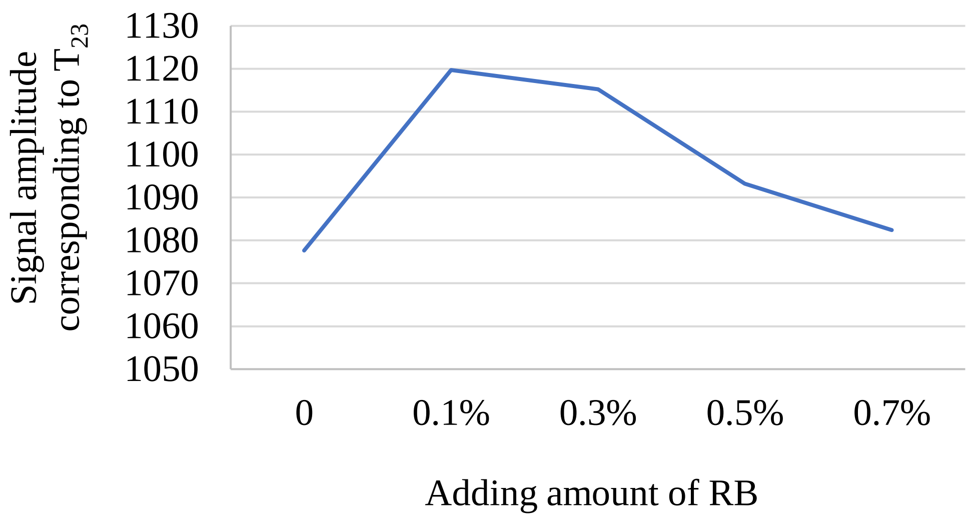


Fig. 3.20 – Signal amplitude corresponding to T_{23} on 0 day

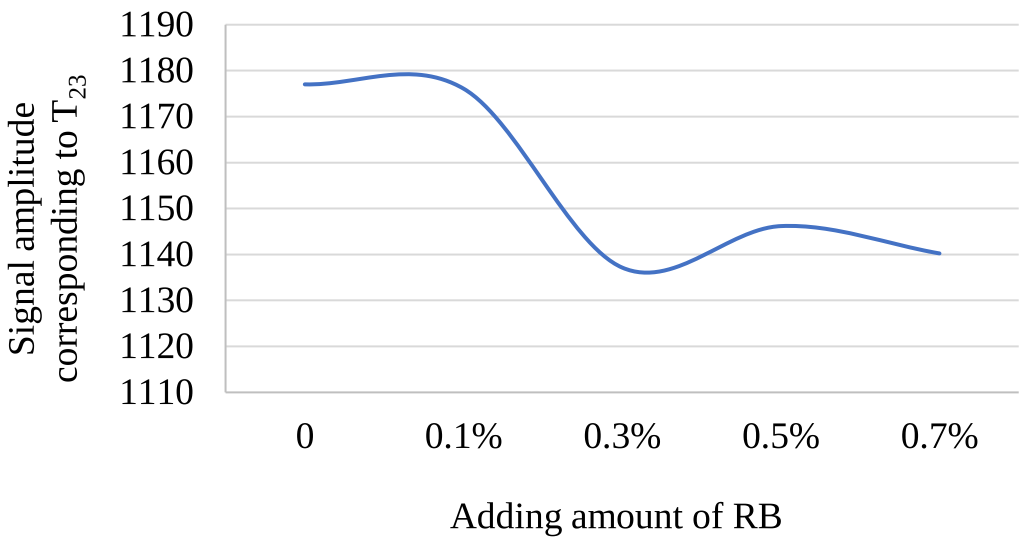


Fig. 3.21 – Signal amplitude corresponding to T_{23} on 7th day

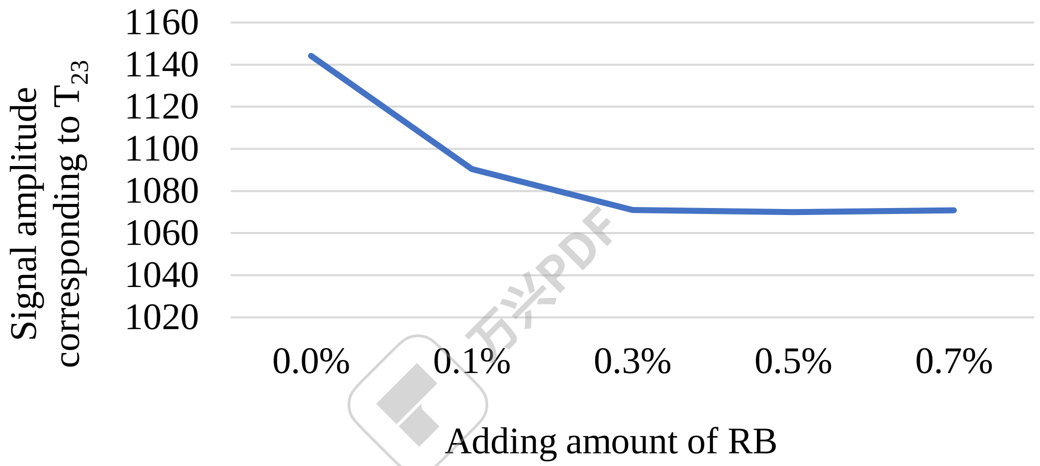


Fig. 3.22 – Signal amplitude corresponding to T_{23} on 14th day

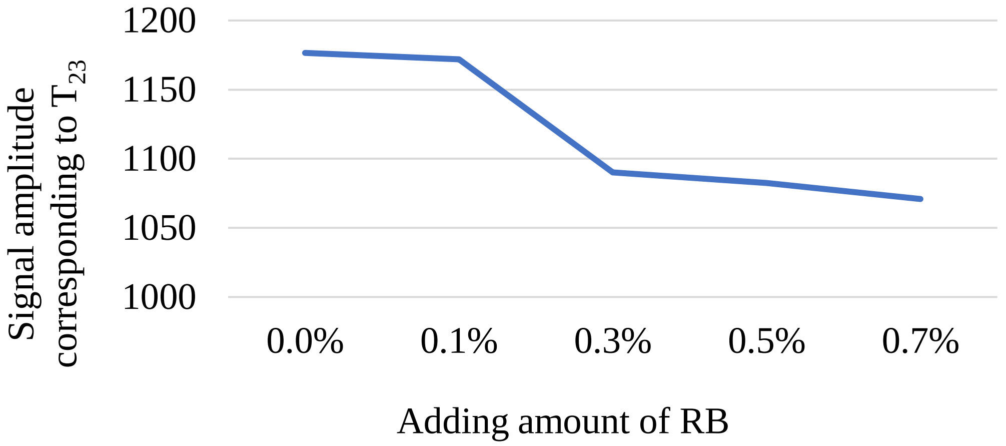


Fig. 3.23 – Signal amplitude corresponding to T_{23} on 21th day

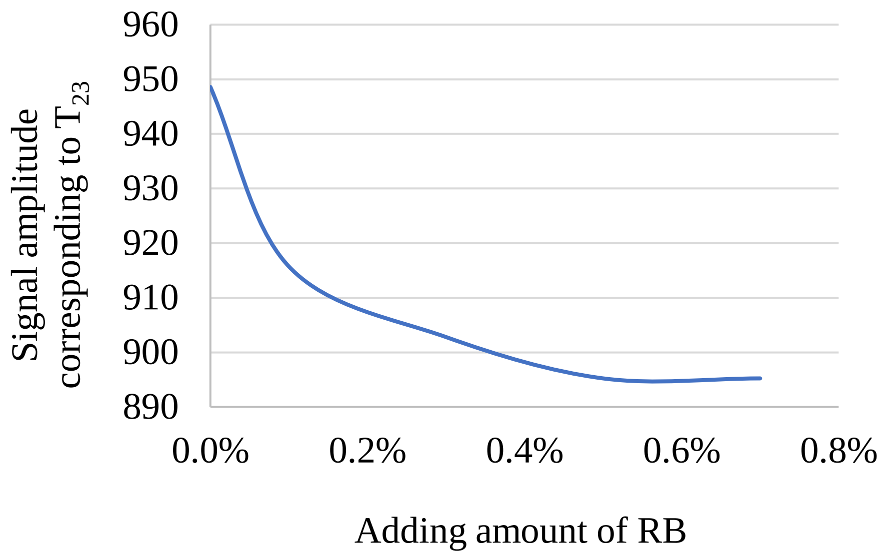


Fig. 3.24 – Signal amplitude corresponding to T_{23} on 28th day

The results of the study of the stability of fermented milk drink enriched with rice bran after 28 days of storage are presented in Fig. 3.25.

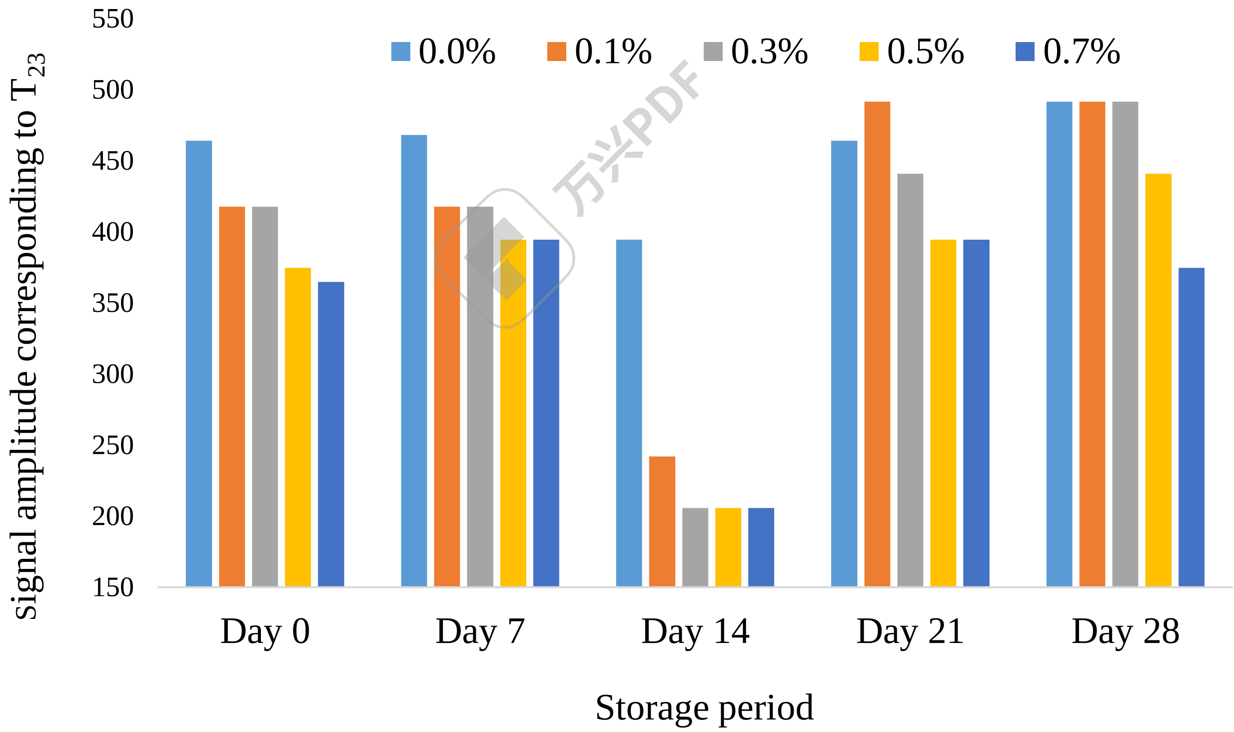


Fig. 3.25 – Signal amplitude corresponding to T_{23} of fermented milk drink samples enriched with rice bran in 28 days

For the control samples, the storage period had no significant difference in the amplitude of the signal corresponding to T23, with the exception of 28th day. For a sample added with RB, the storage period can significantly increase the amplitude of the signal corresponding to T23, indicating a decrease in stability. For the samples with added sesame flour, the storage period could markedly reduce the amplitude of the signal corresponding to T23 on day 7, indicating an improvement in stability. The amplitude of the signal corresponding to T23, which means that the stability remained constant.

Thus, it confirms that the use of the production by-products of additives containing dietary fibers has a positive effect on the rheological properties of the product during storage. At the same time, it is not necessary to use synthetic stabilizers of the structure.

3.2.3 The effect of rice bran on microbiological parameters of fermented milk drinks

The changes of the population of probiotics of fermented milk drink samples were shown in figures from 3.26 to 3.28.

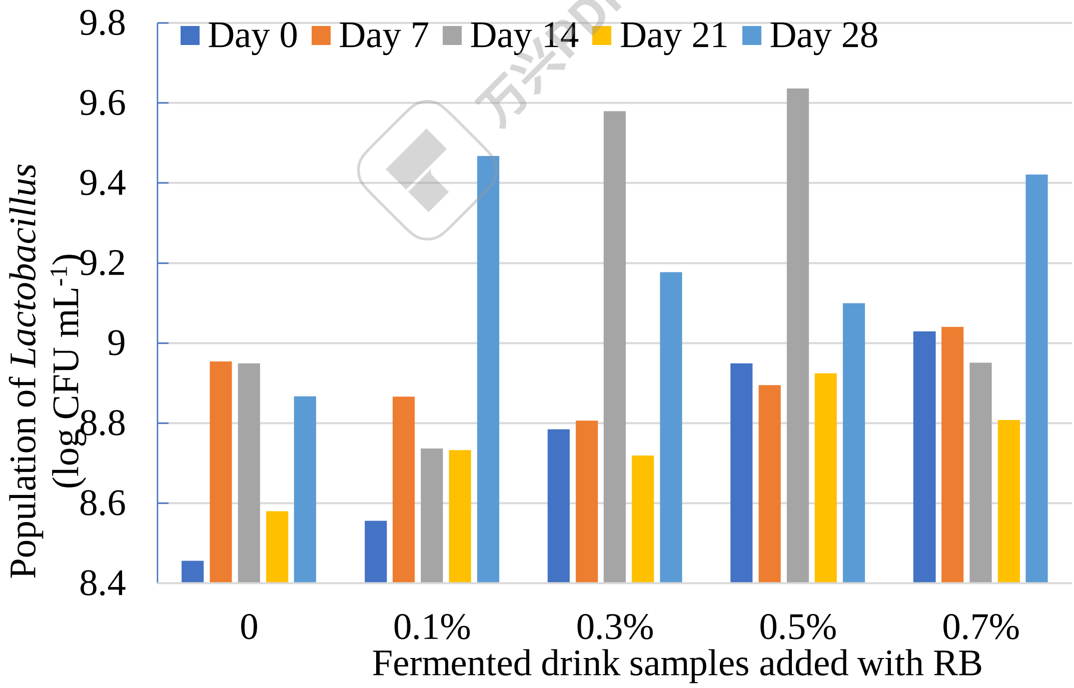


Fig. 3.26 - Population of *Lactobacillus* of fermented milk drink samples enriched with rice bran in 28 days

According to the Codex Standard, fermented milk drink must contain minimum 7 log CFU/g sum of fermented milk drink microorganisms and 4 log CFU/g yeasts.

During storage, yeast (4,3–6,7 CFU/ml, figure 3.27), LAB (8.46–9.64 CFU/g) for Lactobacillus spp. (figure 3.27), and 7.26–10.45 CFU/ml for Lactococcus spp. (figure 3.28) counts of all samples were in accordance with Codex Standard (Commission, 2003).

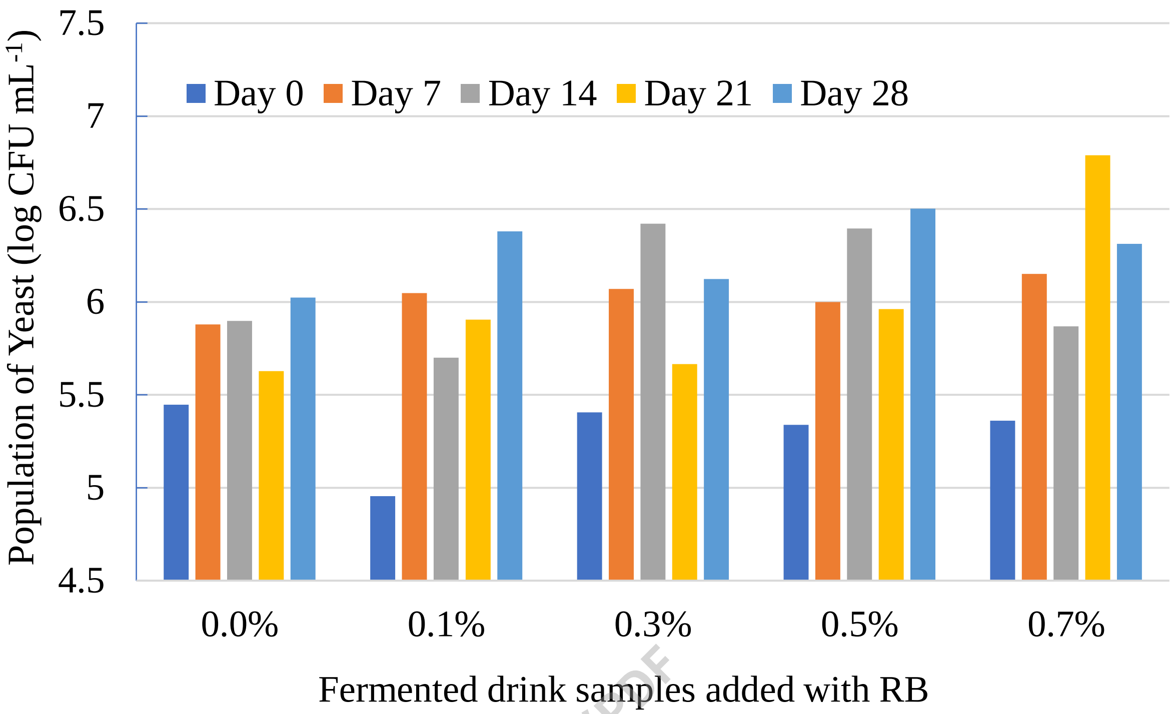


Figure 3.27 – Population of Yeasts of fermented drink samples in 28 days

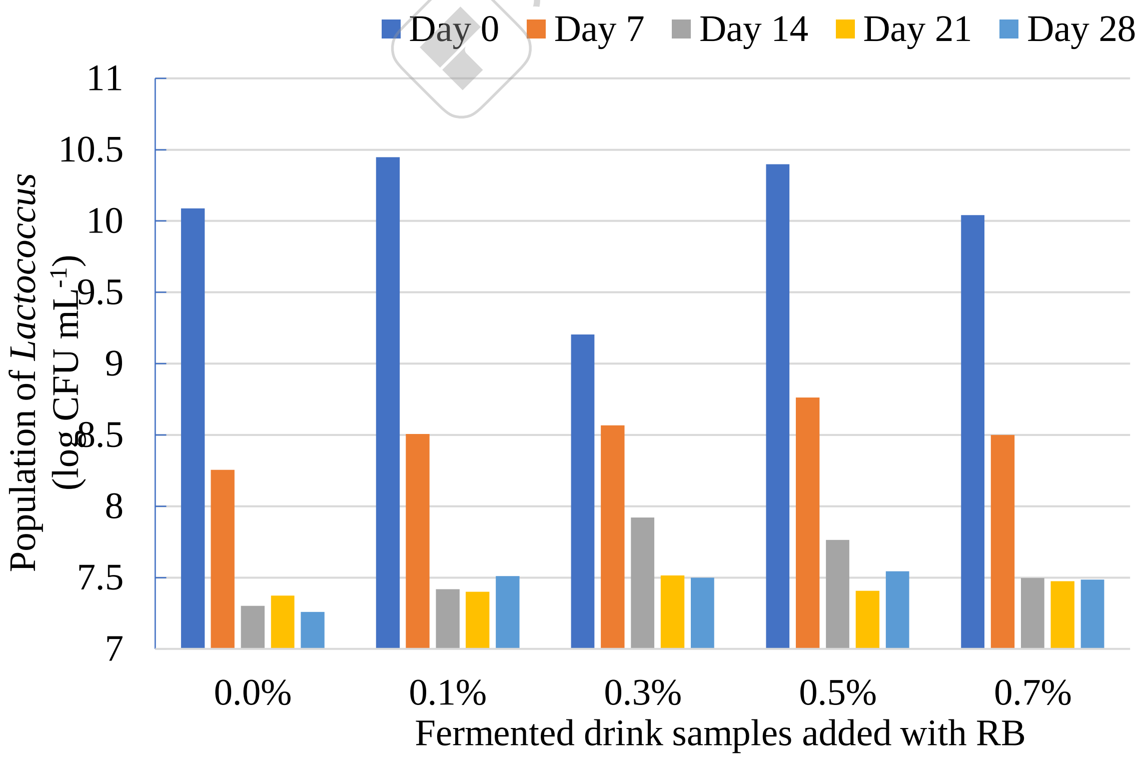


Figure 3.28 – Population of *Lactococcus* of fermented drink samples in 28 days

Changes of population of *Lactobacillus* for 28 days were shown in figure 3.26. Lactobacillus counts were present at 8.5–9.0 log cfu/mL in freshly fermented kefir samples. As the increase of the adding amount of RB, population of Lactobacillus increased too, which indicated the stimulation of RB on lactobacillus. The highest level for *Lactobacillus* was determined in sample D. The lowest value of *Lactobacillus* count was obtained for control sample.

During storage period, the population of *Lactobacillus* increased at first, and then decreased, samples showed the biggest population of *Lactobacillus* on the 14th day, which was due to the post-fermentation during storage. The finding is in agreement with (T. D. A et al., 2017), who reported a very similar decline trend for L. caseiLpc-37 beyond 14 days in yoghurt with RB. The decrease of population of *Lactobacillus* may be related to decrease of suitable nutrients such as sugars, which was used by probiotic culture for improving growth activities.

In contrast to the report of (Lankaputhra et al., 1996), who claimed that yoghurt pH below 4.30 is generally considered adverse effect for growth and survival of probiotics, *Lactobacillus* counts in our study were still high at 9.46 and 9.18 log CFU/g in fermented milk drink enriched with 0.1 and 0.3% RB, respectively. The sensitivity of probiotic bacteria to low pH may be a specific notion of species. These results indicate that RB may have a selective effect on increasing probiotic Lactobacillus counts.

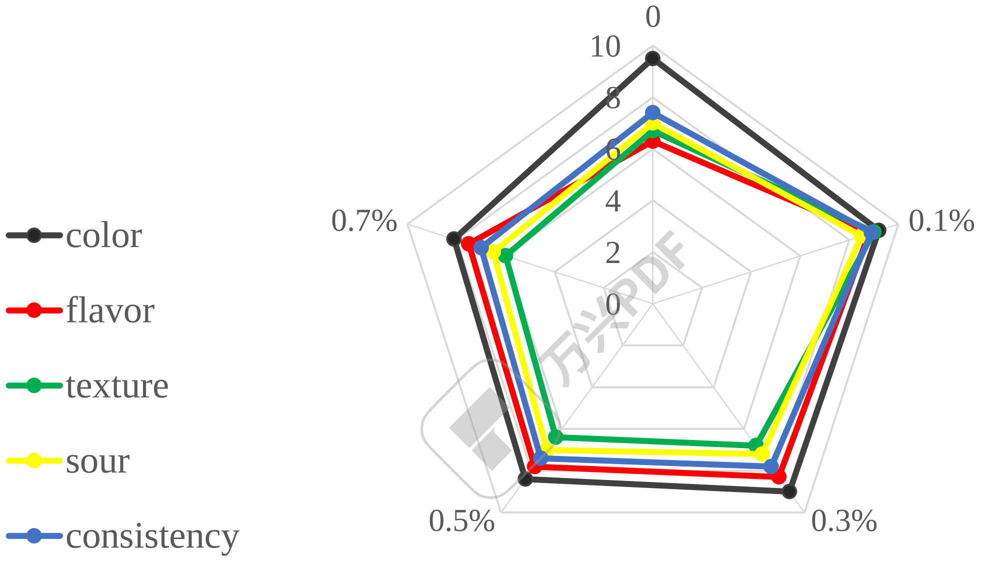
In order to explain the observed a positive influence on probiotic counts, it should be taken into account that RB generally contains 20% oils, 15% protein and 50% carbohydrate, also, it has an number of micronutrients like tocopherols, tocotrienols, oryzanols, dietary fibers such as beta-glucan, pectin, galactooligosaccharide (GOS), hemicellulose, arabinogalactan, xyloglucan and raffinose (Jiang & Wang, 2005).

These important nutritional components of RB, especially raffinose and GOS, have potential for advancing of *Lactobacillus* viability (Zubaidah et al., 2014). These observations are consistent with the findings of (Kurdi & Hansawasdi, 2015), who explored prebiotic potential of oligosaccharide mixtures from RB on some Lactobacilli species.

Also in a previous study, (Ryan & Elizabeth, 2011) pointed out that many RB components were fermented by probiotic yeasts and these components supported the growth and survival of probiotic. Likewise, our results are also in agreement with (Saman et al., 2016), who reported that RB was the suitable substrate for the growth and survival of probiotic *L. plantarum NCIMB 8826.*

3.2.4 The effect of rice bran on organoleptic characteristics of fermented milk drinks

Addition of RB had a significant effect on the sensory characteristics of



fermented milk drinks ($p<0.05$) (Figure 3.29).

Fig.3.29 – Sensory properties of fermented milk drink samples

RB can increase the viscosity of fermented milk drinks, because of the good water holding capacity of dietary fiber and protein in RB. RB gives fermented milk drink its unique rice flavor, too. Meanwhile, the introduction of RB would separate whey, resulting in uneven texture and low product acceptability.

Although (Demirci et al., 2017) reported that all RB fortified fermented milk was assessed with lower values compared to plain samples, increasing proportion of RB led to a lower acceptability of samples.

(Goncu, 2017) reported that dietary fiber addition positively influenced taste and aroma, consistency and general acceptability scores up to at a rate of 0.5%, because of better mouth thickness and pleasant aroma of them. Higher rice bran fiber concentration (1 %) caused lower sensory scores.

The storage period made a significant effect on the sensory quality of fermented milk drink ($P< 0.05$). Generally speaking, the acceptability of fermented milk drink was the highest when 0.1% RB was added, and 0.5% RB added fermented milk drinks had the lowest sensory scores.

The introduction of RB leads to whey separation, rough texture, although it can improve the viscosity, and rich the flavor of fermented milk drink. The problem of whey separation should be solved if RB was introduced into fermented milk drink.

3.3. The study of dietary fibers content in fermented milk drinks enriched with defatted sesame flour and rice bran

The content of the dietary fibers in fermented milk drinks samples enriched with defatted sesame flour and rice bran was given in the table 3.13.

Table 3.13 - The content of dietary fiber in different samples

Samples	Control samples	Samples added with 0.1% RB	Samples added with 2% DSF
Content of fiber, %	0.01±0.00	0.03±0.002	0.68±0.11

The introduction of rice bran and defatted sesame flour improved the content of the dietary fibers in fermented milk drinks.

The results have an agreement with do Espírito Santo, Perego, Converti, and Oliveira (2012) whom produced yoghurt with passion fruit fiber, it could be related to stimulation of lactic acid bacteria by fiber. Previous studies have also demonstrated that kefir as a probiotic (Guzel-Seydim, Z. B., et al., 2011; Mariana, Carmen, et al., 2011) and dietary fiber had a potential prebiotic effect (Shah, B. R., Li, B., Sabbah, H. A., et al., 2020; Wu, W., Hu, J., et al., 2020).

Conclusions to Section 3

Having analyzed the experimental study results as regards to the fermented milk drinks added with defatted sesame flour, it has been established that adding of the additive increases the functional properties and energy value of fermented milk drinks due to increase of the content of fats, proteins, dietary fibers and vitamins.

The optimal amount of defatted sesame flour additive, which has a positive effect on the rheological and physical and chemical properties is from 2 % to 4%.

The introduction of rice bran can increase pH value and decrease significantly acidity of fermented milk drink that can be partially explained by the availability of protein and fiber in rice bran.

The introduction of rice bran increases significantly water holding capacity of fermented milk drink, apparent viscosity.

Signal amplitude of samples decreases that means that rice bran can reduce the content of free water and increase its density.

The adding of addittives containing dietary fibers has a positive effect on population of *Lactobacillus*, Yeasts and *Lactococcus* ($P<0,05$).

Adding of rice bran influences sensory properties of fermented milk drinks.

The introduction of 0,1% rice bran into the product increases the content of the dietary fibers in the product up to 0,03%, the introduction of 2% defatted sesame flour into the product increases the content of the dietary fibers up to 0,68%.

The recommended expiration period of fermented milk drinks enriched with defatted sesame flour and rice bran is 14 days.

SECTION 4

THE MODELLING OF THE PRODUCTION PROCESS OF FERMENTED MILK DRINKS ENRICHED WITH PLANT PROCESSING WASTES

4.1. The optimization parameters of technical - technological indicators of fermented milk drinks with plant raw material

The optimization parameter is the optimal ratio of cow milk and plant additives such as defatted sesame flour and rice bran in the recipe. The cow milk was used in the range from 97,9 to 99,98 g in the recipe, defatted sesame flour was used in the range from 2 to 8 g, rice bran - from 0,1 to 0,7 g, also starters were used in the recipe (table 4.1), that were not subjected to the mathematical analysis.The central compositional plan was used for studying interaction of process variables. There are 3 variables : content of cow milk in the recipe (x_1), content of defatted sesame flour in the recipe (x_2) and content of rice bran in the recipe (x_3). Each variable has 3 coded levels: from low (-1), up to medium (0) and high (+1), as well as star points (-α та +α). For two-factors analysis α = 1, for three-factors analysis α = 1,215. Two central compositional plans of two-factors analysis (x_1x_2 and x_1x_3, respectively) and one three-factors analysis plan ($x_1x_2x_3$) were formed to predict optimal recipe and to reduce the quantity of future experimental studies.

Mathematical modelling allows to determine approximating polinomial factors. The polinomial of two-factors analysis for the recipe with defatted sesame flour adding, taking into account transformation and after opening of the brackets can be converted to the usual view :

$$Y_{sesame}=b_0+b_1x_1+b_2x_2+b_{12}x_1x_2+b_{11}x_1^2+b_{22}x_2^2, \qquad (4.1)$$

The polinomial of two-factors analysis for the recipe with rice bran adding looks in the following view:

$$Y_{rice}=b_0+b_1x_1+b_3x_3+b_{13}x_1x_3+b_{11}x_1^2+b_{33}x_3^2, \qquad (4.2)$$

The polinomial of of three-factors analysis for the recipe with defatted sesame flour and rice bran adding taking into account transformation and after opening of the brackets can be converted to the usual view:

$$Y_{sesame+rice}=b_0+b_1x_1+b_2x_2+b_3x_3+b_{12}x_1x_2+b_{13}x_1x_3+b_{23}x_2x_3+b_{123}x_1x_2x_3+b_{11}x_1^2+b_{22}x_2^2+b_{33}x_3^2 \quad (4.3)$$

The central compositional plan fixes each factor at 5 levels, in the view of maximum and minimum quantity of ingredients when the main properties of food is remained not changed that is given in table 4.1-4.3 for three central compositional plans according to the mathematical modelling targets.

Table 4.1 - Recipe components and levels of factor fixation for two-factors analysis for the recipe with defatted sesame flour adding

Factors influencing optimization		Levels of factor fixation and their natural values, g				
		α(-1)	-1	0	1	α(1)
x_1	Cow milk	98	98	98,5	99	99
x_2	Defatted sesame flour	2	2	5	8	8

Table 4.2 - Recipe components and levels of factor fixation for two -factors analysis for the recipe with rice bran

Factors influencing optimization		Levels of factor fixation and their natural values, g				
		α(-1)	-1	0	1	α(1)
x_1	Cow milk	99,9	99,9	99,94	99,98	99,98
x_3	Rice bran	0,1	0,1	0,4	0,7	0,7

Table 4.3 - Recipe components of levels of factor fixation for three-factors analysis for the recipe with defatted sesame flour and rice bran

Factors influencing optimization		Levels of factor fixation and their natural values, g				
		α(-1,215)	-1	0	1	α(1,215)
x_1	Cow milk	97,9	97,918	98,0	98,082	98,1
x_2	Defatted sesame flour	2,0	2,531	5,0	7,469	8,0
x_3	Rice bran	0,1	0,153	0,4	0,647	0,7

4.2. Experiment plan and results of measurements of fermented milk drinks with plant raw materials

9 experiments are performed for two-factors recipe analysis and 15 - for three-factors analysis. According to the central compositional plan constant value d exists that depends on the quantity of factors. For two-factors analysis d=0,667, for three-factors analysis d=0,73. Experiment results of the most significant reviews as regards to the fermented milk drinks with plant raw materials are presented by physical and chemical and organoleptic analysis.

Fiber values (y_1), pH (y_2) and organoleptic indicators of fermented milk drinks with plant raw materials were determined. The fiber values are given in table 4.1 (defatted sesame flour) and 4.2 (rice bran), pH in table 3.3 (defatted sesame flour) and 3.10 (rice bran) and organoleptic indicators are given in fig. 3.23 (defatted sesame flour) and 3.27 (rice bran).

The experiment plan and results of measurements for the two-factors analysis recipe with defatted sesame flour are given in table 4.4 and 4.5, accordingly.

Table 4.4 - Experiment planning matrix for two-factors recipe analysis with defatted sesame flour

No.	x_0	x_1	x_2	$x_1^2 - 0,667$	$x_2^2 - 0,667$	x_1x_2
1	1	1	1	0,33	0,33	1
2	1	-1	1	0,33	0,33	-1
3	1	1	-1	0,33	0,33	-1
4	1	-1	-1	0,33	0,33	1
5	1	-1	0	0,33	-0,67	0
6	1	1	0	0,33	-0,67	0
7	1	0	-1	-0,67	0,33	0
8	1	0	1	-0,67	0,33	0
9	1	0	0	-0,67	-0,67	0

Table 4.5 - The results of measurements for two-factors recipe analysis with defatted sesame flour

No.	y_1	y_2	y_3	$\bar{y}_j$	s_j^2	$\hat{y}$	$\bar{y}$	Sad^2
1	4,52	4,14	7,2	5,3	2,73	5,28	5,03	0,064
2	5,20	4,2	7,1	5,5	2,17	5,50	5,40	0,011
3	1,92	4,05	8,2	4,7	10,20	4,72	4,70	0,000
4	0,68	4,38	8,5	4,5	15,20	4,51	4,64	0,016
5	3,05	4,2	7,8	5,0	6,14	5,02	4,99	0,001
6	2,25	4,15	7,3	4,6	6,51	4,57	4,83	0,071
7	1,14	4,25	8,1	4,5	12,25	4,51	4,39	0,013
8	2,12	4,11	7,5	4,6	7,47	4,59	4,93	0,121
9	2,38	4,18	8,0	4,9	8,24	4,85	4,63	0,049

Based on the results of the research, the coefficients of the regression equation (bi) were obtained and a statistical analysis of the model and its coefficients, which are listed in the table 4.6, was carried out.

Table 4.6 - The results of the experiment statistical analysis for two -factors recipe analysis with defatted sesame flour

	x_0	x_1	x_2	$x_1^2 - 0{,}667$	$x_2^2 - 0{,}667$	$x_1 x_2$
∑xi*ycp	43,5	-0,5	1,6	0,6	0,1	-0,4
∑xi^2	9	6,0	6,0	2,0	2,0	4
b_i	4,84	-0,08	0,27	0,28	0,03	-0,11
S_y^2	7,88	*G*	0,21	Sad^2		0,0576
α	0,05	*m-1*	2,00	F		0,007316
f_1	8,00	*N*	9,00	k1		2
t_T	2,31	G_{cr}	0,47	k2		6,00
		$G – G_{cr=}$	-0,26	F_{cr} (table)		5,14

The end of the table 4.6

$G < G_{cr}$ Homogeneous dispersion	F-Fcr=	-5,132683638
	F<Fcr The statistical model is significant, the regression equation is reliable	

After mathematical modelling, calculations and determination of the regression equation as reliable, the coefficients in formula 4.1 are replaced by those determined in research, which makes it possible to determine the interdependence of recipe components and their influence on optimization indicators. As a result, the obtained regression model in coded units has the following form:

$$Y_{sesame}=4{,}84-0{,}08x_1+0{,}27x_2-0{,}11b_{12}x_1x_2+0{,}28x_1^2+0{,}03x_2^2, \quad (4.4)$$

In order to determine the optimal recipe composition according to the given parameters, 3D model was built by means of the software package for statistical analysis Statistica (Fig. 4.1).

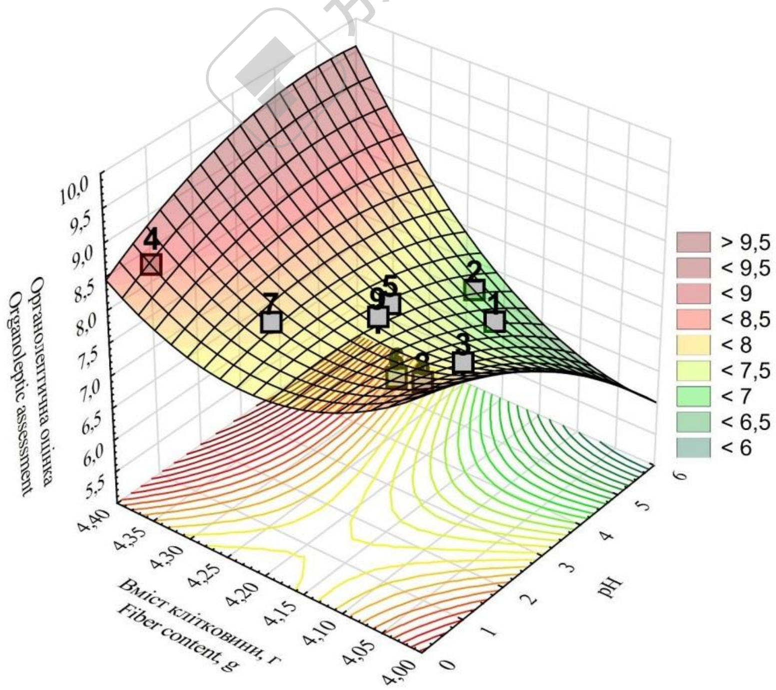


Fig. 4.1 - 3D optimization model for two-factors recipe analysis with defatted sesame flour

After study of all mathematical modelling results, the 4th sample was determined as the optimal recipe composition, that corresponds to the content of defatted sesame flour in the amount of 2% in the recipe.

The experiment plan and results of measurements for two-factors recipe analysis with rice bran are given in table 4.7 and table 4.8, accordingly.

Table 4.7 - Experiment planning matrix for two-factors recipe analysis with rice bran

No.	x_0	x_1	x_3	$x_1^2 - 0{,}667$	$x_3^2 - 0{,}667$	x_1x_3
1	1	1	1	0,33	0,33	1
2	1	-1	-1	0,33	0,33	1
3	1	1	-1	0,33	0,33	-1
4	1	-1	1	0,33	0,33	-1
5	1	-1	0	0,33	-0,67	0
6	1	1	0	0,33	-0,67	0
7	1	0	-1	-0,67	0,33	0
8	1	0	1	-0,67	0,33	0
9	1	0	0	-0,67	-0,67	0

Table 4.8. The results of measurements for two-factors recipe analysis with rice bran

No.	y_1	y_2	y_3	$\bar{y}_j$	s_j^2	$\hat{y}$	$\bar{y}$	Sad^2
1	0,100	4,57	7,0	3,9	12,25	3,89	3,90	0,000
2	0,029	4,92	8,9	4,6	19,74	4,62	4,54	0,005
3	0,110	4,72	8,3	4,4	16,86	4,38	4,35	0,001
4	0,025	4,95	8,0	4,3	16,19	4,33	4,29	0,001
5	0,095	4,65	8,1	4,3	16,12	4,28	4,38	0,010

The end of the table 4.8

No.	y_1	y_2	y_3	$\bar{y}_j$	s_j^2	$\hat{y}$	$\bar{y}$	Sad^2
6	0,127	4,71	7,4	4,1	13,52	4,08	4,09	0,000
7	0,086	4,74	7,8	4,2	15,09	4,21	4,30	0,009
8	0,210	4,30	7,3	3,9	12,67	3,94	3,95	0,000
9	0,075	4,70	7,8	4,2	15,11	4,19	4,09	0,010

Based on the results of the research, the coefficients of the regression equation (bi) were obtained and a statistical analysis of the model and its coefficients, which are listed in the table 4.9, was carried out.

Table 4.9 - The results of the experiment statistical analysis for two-factors recipe analysis with rice bran

	x_0	x_1	x_3	$x_1^2 - 0{,}667$	$x_3^2 - 0{,}667$	$x_1\ x_3$
∑xi*ycp	37,9	-0,9	-1,1	0,3	0,1	-0,2
∑xi^2	9	6,0	6,0	2,0	2,0	4
b_i	4,21	-0,15	-0,18	0,14	0,04	-0,05

S_y^2	15,28	G	0,14	Sad^2	0,0061
α	0,05	m-1	2,00	F	0,000398
f_1	8,00	N	9,00	k1	2
t_T	2,31	G_{cr}	0,47	k2	6,00
		$G - G_{cr}$=	-0,33	Fcr (table)	5,14
$G < G_{cr}$ Homogeneous dispersion				F-Fcr=	-5,139601864
				F<Fcr The statistical model is significant, the regression equation is reliable	

After mathematical modelling, calculations and determination of the regression equation as reliable, the coefficients in formula 4.5 are replaced by those determined in research, which makes it possible to determine the interdependence of recipe components and their influence on optimization indicators. As a result, the obtained regression model in coded units has the following form:

$$Y_{rice}=4,21-0,15x_1-0,18x_3-0,05x_1x_3+0,14x_1^2+0,04x_3^2, \quad (4.5)$$

In order to determine the optimal recipe composition according to the given parameters, 3D model was built by means of the software package for statistical analysis Statistica (Fig. 4.2).

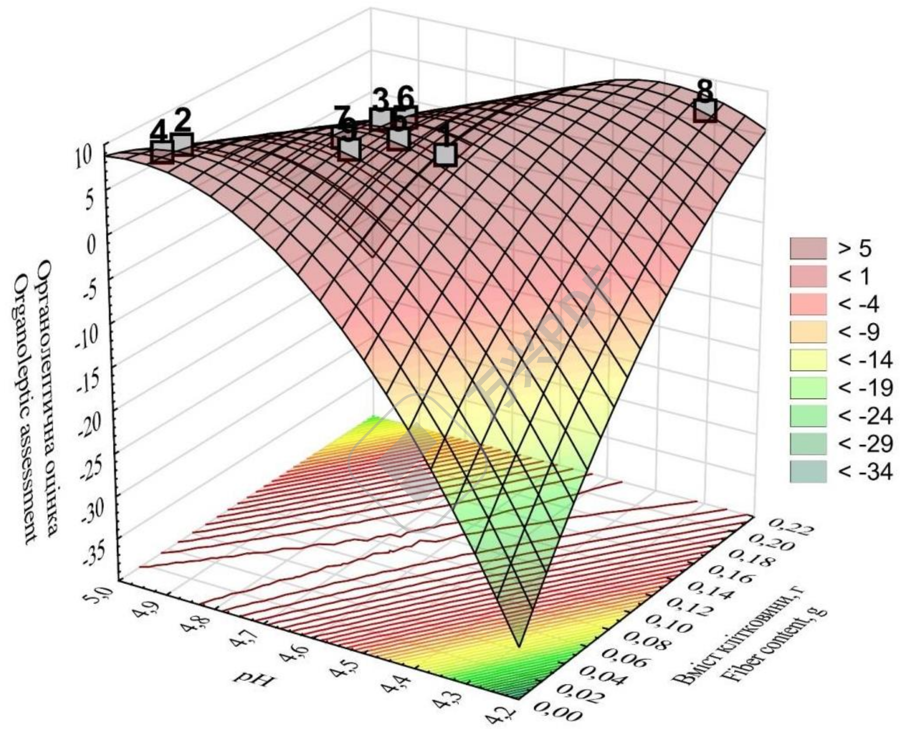


Fig. 4.2 - 3D optimization model for two -factors recipe analysis with rice bran

After study of all mathematical modelling results, the 2nd sample was determined as the optimal recipe composition, that corresponds to the content of rice bran in the amount of 0,1% in the recipe.

The experiment plan and results of measurements for three-factors recipe analysis with defatted sesame flour and rice bran are given in table 4.10 and table 4.11, accordingly.

Table 4.10 - Experiment planning matrix for three-factors recipe analysis with defatted sesame flour and rice bran

No.	x_0	x_1	x_2	x_3	$x_1^2 - 0{,}73$	$x_2^2 - 0{,}73$	$x_3^2 - 0{,}73$	$x_1 x_2$	$x_1 x_3$	$x_2 x_3$	$x_1 x_2 x_3$
1	1	1	1	1	0,27	0,27	0,27	1	1	1	1
2	1	-1	1	1	0,27	0,27	0,27	-1	-1	1	-1
3	1	1	-1	1	0,27	0,27	0,27	-1	1	-1	-1
4	1	-1	-1	1	0,27	0,27	0,27	1	-1	-1	1
5	1	1	1	-1	0,27	0,27	0,27	1	-1	-1	-1
6	1	-1	1	-1	0,27	0,27	0,27	-1	1	-1	1
7	1	1	-1	-1	0,27	0,27	0,27	-1	-1	1	1
8	1	-1	-1	-1	0,27	0,27	0,27	1	1	1	-1
9	1	-1,215	0	0	-0,73	-0,73	-0,73	0	0	0	0
10	1	1,215	0	0	-0,73	-0,73	-0,73	0	0	0	0
11	1	0	1,215	0	-0,73	0,75	0,75	0	0	0	0
12	1	0	1,215	0	-0,73	0,75	0,75	0	0	0	0
13	1	0	0	-1,215	0,75	-0,73	-0,73	0	0	0	0
14	1	0	0	1,215	0,75	-0,73	-0,73	0	0	0	0
15	1	0	0	0	-0,73	-0,73	-0,73	0	0	0	0

Table 4.11 - The results of measurements for three-factors recipe analysis with defatted sesame flour and rice bran

No.	y_1	y_2	y_3	$\bar{y}_j$	s_j^2	$\hat{y}$	$\bar{y}$	Sad^2
1	4,62	4,36	7,40	5,5	2,85	5,46	5,38	0,006
2	5,23	4,56	7,20	5,7	1,88	5,66	5,43	0,056
3	2,03	4,39	8,40	4,9	10,37	4,94	5,02	0,007
4	0,71	4,67	8,60	4,7	15,58	4,66	4,58	0,006
5	2,33	4,21	7,40	4,6	6,57	4,65	4,71	0,004
6	4,81	4,45	7,80	5,7	3,38	5,69	5,59	0,009
7	0,61	4,54	8,40	4,5	15,17	4,52	4,74	0,051

The end of the Table 4.11

No.	y_1	y_2	y_3	$\bar{y}_j$	s_j^2	$\hat{y}$	$\bar{y}$	Sad^2
8	0,65	4,6	8,10	4,5	13,89	4,45	4,52	0,005
9	0,65	4,43	7,80	4,3	12,79	4,29	4,57	0,076
10	2,38	4,43	7,40	4,7	6,38	4,74	4,49	0,061
11	0,71	4,65	9,00	4,8	17,20	4,79	5,28	0,241
12	1,23	4,50	8,20	4,6	12,18	4,64	4,60	0,002
13	2,20	4,50	8,00	4,9	8,53	4,90	4,68	0,047
14	2,45	4,21	7,40	4,7	6,30	4,69	4,94	0,065
15	2,46	4,44	8,10	5,0	8,20	5,00	4,53	0,221

Based on the results of the research, the coefficients of the regression equation (bi) were obtained and a statistical analysis of the model and its coefficients, which are listed in the table 4.12, was carried out.

Table 4.12 - The results of the experiment statistical analysis for three-factors recipe analysis with defatted sesame flour and rice bran

	x_0	x_1	x_2	x_3	$x_1^2-0,73$	$x_2^2-0,73$	$x_3^2-0,73$	$x_1 x_2$	x_1x_3	$x_2 x_3$	$x_1x_2x_3$
Σxi *ycp	73,1	-0,4	3,1	1,2	0,8	0,6	0,6	-1,6	1,1	0,2	0,6
Σxi ^2	15	11,0	11,0	11,0	4,4	4,4	4,4	8	8	8	8
b_i	4,87	-0,03	0,28	0,11	0,19	0,14	0,14	-0,20	0,13	0,02	0,08

S_y^2	9,42	G	0,12	Sad^2	0,0779
α	0,05	$m-1$	2,00	F	0,008276
f_1	14,00	N	15,00	k1	3
t_T	2,14	G_{cr}	0,33	k2	11,00
		$G-G_{cr}=$	-0,21	Fcr (table)	3,59
				F-Fcr=	-3,581724219
$G< G_{cr}$ Homogeneous dispersion				F<Fcr The statistical model is significant, the regression equation is reliable	

After mathematical modelling, calculations and determination of the regression equation as reliable, the coefficients in formula 4.6 are replaced by those determined in research, which makes it possible to determine the interdependence of recipe components and their influence on optimization indicators. As a result, the obtained regression model in coded units has the following form:

$$Y_{sesame+rice}=4,87-0,03x_1+0,28x_2+0,11x_3-0,20x_1x_2+0,13x_1x_3+0,02x_2x_3+0,08x_1x_2x_3+0,19x_1^2+0,14x_2^2+0,14x_3^2, \quad (4.6)$$

In order to determine the optimal recipe composition according to the given parameters, 3D model was built by means of the software package for statistical analysis Statistica (Fig. 4.3).

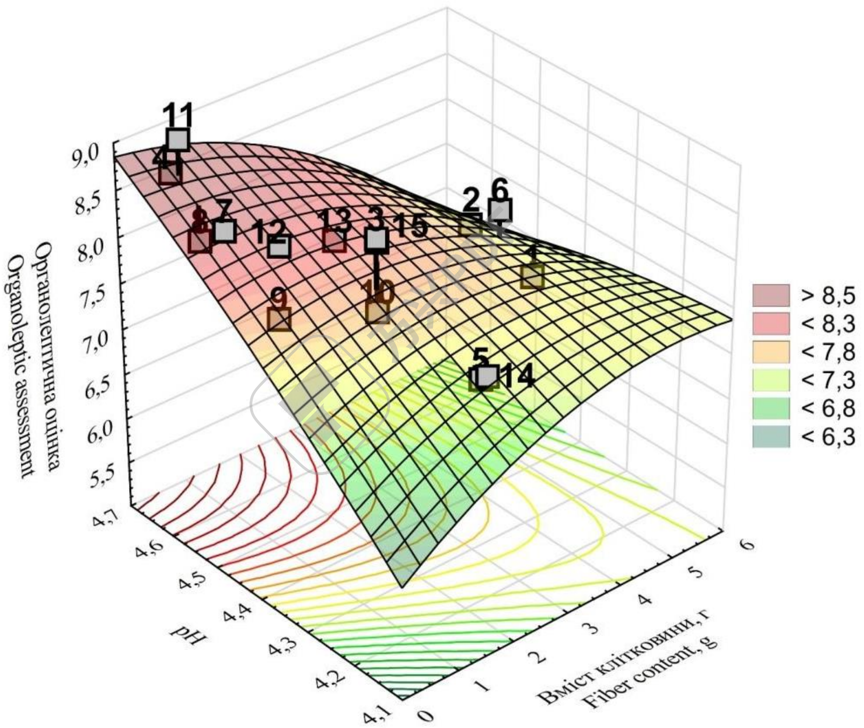


Fig.4.3 - 3D optimization model for three -factors recipe analysis with defatted sesame flour and rice bran

After study of all mathematical modelling results, the 11th sample was determined as the optimal recipe composition, that corresponds to the content of defatted sesame flour in the amount of 2% and to the content of rice bran in the amount of 0,4% in the recipe.

SECTION 5

RECOMMENDATIONS REGARDING THE RATIONAL MODE OF MANUFACTURE OF FERMENTED MILK BEVERAGES ENRICHED WITH DIETARY FIBER IN INDUSTRIAL CONDITIONS

5.1. Quality indicators of fermented milk drinks with added additives containing dietary fibers

Kefir shall comply with national standard GB 2746. As follows:

Sensory indicators: milky white or slightly YELLOWish, with fresh and pure lactic acid taste, dense and firm clots, no bubbles, a small amount of whey precipitation was allowed.

Physical and chemical indexes were shown in table 5.1.

Table 5.1- Physical and chemical indexes

Items	Index
Fat, %	3,10
Acid (In lactic acid), %	0.63 ~ 0.99

Bacterial indexes were shown in table 5.2.

Table 5.2 - Bacterial indexes

Items	Index
coliform group (PCs /mL)	≤90
pathogen	0.63 ~ 0.99
Refers to intestinal pathogenic bacteria and pathogenic cocci	

5.2. Production technology of fermented milk drinks with additives containing food fibers

We have developed a recipe for natural fermented milk drink with the addition of sesame flour and rice bran (table 5.3).

Table 5.3 - The recipe of fermented milk drink

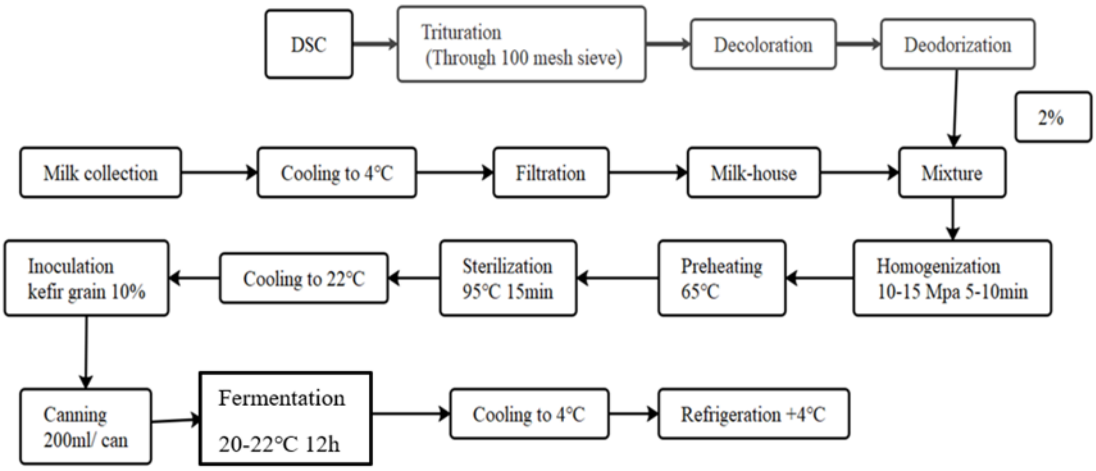
Name of the component	Component quantity, кг	
	Fermented milk drink enriched with sesame flour	Fermented milk drink enriched with rice bran
Milk	880	896
RB	0	4
DSF	20	0
Leaven	100	100
Together	1000	1000

When fresh milk went into the factory acceptance link, not only density determination and alcohol test were necessary, but also the total dry matter content of fresh milk should be not less than 11.5%, non-fat dry matter should be not less than 8.5%. The acidity of raw milk should be below 18°T.

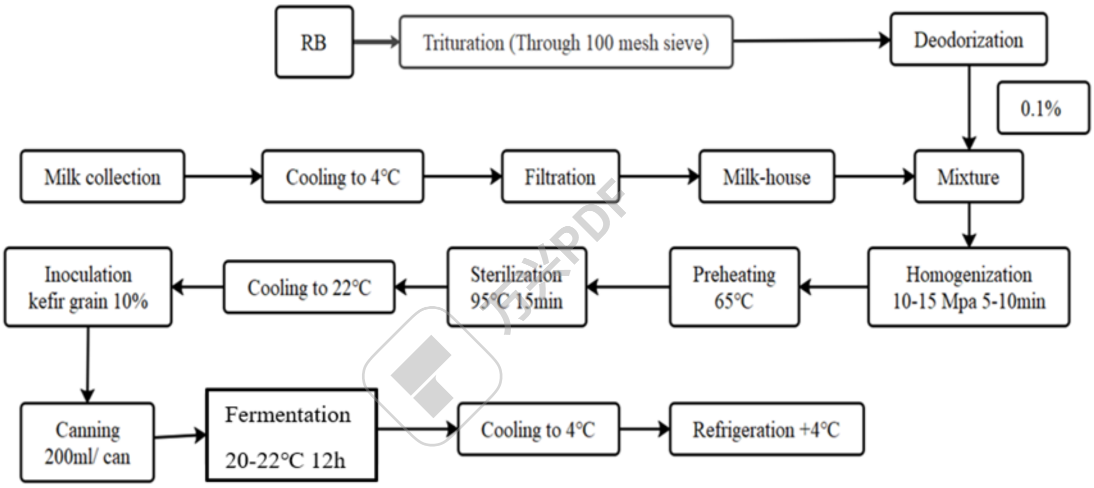
Do not use fresh milk containing antibiotics or residual chlorine and other fungicides. It is strictly forbidden to use milk suffering from mastitis and milk injected with antibiotics, and the number of miscellaneous bacteria per milliliter should be not higher than 5 million. In addition, according to the requirements of the Chinese national standard of fermented milk (GB2746-1999), raw fresh milk should be standardized to ensure the stability of the quality of each batch of products.

The pretreatment of DSF include: sieving, decoloration and deodorization, and adding ratio was 2%; RB should be sieved before used, and the adding ratio was 0.1%.

The working flows for manufacturing fermented milk drink was shown in figure 5.2.



a



b

Figure 5.1 - Technological schemes for the production of fermented milk drinks enriched with dietary fibers: *a* - working flows of kefir added with DSF; *b* - Working flows of kefir added with RB

The raw material liquid should be preheated at 55 ~ 65°C before entering the homogenizer. Through medium pressure homogenization at 8.0-10.0 mpa, fat globule in raw material liquid can be broken finely, which can prevent fat particles floating. Then the raw material liquid went into the sterilizer.

Higher temperature and long time heat treatment are beneficial to the acid production and curd state of kefir. After homogenization, the raw material liquid was heated in a sterilizer to 90 ~ 95 °C for 3~5 min, or 85 °C for 30 min and then cooled.

After sterilization, the raw material liquid should be cooled to 26 ~ 28°C, and should be not more than 30°C, otherwise it would have adverse effects on acid production and curd state, even bring serious whey precipitation. The start culture was obtained from kefir grain. After multiple activation, kefir grain was added into aseptic cow milk, when the pH was up to 4.7, filtered the grain, the left liquid was used as start culture, and added to the milk tank at 10%, and the mixture is evenly stirred.

The cooled raw material liquid is fed into milk tank through a changeover valve. The starter culture is continuously added to the raw material liquid by means of a metering pump. During the inoculation process, the milk tank was kept in a state of agitation. Aseptic operation was used to inoculate to prevent microbial contamination. Stir thoroughly for 10 min after inoculation, so that the thallus and raw material liquid could mixed evenly. Also keep the milk warm to avoid prolonged fermentation.

The inoculated milk should be filled immediately and continuously. Kefir containers generally have 4 categories: porcelain jars, glass bottles, plastic cups, paper boxes. Filling and capping can be operated by automatic aseptic filling machine. Asepsis control must be carried out on filling equipment, filling containers and filling chambers. When plastic bottles are used as small containers for sale, the check of the lid of the container should be carried out to prevent leak. Small containers must be incubated immediately after leaving the filling machine in the culture chamber.

Fermentation was carried out in a special fermentation room, which is an important process affecting product quality. The temperature of fermentation is generally 26~28°C, and the fermentation temperature is related to equipment performance, cooling rate, acid production capacity, incense production capacity, proliferation rate, kefir species and other factors. The general fermentation time for kefir production is 26-28°C for 12-14h.

The fermentation time is related to many factors such as inoculum amount, starter activity, incubation temperature, dairy processing time, cooling rate, container type, fermentation season and climate conditions, and density of kefir pile. The fermentation time cannot be defined as unchangeable.

The equipment and technological scheme for the production of fermented milk drinks enriched with dietary fibers is presented in fig.5.2

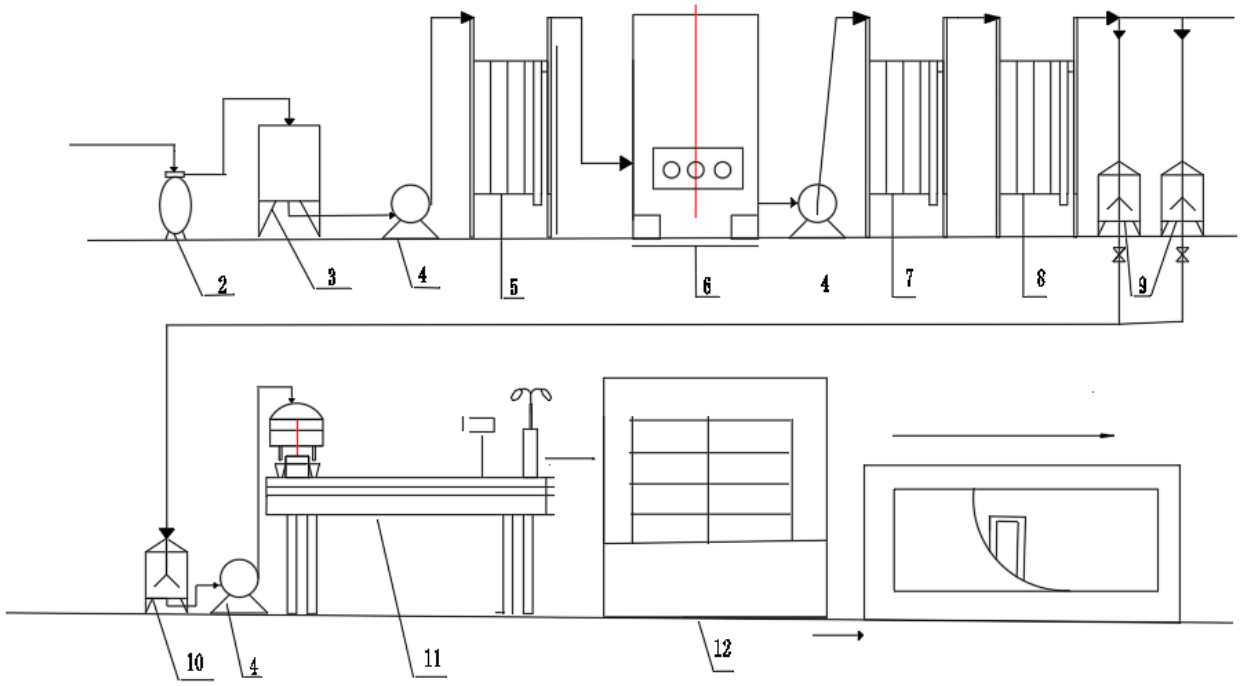


Fig. 5.2 - Equipment and technology scheme for the production of fortified fermented milk drinks: 1 - Cold storage; 2 - Joint filter; 3 - Mixing tank; 4 – Pump; 5 - Plate heat exchanger for preheating; 6 – Homogenizer; 7 – Sterilizer; 8 - Plate heat exchanger for cooling; 9 - Vaccination tank; 10 - Stirred tank; 11 - Filling machine; 12 - Fermentation room; 13 - Cooler

The total fermentation time is generally about 12h, even 14h, and the end of fermentation time range is relatively small. If stop fermentation too early, the kefir tissue is soft and tender, and the flavor is poor; Too late, kefir was in high acidity, excessive whey precipitation, and the flavor is poor. Therefore, the determination of fermentation end is one of the key technologies for kefir production.

In addition to the experienced personnel in the production process, the following methods can be used to determine the end point of fermentation:

- pH. sampling and test pH, when pH reached to 4.7 the fermentation could be stopped;

- Pay attention to the time that samples enter the fermentation room, the above several classes of fermentation time should prevail under the same production conditions;

- Sampling timely and observed, open the cap slowly, tilt the bottle, observe the fluidity and tissue state of kefir, if the fluidity became poor, and small particles appear, fermentation could be terminated;

- Record the fermentation time and temperature of each batch in detail, take the record as a reference for nest batch;

- Check the pH value and titration acidity every half an hour in the production process.

The purpose of cooling is:

- to inhibit the growth of lactic acid bacteria rapidly and effectively to prevent excessive acid production;

- promote the formation of good state of kefir;

- reduce and stabilize the speed of kefir fat floating and whey precipitation;

- extend the shelf life of kefir.

Cooling method is divided into direct cooling method and pre-cooling method. The key to the cooling process is to handle gently and prevent vibration, even for fully mature samples.

Refrigeration could promote the production of fragrance substances, improve the hardness of kefir. The peak of flavor substances is generally 12 ~ 14 h after the completion of production, which is the result of the balance of a variety of flavor substances.

Equipment selection is based on production technology, can ensure the quality and quantity of production, at the same time, it was investment saving, less energy consumption, low maintenance cost, easy operation and maintenance, and a certain amount of production capacity margin was left. In this design, 2 batches are produced per day, and each batch is calculated as 30 tons of kefir.

5.3 Technical and economic calculations of the project

5.3.1 Production costs

Consumption of raw materials and packaging materials

Table 5.4 - Consumption of raw materials and packaging materials/ day

Cost	Items	Rate of consumption	Price per unit	Total
		day	USD	
Raw material	Cow milk	8.7(t)	700	3480
Raw material	DSF	44.8 (kg)	0.3	13
Raw material	RB	2.24 (kg)	0.29	0.65
Package	Bottles	2000 (kg)	0.07	140
Package	Carton	200 (kg)	0.07	14

Total cost of the rest of the raw material: about 54,286 USD. Total: (3480+13+0.65+140+14)×300+54,286=1,110,543 USD, the total investment is 1,111,742 USD/ year.

5.3.2 Fixed-asset investment

Through the inquiry to the equipment supplier and summary, investment in fixed assets was estimated, which was shown in table 5.5. A total of about 16 million yuan of investment in fixed assets.

Table 5.5 - Investment in fixed assets

Equipment	number	Sum up (USD)
CIP tank	5 PCs	65,714
Mixing tank system	4 PCs	48,571
Fermentation tank	8 PCs	131,429
Plate heat exchanger	3 groups	35,714
Centrifugal pump	24 PCs	72,857
All kinds of valve	180 PCs	171,428

The end of the Table 5.5

Equipment	number	Sum up (USD)
High shear mixer	1 PCs	128,571
homogenizer	1 PCs	1,214,285
Filling machine	2 PCs	2500000
Stainless steel tube for products	5000 m	542,857
Seamless carbon steel pipe of steam and insulation	500 m	142,857
The boiler	2 PCs	13,571,428
Water treatment system	1 set	214,285
Refrigeration units	1 set	142,857
Refrigeration house	1 set	28,571
Sewage treatment	1 set	214,285
Installation and debugging cost	1 set	142,857
Distribution, pressure air, inspection, and other auxiliary facilities	1 set	142,857
Office building renovation and new plant construction	1 set	285,714
Total		2,285,714

5.3.3 Depreciation of equipment

Investment in fixed assets was 16 million yuan, was expected to use 10 years, the residual value after depreciation is 5% of the original investment, the cost of cleaning is 1% of the original investment, the annual depreciation was: (1-5% + 1%) x 2,285,714/ 10 = 219,429 USD/year.

5.3.4 Energy consumption cost

Energy consumption such as water, electricity and coal were shown in table 5.6, the total energy consumption cost was 251,486 USD/year.

Table 5.6 - Energy consumption cost

Items	rate of expenditure	unit price	Total
Water	22 (t/shift)	0.285 (USD/t)	6.29 (USD/ shift)
Electricity	200 (kw/ shift)	0.285 (USD /kw)	85.71 (USD/ shift)
Coal	2.90 (t/ shift)	28.57 (USD /t)	331.43 (USD/ shift)
Total			251,486 (USD/ year)

5.3.5 Wages for workmen

Estimated according to the average salary is 570 USD per person, the total cost for human resource cost was 570×160×12=1,097,143 (USD / year). Profit analysis and payback period

Calculated as 0.7 USD per bottle (200g), the sales revenue = (2,500,000,000/ 200) · 0.7 = 8,928,571 USD.

Sales expenses are expected to be 40% of sales revenue, so it is 8,928,571· 40%=3,571,428 USD.

The production operation cost is = 1,111,742+219,429+251,486+1,097,143 = 2,679,800 USD. Liquidity is expected to account for 20% of the assets invested, and the monthly interest rate on bank loans was 0.6%.

Then the year financial cost is: 2,285,714×1.2×0.6%×12=197,486 USD.

The year profit = sales revenue - the cost of production - sales - finance charges=8,928,571 - 3,571,428-2,679,800-197,486=2,479,857 USD.

Payback period of investment profit = total fixed assets/annual profit =2,285,714/ 2,479,857= 0.92 year= 11.1 months

CONCLUSIONS

1. Defatted sesame flour and rice bran are by-products obtained as a result of grain processing. The content of dietary fibers was studied and it was established that rice bran contains 28.57%, and sesame flour - 10.8%. Adding 0.1% of rice bran to a fermented milk drink increases the dietary fiber content in it to 0.03%, and adding 2% of sesame flour to 0.68%.

2. The addition of defatted sesame flour and rice bran affected the sensory characteristics of the product. A change in the color of both samples was observed (from white to light cream). A characteristic taste and aroma of the corresponding additive appeared. The recommended amount of an additive that does not have a negative effect on the organoleptic properties of the product: defatted sesame flour - 2%, rice bran - 0.1%.

3. The research showed that adding od defatted sesame flour led to an increase of mass fraction of proteins, fats, compared with control sample of 2,7% and 2,5%, respectively.

4. The introduction of defatted sesame flour can improve the total content of amino acids of fermented milk drink, content of Glu and Arg in all amino acids.

5. While adding of defatted sesame flour, pH value reduces and acidity of fermented milk drinks increases. The optimal value of titrated acidity is observed in fermented milk drinks enriched with sesame flour, at -14 days of storage. Similar results were obtained the addition of rice bran. The addition of rice bran leads to a decrease in the pH level (0.14), an increase in acidity by 5.81 ºT, compared to the control.

6. Compared with control sample, even 2% of added defatted sesame flour increase significantly apparent viscosity (up to 4,87 Pa*s) and water holding capacity up to - 67,76%. Adding of rice bran (0,1%) leads to an increase of water holding capacity up to 11,1%. Rice bran can improve apparent viscosity of fermented milk drink compared with control sample. The apparent viscosity of fermented milk drink

with rice bran was the highest on the 14th day.

7. The introduction of defatted sesame flour significantly deepened the yellow and red colors of kefir. The storage period did not affect the brightness of all samples. With the extension of the storage period, the red color of the samples showed a tendency first to deepen, and then to weaken, and the red color was the strongest on the 14th day. Similar results were obtained with the addition of rice bran. It was established that the best shelf life of fermented milk drinks with the addition of rice bran and sesame flour is 14 days.

8. It was found that the introduction of defatted sesame flour significantly increased the level of DDPH radical scavenging activity and ·OH radical scavenging activity. DDPH radical scavenging activity in all samples decreased after 7 days of storage, while ·OH radical scavenging activity decreased for all samples after 14 days of storage. This indicates an improvement in the antioxidant properties of the drink with the addition of sesame flour. As a result, the shelf life increases to 14 days without the use of preservatives and stabilizers.

9. The results of microbiological indicators of kefir showed that the introduction of defatted sesame flour did not change the population of *Lactobacillus*, *Yeasts* and *Lactococcus* A significant reduction of lactobacilli occurred in some samples after 14 days of storage, a significant decrease of *Lactococcus* occurred in some samples after 7 days of storage, a significant decrease of Yeasts occurred in some samples after 14 days of storage.

10. The results of microbiological indicators of fermented milk drinks with rice bran showed that the population of *Lactobacillus* (the highest - on the 14th day of storage) and *Yeasts* are growing. The number of *Lactococcus* decreased significantly in the first two weeks and remained stable during the following two weeks.

11. Optimization using of the software package established that the optimal quantity of the additives are the following: 2% of defatted sesame flour and 0,4% of rice bran. Under these conditions the products have the best quality sensory indicators.

12. The technology for production of fermented milk drinks enriched with the

dietary fibers (defatted sesame flour and rice bran) and recommendations as regards to their implementation into production process was developed.

13. The economic calculations showed that the payback period for the implementation of the proposed technologies using sesame flour and rice bran is 11 months.

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APPLICATIONS

Appendix 1

NOTES

Links to the materials of your dissertation work are indicated in gray in the text

Items, tables and figures that need to be renumbered according to your work are marked in yellow

Errors in organoleptic studies that need to be changed in the work are marked in red. The correct indicators were provided by your academic supervisor.

Appendix 2

Act of industrial testing in China

批准

董事 "珠海市宝贤企业有限公司"

冯海志

八月 16， 2022

添加膳食纤维的发酵乳的产品研究及生产应用

根据标准 DSTU 4417：2005 "开菲尔生产技术"

2022 年 8 月 5 日，宝贤餐饮管理有限公司的员工和苏梅国立农业大学的代表按照 DSTU 4417：2005 "开菲尔的开发工艺"。生产了一批含有膳食纤维的发酵乳饮料，使用全脂牛奶，米糠（TU U 10.8-37756905-001：2015），干细菌发酵剂" Kefir VIVO "（TU U 15.5-3060300036-001：2009）生产，在宝贤餐饮管理有限公司的条件下和生产场所。

根据生产测试结果，本产品符合要求（DSTU 4417：2005 "开菲尔的开发工艺"），并已于 2022 年 8 月 17 日投入生产。1 升产品的既定成本是人民币 10 元，售出 1000L，这使得获得 3690 元人名币的净利润成为可能。

苏梅国立农业带小额技术与食品安全部主任

萨米利克

珠海市宝贤企业有限公司董事

冯海志

Appendix 3

Act of industrial testing in Ukraine

ЗАТВЕРДЖУЮ
Директор ФОП «ОпришкоД.В.»
Опришко Д.В.

16 липня 2022 р.

Акт виробки
дослідно-промислової партії
кисломолочного напою із харчовими волокнами
згідно з ДСТУ 4417:2005«Кефір. Технічні умови»

Співробітниками ФОП «Опришко Денис Володимирович»(ТМ O'BEREG)та представниками Сумського національного аграрного університету 05.07.2022 року згідно з розробленими технологічними рецептурами у відповідності зДСТУ 4417:2005«Кефір. Технічні умови»було виготовлено партіюкисломолочного напою із харчовими волокнами в кількості 1000 кгіз використанням молока незбираного, кунжутного борошна (ТУ У 10.8-37756905-001:2015), заквасксухої бактеріальної «Кефір VIVO» (ТУ У 15.5-3060300036-001:2009), в умовах та на виробничих площах ТМ O'BEREG.

За результатами виробничих випробувань дана продукція відповідає вимогам (ДСТУ 4417:2005 «Кефір. Технічні умови») та поставлена на виробництво з 17.07.2022 року.Встановлена вартість 1 л продукції – 35 грн, реалізовано – 900 шт., що дозволило отримати чистий прибуток в розмірі 18950 грн.

Завідувач кафедри технологій та безпечності харчових продуктів СНАУ
Самілик М.М.

Директор ФОП Опришко Д.В.
Д.В.Опришко