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International Journal of Food Engineering Research (IJFER) is peer-reviewed journal which provides a platform for publication of original scientific research and applied practice studies. Positioned as a vehicle for academics and practitioners to share field research, the journal aims to appeal to both researchers and academicians.

Aims and Scope

International Journal of Food Engineering Research (IJFER) is an international , peer-reviewed journal devoted to the publication of high quality original studies and reviews concerning a broad and comprehensive view of fundamental and applied research in food science&technology and their related subjects as nutrition, agriculture, food safety, food originated diseases and economic aspects.

IJFER is an international periodical published twice a year (April and October). The journal is published in both print and electronic format.

From The Editor

International Journal of Food Engineering Research (IJFER) has been publishing by Istanbul Aydın University Faculty of Engineering Department of Food Engineering since 2015. The journal covers wide ranges of area such as Food Processing, Food Preservation, Food Microbiology, Food Chemistry, Biotechnology, Nanotechnology, Novel Technologies, Food Safety, Food Security, Food Quality and their related subjects as nutrition, food and health, agriculture, economic aspects and sustainability in food production.

Food Engineering is getting more and more attention because it is directly related to human health. While the food and drinks we eat help to protect our health, on the other hand, improper conditions during the conversion of the raw material to the product, the use of poor quality raw materials, and the employees not working under hygienic conditions can cause the food harmful to health. Our aim in this journal is to include the recent research and reviews on food and beverages from field to fork. Articles submitted to the journal are accepted for publication after being reviewed by expert referees.

In the following years, the journal will include scientific activities such as symposiums, congresses, conferences and workshops held in the field of food science and technology, and information about the books published in this field. We hope that the journal will be a good resource for engineers, experts, researchers and students working in the food industry.

Prof. Dr. Z. Dilek Heperkan

Editor

International Journal of Food Engineering Research (IJFER)

CONTENTS

DETERMINATION OF THE PRESENCE OF LIVING MICROORGANISMS IN KEFIR GRAIN

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ABSTRACT
Kefir, defined as fermented milk product, is a drink that lactic acid bacteria and yeasts formed together with kefir grain obtained by adding milk and waiting at room temperature for one day. Kefir is being investigated with increasing interest in recent years as it contains micro-organisms useful for health. Although kefir is produced on an industrial scale, consumers have traditionally been producing kefir in homes. This study aimed to determine the presence of living lactic acid bacteria and yeast in kefir grain. Man Rogosa and Sharp (MRS) media were used for isolation and counting of lactic acid bacteria and Yeast extract Glucose Chloramphenicol agar (YGC) was used for yeasts. Kefir grain was found to contain $5x10^4$ CFU/g of living lactic acid bacteria and yeast.

Keywords: *Probiotic, Lactobacillus, Yeast, Health benefits, Saccharomyces*

INTRODUCTION

Kefir is a milk-based fermented beverage produced using a micro-organism community called kefir grains or kefir cultures. Based on Turkish Food Codex **[2],** kefir is defined as a fermented milk product in which starter cultures or kefir grains are used in fermentation containing different strains of *Lactobacillus, Leuconostoc, Lactococcus* and *Acetobacter* species and strains of yeast species that are able to ferment (*Kluyveromyces marxianus*) or non-ferment(*Saccharomyces unisporus, S. cerevisiae, S. exiguus*) lactose. Nowadays, with the increasing importance of consumers to eat natural and healthy foods, the demand for kefir also increases. This increase in consumer demand has accelerated the commercialization of kefir, which was previously produced only in individually at homes. The source of kefir is Caucasus and Tibet, it started to be produced in Eastern and Central European countries towards the end of the century in 19th century and today it has spread all over the world.

The production of fermented foods has been done since ancient times (A.C. 7000-A.C. 8000) is known. However, commercially the production of meat and milk-based fermented foods increased after 1970. Kefir is one of the commercially produced fermented beverages in our country.

Fermented foods are produced using a wide variety of raw materials. These include a large number of fermented foods produced using vegetables and fruits, milk, meat, fish, and cereals. Examples of some fermented foods produced in our country are given in Table 1. Some foods, such as cheese, pickles, and wine, are more or less different in their sensory and structural properties but have been accepted all over the world. However, fermented fish is more preferred in northern Europe and America and Asian countries, while fermented rice is produced in Asia, fermented cereals (boza) in our country, the Caucasus, and the Balkans. Fermented foods such as pickles, vinegar, olives, sausage, and turnip, especially yoghurt, are traditionally produced individually at homes for years.

Table 1: Examples of fermented food and beverages and their raw material

Fermented Foods	Raw Materials
Boza	Wheat, barley, corn, millet, rice
Hardaliye	Black grapes, cherries, mustard (grain)
Kefir	Milk
Cheese	Milk (cow, sheep, goat)
Feta cheese Cheddar cheese Sheep cheese (Pergamon Erzincan, Izmir) Blue mould cheese Pot cheese Herbed cheese Hellim cheese	
Sausage	Meat
Vinegar	Greengrocery
Turnip	Turnips, black carrot
Pickle	Fruits and vegetables
Yoghurt	Milk
Olive	Black and green olives

Fermented foods in our country as a variety of the highest number of foods produced are cheese and pickles. The most common types of cheese are feta cheese, tulum cheese, and cheddar cheese, while cucumber, cabbage, and pepper pickles are the examples of pickles produced in the greatest amount of time. It is observed that vinegar, which is added as flavoring to food and sauces, has been consumed as a beverage in recent years considering it to be beneficial to health.

Literature related fermented foods have been found in numerous studies examined kefir. While some studies have examined the effect of the milk variety on the physical, chemical and sensory properties of kefir, the majority of research has focused on microbiota and kefir micro-organisms.

In addition to the milk of animals such as cows, sheep, and goats, there are also studies where camel and horse milk are used in the production of kefir. Vegetable-derived milk substitutes such as soy, rice, and coconut milk can also be used. The Kefir grain consists of a structure in which a group of micro-organisms, lactic acid bacteria and yeasts, cling to each other with their own polymeric substances. When a kefir grain is added into a liquid medium such as milk, the micro-organisms that make up the kefir grain act out of the cell by releasing various flavorings, especially organic acids such as lactic acid, acetic acid and CO_2. Thus, kefir is formed with a slightly acidic, distinctive taste, slightly frothy and creamy-white color. An article published by Altay et al. **[3]** about non-alcoholic fermented Turkish beverages has included a broad literature review on micro-organisms found in kefir. In the studies carried out in Table 2, the species belonging to the *Lactobacillus* genus and yeast species in Table 3, which were found in kefir samples.

Table 2: *Lactobacillus* species found in kefir

***Lactobacillus* spp. in Kefir**	**References**
Lactobacillus acidophilus	**[4, 5]**
L. brevis	**[6]**
L. casei	**[4]**
L. casei subsp. *pseudoplantarum*	**[7]**
L. delbrueckii subsp. *bulgaricus*	**[7]**
L. fermentum	**[5]**
L. helveticus	**[5]**
L. kefiranofaciens	**[6]**
L. kefiri	**[6]**
L. otakiensis	**[5]**
L. plantarum	**[8, 9]**
L. sunkii	**[5]**

Kefir contains different species of lactic acid bacteria belonging to the genus *Lactobacillus, Lactococcus, Pediococcus, Streptococcus* and *Leuconostoc*. Lactic acid bacteria is the name given to a group of bacteria that produce lactic acid and are proven not to be harmful to health. They are considered GRAS (Generally Recognized as Safe) status because they are not harmful to health. The two new species as *Lactobacillus kefiranofaciens* and *L. kefiri*, which are not found in other fermented foods, have been found **[6].**

Table 3: Yeasts species found in kefir

Yeast species in Kefir	References
Candida inconspicua	**[7]**
C. kefyr	**[10]**
C. krusei	**[10]**
C. lambica	**[10]**
C. maris	**[7]**
Cryptococcus humicolus	**[10]**
Geotrichum candidum	**[10]**
Kazachstania aerobia	**[11]**
Kluyveromyces marxianus	**[9]**
Lachancea meyersii	**[11]**
Pichia fermentans	**[12]**
Saccharomyces cerevisiae	**[11]**
S. turicensis	**[12]**

The kefir composition is given in Table 4.

Table 4: Kefir composition and micro-organisms load **[1]**

Property	Quantity
Milk protein (w/w %)	min. 2,7
Milk fat (w/w %)	max. 10
Titration acidity (as lactic acid %)	min. 0,6
Ethanol (v/w %)	-
Total specific micro-organisms (CFU/g)	min. 10^7
Total additional micro-organisms indicated on the label (CFU/g)	min. 10^6
Yeasts (CFU/g)	min. 10^4

In a study by Renner and Renz **[13]**, amino acids, vitamins, and trace metals found in kefir were determined as well as the composition of kefir and given in Table 5.

Table 5: Chemical composition of kefir **[13].**

Components			
Fat (%)	3.5	**Trace metals (mg)**	
Protein (%)	3.3	Iron	0.05
Lactose (%)	4.0	Copper	12
Water (%)	87.5	Molybdenum	5.5
Essential amino acids		Zinc	0.36
Tryptophan	0.05	Manganese	5
Phenylalanine + Tyrosine	0.35	**Vitamins**	
Leucine	0.34	A (mg)	0.06
Isoleucine	0.21	Carotin (mg)	0.02
Threonine	0.17	B1 (mg)	0.04
Methionine + Cystine	0.12	B2 (mg)	0.17
Lysine	0.27	B6 (mg)	0.05
Valine	0.22	B12 (mg)	0.5
Mineral Matter		Niacin (mg)	0.09
Calcium (g)	0.12	C (mg)	1
Phosphorus (g)	0.1	D (mg)	0.08
Magnesium (mg)	12	E (mg)	0.11
Potassium (g)	0.15	Lactic acid (%)	0.8
Sodium (g)	0.05	Phosphatides (mg)	40
Chlorine (g)	0.1	Cholesterin (mg)	13
		Ethyl alcohol (mg)	0.2

It has been determined that kefir is rich in phenylalanine, leucine, lysine, valine and isoleucine amino acids and in vitamins B2, B12, E and its energy value is 65 kcal at 100 mL **[13]**.

Kefir is also rich in vitamin C. There are numerous articles about the positive effects of kefir on health. But some of these positive effects have not yet been proven. The beneficial effects of kefir on health are summarized below. The most important benefit of kefir besides its rich mineral and nutrient content is that it can be consumed by individuals with lactose intolerance. Kefir contains all the nutrients found in milk. Due to its unique composition and structure, milk is also a suitable environment for micro-organisms. Micro-organisms provide nutrients more easily in aqueous environments and multiply faster. A major part of lactose found in milk is biodegrading by bacteria. As a result, the amount of lactose decreases on the one hand, while the amount of lactic acid increases on the other. Similarly, some of the proteins are also biodegrading by bacteria. Thus, kefir becomes a product with properties that can be consumed by individuals who are lactose intolerant and allergic to milk proteins. Furthermore, biochemical changes that occur during fermentation enable kefir to have its own unique flavor properties.

The probiotic properties of some of the kefir bacteria also contribute positively to the intestine microbiota. Most of the health benefits attributed to kefir are associated with kefir containing high amounts of probiotic micro-organisms. Studies on the control and improvement of many diseases with the consumption of kefir are still ongoing and it is stated that it creates protective and therapeutic effects against these ailments by providing the balance of the stomach and intestinal flora with antimicrobial and anticancer effects **[14, 15, 16]**.

Kefir is receiving increasing interest from consumers due to its beneficial health effects. Although kefir is commercially produced, traditionally kefir production is also very common in homes. In this study, the presence of living bacteria in the kefir grain used to produce kefir at home was examined and the total number of bacteria, lactic acid bacteria, and yeast was determined for this purpose.

MATERIAL AND METHOD

Material

As a material, kefir grain that used in making kefir individually at home was used. Kefir grain of approximately walnut-size was added to 500 ml UHT milk and incubated at 25°C.

At the beginning of incubation and 3 and 6 hours after incubation, samples were taken and microbiological inoculation was done.

Determination of Total Number of Bacteria

Plate Count Agar (PCA, Oxoid) was used to determine the total number of bacteria. After the medium sterilized in an autoclave at 121°C and 15 min. it is cooled to 50°C and poured into sterile petri plates. Inoculation was done by the spread plate technique. The total number of bacteria (CFU/mL) was determined by incubating at 30°C.

Determination of The Number of Lactic Acid Bacteria

Man Rogosa and Sharp (MRS) agar were used to identify lactic acid bacteria. It was prepared as PCA and inoculation were done by pour plate technique.

Determination of The Number of Yeast

YGC medium is used to determine the number of yeasts. This medium was prepared as PCA. Inoculation was done by the spread plate technique.

RESULTS

The total number of bacteria, the number of lactic acid bacteria and the number of yeast were given in Table 7, in the kefir grain that was inoculated in milk initially, 3 and 6 hours after incubation.

Table 6: Total number of bacteria, lactic acid bacteria and yeast in kefir grain

Media	Incubation time (h)		
	0	3	6
PCA (CFU/g)	$2x10^5$	$6x10^5$	$6x10^6$
MRS (CFU/g)	$5x10^4$	$3x10^4$	$2x10^5$
YGC (CFU/g)	$5x10^4$	$3x10^4$	$5x10^5$

As can be seen, by the examination of Table 7, the total number of bacteria per gram was 10^5 CFU, while 6 hours later it increased to $6x10^6$ CFU. The change in the total number of bacteria due to time is given in Figure 1.

The number of lactic acid bacteria in kefir (Table 6) was initially $5x10^4$ CFU/g, but after 6 hours it increased to $2x10^5$ CFU/g, while the number of yeast increased from $5x10^4$ CFU/g to $5x10^5$ CFU/g.

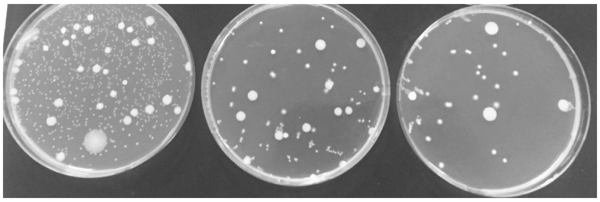

Figure 1: Colonies after 0, 3. and 6. hours incubation in PCA

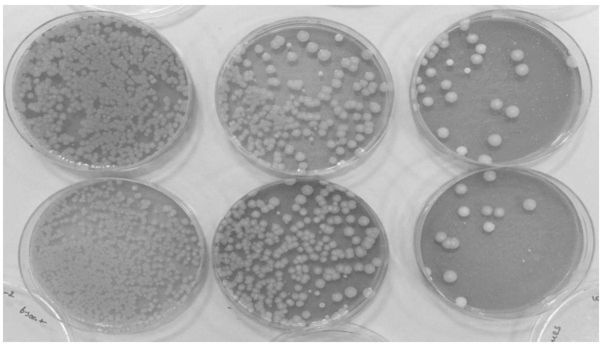

Figure 2: Lactic acid bacteria and yeast colonies at the end of 6 hours in MRS agar media

Figure 3 shows yeast colonies in YGC Agar media and Figure 4 shows yeast cells in light microscopy (40x).

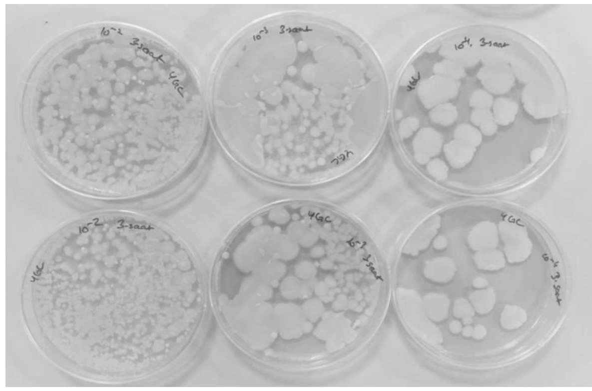

Figure 3: Yeast colonies in YGC (Parallel Inoculation)

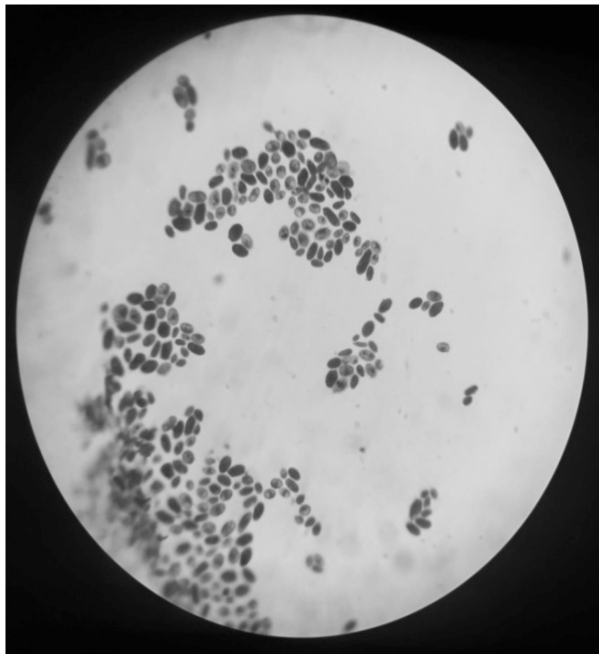

Figure 4: Yeast cells under a microscope (40x)

CONCLUSION

In this study, the presence of living lactic acid bacteria and yeasts in the kefir grain used to produce kefir was determined. In order for kefir grain to be used safely in kefir production, testing whether pathogenic bacteria are contaminated to the grain should be carried out. Furthermore, to determine which yeast and lactic acid bacteria are a presence in the kefir grain, it is necessary to isolate the species as pure, to perform biochemical tests for lactic acid bacteria, and to identify all isolates with PCR at the molecular level.

REFERENCES

[1] TGK, (2009). Türk Gıda Kodeksi, Fermente Süt Ürünleri Tebliği, Madde 4. Tarım ve Köy İşleri Bakanlğı, Tebliğ No:2009/25)

[2] TGK, (2017). Türk Gıda Kodeksi, Fermente Süt Ürünleri Tebliği, Madde 4. Tarım ve Köy İşleri Bakanlğı, Tebliğ No:Taslak/2017.

[3] Altay, F., Karbancıoglu-Güler, F., Daskaya, C. & Heperkan, D. (2013). A review on traditional Turkish fermented non-alcoholic beverages: Microbiota, fermentation process and quality characteristics. International Journal of Food Microbiology. 167: 44–56.

[4] Sabir, F., Beyatli, Y., Cokmus, C. & Onal-Darilmaz, D., (2010). Assessment of potential probiotic properties of *Lactobacillus* spp., *Lactococcus* spp., and *Pediococcus* spp. Strains isolated from kefir. Journal of Food Science. 75: 568-573.

[5] Kesmen, Z & Kacmaz, N. (2011). Determination of lactic microflora of kefir grains and kefir beverage by using culture-dependent and culture-independent methods. Food Science. 76 (5): M276-M283

[6] Leroy, F. & De Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. Trends in Food Science and Technology. 15: 67-78.

[7] Simova, E., Beshkova, D., Angelov, A., Hristozova, Ts, Frengova, G. & Spasov, Z., (2002). Lactic acid bacteria and yeasts in kefir grains and kefir made from them. Journal of Industrial Microbiology and Biotechnology. 28: 1-6.

[8] Powell, J. E., Witthuhn, R. C., Todorov, S. D., & Dicks, L. M. T. (2007). Characterization of bacteriocin ST8KF produced by a kefir isolate *Lactobacillus plantarum* ST8KF. International Dairy Journal, 17(3), 190-198.

[9] Wang, J., Zhao, X., Tian, X. Z., He, C., Yang, Y. & Yang, Z. (2015). isolation and characterization of exopolysaccharide-producing *Lactobacillus plantarum* SKT109 from Tibet kefir. Polish Journal of Food and Nutritional Sciences. 65: 269-279.

[10] Witthuhn, R. C., Schoeman, T., & Britz, T. J. (2005). Characterisation of the microbial population at different stages of kefir production and kefir grain mass cultivation. International Dairy Journal, 15(4), 383-389.

[11] Magalhaes, K.T., Pereira, G.V.M., Campos, C.R., Dragone, G. & Schwan, R.F. (2011). Brazilian kefir: structure, microbial communities and chemical composition. Brazilian Journal of Microbiology. 42: 693-702.

[12] Wang, S., Chen, K., Lo, Y., Chiang, M., Chen, H. & Liu, J. (2012). Investigation of microorganisms involved in biosynthesis of the kefir grain. Food Microbiology. 32: 274-285.

[13] Renner, E. & Renz, S. (1986). Nahrewettabellen fur milch und milchprodukte. Verlag B. Renner, Köhler K. G. Gieben, Germany.

[14] Alpkent Z. & Küçükçetin A. (2003). Farklı sıcaklıklarda muhafaza edilen kefirlerin duyusal, fiziksel, kimyasal ve mikrobiyolojik özelliklerinde meydana gelen değişmeler. VI. Süt ve Süt Ürünleri Sempozyumu, Tekirdağ, Türkiye, 22-23 Mayıs, p.363-373

[15] Karagözlü C. (2003). Kefir-probiotic fermented milk product, 50th Anniversary of The University of Food Teknology HIFFI 15-17 Oct. Collection of Scientific Works of The HIFFI Plovdiv, Bulgaria. 50: 404-409.

[16] Chen H. C., Wang S. Y. & Chen M. J. (2008). Microbiological study of lactic acid bacteria in kefir grains by culture-dependent and culture-independent methods. Food Microbiology. 25: 492–501.

LINEAR INCREASE BETWEEN POVERTY AND FOOD WASTE IN THE WORLD

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ABSTRACT

The problem of hunger has been the primary concern of human beings for centuries. With calamity, poverty, and death, starvation chaos is one of the biggest dreads of human beings. There are main reasons for this. People have to eat their fills to survive and continue their generation. The competence of people to participate, produce and create economic, political, cultural, and artistic activities that will make their lives meaningful depends on being healthy, and for to be healthy, they must be well fed. If food is consumed without wasting, the resources of the earth are sufficient to feed more people than the world population today.

Keywords: *Poverty, Starvation Chaos, Food Waste, Waste Policies*

INTRODUCTION

According to the results of the studies, there have been serious increases in the hunger rates, which is taking into account the increasing world population. In 2000, 900 million people were living below the hunger limit, while in 2016 this rate was around 815 million. A rapid acceleration in food waste is observed in proportion to these statistics. In 2018, our country, which has a population of 82 million, is wasting 214 billion of food waste annually. According to the Consumer Rights Association report in 2018, there are over 16 million people in Turkey can not be adequately nourished. Some activities determined to achieve the dream of zero starvation in the world by targeting 2030 are listed below **[1,5].**

Universal access to safe and nutritious food

Terminating all forms of malnutrition

Doubling the efficiency and revenues of small-scale food producers

Sustainable food production and durable agriculture practices

Protection of genetic diversity in food production

Investing in rural infrastructure, agricultural research, technology, and gene banks

Avoiding agricultural trade restrictions, market disturbances and export subsidies

Stable food markets and timely access to information **[2].**

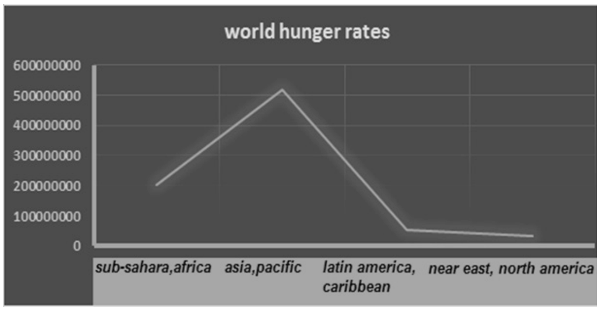

Figure 1: World Hunger Rates **[3]**

In the brochure of the United Nations Food and Agriculture Organization (FAO), various numbers were given by drawing attention to the hunger situation in the world. Accordingly, the world produces enough food to feed everyone, but still, 1 out of 9 people in the world have struggled with chronic hunger. In recent times, it is thought that the number of chronically hungry people, calculated to be 815 million in 2016, has increased more. The fact that the living of the hungriness fact 60% are women adds another dimension to the magnitude of the problem. According to the data, hunger causes more people to die from epidemic diseases such as malaria, tuberculosis, and AIDS in the world. It is stated that 45% of child deaths are caused by malnutrition, while 155 million children under the age of 5 are reported to have developmental retardation **[1, 4, 5, 6].**

Hunger and Obesity Compete

In other respects, it is stated that more than a quarter of the world's population, 1.9 billion people are overweight, and 600 million of them are obese. According to the literature, about 3.5 million people die every year due to excess weight. It is also among the data that the costs of malnutrition or overfeeding have reached the world economy of 3.5 trillion US dollars **[7].**

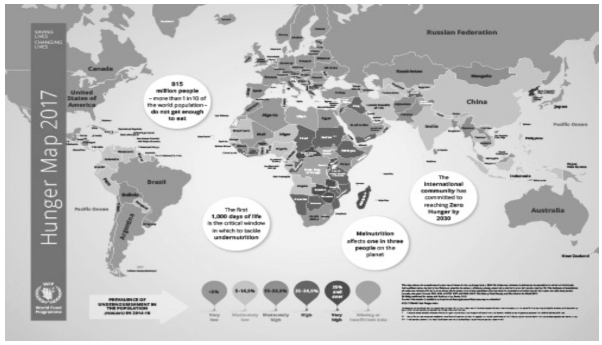

Figure 2: Hunger Map of 2017 **[8]**

Blue zones <5,% Yellow zones 5-14.9%, Orange zones 15-24.9%, Red zones 25-34.9%, Burgundy zones 35%, Values of gray zones are unknown.

Five Innovative Solution Suggestions for Waste Foods

According to the United Nations Report, if waste foods were a country, this country would be third in the world greenhouse gas emissions ranking, after the United States and China. According to the United Nations Report, innovative solution titles for waste foods are listed below.

Food Shift: It is based on the establishment of a more sustainable system that all produced foods can be processed and preserved.

Food Cowboy: The surplus production is based on the principle of networking between food owners (farmers) and charities providing free food for people in need.

Spoiler Alert: Large farms and wholesalers are based on sharing real-time information about surplus food and providing access to potential buyers.

Imperfect Produce: It is based on the principle of replacing the traditional food system and providing a more positive perspective to the rejected products.

For Solutions: It is based on the principle of saving food waste with a type of fertilizing system.

CONCLUSION

Even though food waste prevention becomes a global priority, government policies alone will not be enough and individual waste prevention policies can be implemented only by everyone, which can create permanent solutions. It is necessary to increase personal awareness to increase health levels with equal opportunities in all countries of the world, where healthier opportunities can be used as an equality principle.

REFERENCES

[1] Blesh, J., Hoey, L., Jones A.D., Friedmann, H. & Perfecto, I. (2019). Development pathways toward ‘‘zero hunger”. World Development, 118, 1–14.

[2] Şefik, O. (2015). World Hunger, Journal of Gezgin.

[3] Eksi, A. & Isci, A. (2012). The phenomenon of hunger in the world and search for solutions. Food, 37 (1): 39-45.

[4] Kim, Y., Sohn, H.S. & Park, B. (2019). Make the village better: An evaluation of the Saemaul Zero Hunger Communities Project in Tanzania and Bangladesh. World Development, 124, 104652.

[5] Djekic, I., Miloradovic, Z., Djekic, S. & Tomasevic, I. (2019). Household food waste in Serbia e Attitudes, quantities, and global warming potential. Journal of Cleaner Production, 229, 44-52.

[6] Ribeiro, A.P. Rok, J., Harmsen, R., Rosales Carreon, J. & Worrell, E. (2019). Food waste in an alternative food network -A case-study. Resources, Conservation & Recycling 149, 210–219.

[7] Adıyaman, F. (2016). Dünya Sağlık Örgütü Avrupa Obezite ile Mücadele Bakanlar Konferansının Öncesi Ve Sonrasında Obezitenin Gazetelerde Sunumu. Selçuk Üniversitesi İletişim Fakültesi Akademik Dergisi, 9(3).

[8] Anonymous (2018). Hunger Map of 2018. World Food Programme. https://www.wfp.org/publications/2018-hunger-map (Erişim tarihi:11.07.2020)

INVESTIGATION OF THE EFFECTS OF LACTIC ACID BACTERIAL EXOPOLYSACCHARIDES ON THE RHEOLOGICAL PROPERTIES OF WHEAT BEVERAGE

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ABSTRACT

Exopolysaccharides (EPS) which are produced by lactic acid bacterias are principally used in various fields such as the food industry, medicine, pharmacy, and cosmetics. Along with being colorless and odorless, bacterial EPSs have been subject to intensive research in recent years due to their various technological features and non-toxicity. In this study, changes in the rheological properties of wheat water of EPS, produced by eight different bacterial species and strains in soybean water were investigated. The species used in the study are *Lactobacillus coryniformis* C55, *L. paracasei* D41, *L. Brevis* 25A, *Lactococcus lactis* A47 and F39, *Pediococcus parvulus* E42, *Streptococcus macedonicus* A15 and *Weisella confusa* C19. In this study, the effect of EPS on the viscosity of wheat water was determined with the Ostwald-de Waele Model using Parallel Plate Sensor. At the same time, the pH of wheat water and distilled water was adjusted to 6.0 to eliminate the variables and viscosity behavior was examined at room temperature. Viscosity values appearing for 120 seconds at different shear rates from 0 s^{-1} to 300 s^{-1} were read in the study. The highest viscosity increase in wheat juice among the EPSs of the studied species was determined in the EPS produced by *L. coryniformis* C55 species. The findings obtained in the study showed that *Lactobacillus coryniformis* C55 has the potential to be used as a stabilizer in grain-borne foods.

Keywords: *Exopolysaacharides, Reology, Lactic Acid Bacteria, Wheat, MRS*

INTRODUCTION

Exopolysaacharides are the components that have high molecular weight (>10^6mol/g) which is produced by micro-organisms **[1]**. EPS, created by lactic acid bacteria in microbial EPSs, is used in the food industry as an emulsifier for various purposes such as increasing viscosity, improving texture properties and improving taste. Apart from the food sector, it is used in different fields such as medicine, pharmacy and cosmetics due to its anti-coagulant and antioxidant properties, preventing tumor development, stimulating the immune system, and anticoagulant **[2, 3].**

Lactic acid bacteria (LAB) is used to identify a group of bacteria that have Gram positive (+) metabolically and physiologically similar properties. It is mostly used for bacteria that form lactic acid by metabolizing lactose. LAB has a special status which is generally accepted as safe (GRAS, Generally Recognized as Safe) and is suitable for use in food. Lactic acid bacteria are not limited to a certain genus or ambient conditions. They are also found in herbal products, food processing environments, humans or animals. LAB are asporogenic, immobile bacteria. In addition to these common features, morphologically it shows significant differences depending on the genus. All species of the genus All species of the genus *Lactobacillus* are rod-shaped, the genus *Lactococcus* are coccus and the genus *Leuconostoc* are mostly cocobacillus. LAB is divided into two groups as homofermantative and heteroferemantative depending on the pathway they follow to break down lactose. In Table 1, some fermentative properties are given according to some lactic acid bacteria types and glucose metabolism.

Table 1: Lactic acid bacteria genus and fermentative specifications

The shape of the bacterium	Homofermantative LAB	Heterofermantative LAB
Rod-shaped	*Lactobacillus acidophilus*	*Lactobacillus brevis*
	L. delbrueckii	*L. kefir*
	L. helveticus	*L. paracasei*
		L. plantarum
		L. coryniformis
Round-shaped	*Lactococcus lactis*	
	Pediococcus parvulus	
	Streptococcus macedonicus	
Oval (coccobacillus)		*Leuconstoc mesenteroides*
		Weisella confusa
		Oenococcus oeni

Some of the species of genus *Lactobacillus* have homofermantative specifications while another part is heterofermantative. In spite of that, *Lactococcus, Streptococcus* and *Pediococcus* are always homofermantative, *Leuconstoc, Weisella* and *Oenococcus* species are heterofermantative (Table 1).

Some lactic acid bacteria produce EPS in the form of capsules in the cell, while others produce EPS, which can be released outside of the cell **[4]**. Some LAB types are known as major EPS producers. For example, *Leuconostoc* spp. Due to the diversity of their structure and physico-chemical properties, they are the primary dextran producers. Dextran, synthesized by *Leuconostoc mesenteroides* (NRRL B-640), contains D-glucose surplusing in the form of a flat chain and shows psedoplastic behavior. The dextran synthesized by *Leuconostoc citreum* (SK24.002) consists of -1,3 and -1,6 D-glucopyranose units and it is water soluble.

The composition and specifications of bacterial EPS vary greatly depending on the producer bacteria. However, EPSs consisting of monosaccharide are classified as homopolysaccharide. Homopolysaccharides (fructan or glucan) are synthesized from high amounts of sucrose **[4].** The acidic property of polymers is usually stem from the presence of uronic acid. However, other acidic compounds such as lactate, pyruvate and acetate also support this acidic property **[2].** Heteropolisaccharide EPSs, on the other hand, are synthesized from multiple types of monosaccharides such as glucose, galactose, fructose and rhamnose, and also contain some non-acidic components. For example, EPS obtained from *Lactobacillus sakei* 0-1 isolated from meat products contain D-glucose and L-rhamnose **[5].** The EPSs obtained from *L. acidophilus* LMG 9433 and *L. helveticus* NCDO 766 contain D-galactose and D-glucose, but their proportions are different. Many EPS originated from LAB, with different composition and properties, have broad field of application in the food industry. They are mostly used as additives to prevent syneresis and improve texture and viscosity in fermented milk products **[3].** EPS produced by *Pediococcus parvulus* in fermented oat-based products has been used as a stabilizer and has also been shown to improve tissue and palate **[6].** The aim of this study is to investigate the effect of the EPS produced by LAB species in soybean on viscosity and rheological behaviors in wheat water.

MATERIAL AND METHOD

Material

In this study, EPSs dependent to 8 lactic acid bacterial species were studied. The EPS produced by bacteria in soy water was added to wheat water and the rheological properties of wheat water were examined. Distilled water was used as a control. In the study, 8 species were examined. Lactic acid bacteria species used in the study are given in Table 2.

Table 2: Species of bacteria used in the study

Species of bacteria	Strain Number
L. coryniformis	C55
L. paracasei	D41
L. brevis	25-A
Lactococcus lactis	A47 ve F39
Pediococcus parvulus	E42
Streptococcus macedonicus	A15
Weisella confusa	C19

Method

In the study, wheat water and distilled water were used to examine the rheological properties. Both samples were adjusted to have pH 6 at room temperature. Test tubes containing 10 mL of wheat water and 10 mL of test tube were heated in the water bath for 15 minutes and then 0.1 g EPS was added. It is homogenized with vortex and kept in a water bath until it dissolves completely. Control samples were prepared in the same way for each sample. After obtaining a viscous solution, the samples are removed from the water bath and be waited for about 30 minutes at room temperature. Viscosity properties of EPS in wheat water were measured by using Parallel Plate Sensor (Plate PP35 Ti, 1.0 mm gap, D = 35 mm) with HAAKE RheoStress 1 (Thermo electron, Typ003-7370, Germany) device. The measurement was made at room temperature (25̊C) and each measurement lasted 120 seconds. The shear rate was adjusted from 0.01 s^{-1} to 300 s^{-1} and the viscosity appearing at varying shear rates was measured. With the Ostwald-de Waele Model, viscosity behavior, consistency coefficient and flow behavior index were calculated.

η: Viscosity [Pa.s]

K: Consistency coefficient [Pa. Sn.]

γ: Shear rate [s^{-1}]

n: Flow behavior index

$$\eta = K\,\gamma^{n-1}$$

RESULT AND DISCUSSION

Viscosity values of EPS obtained from bacteria in wheat water are given in Figure 1.

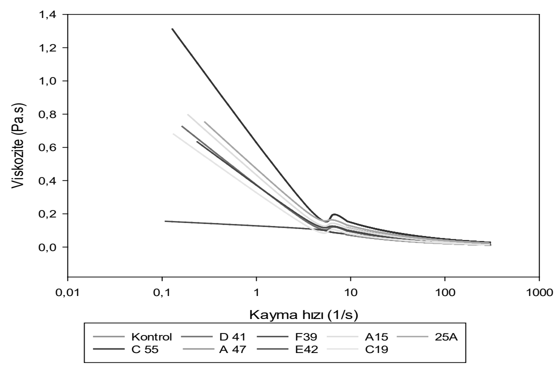

Figure 1: Viscosities of all wheat water samples through different shear rates.

When the viscosities of the EPS and control samples obtained from each bacterial species were calculated with the Ostwald-de Waele Model, it was observed that the control samples had the lowest viscosity value. The species with the highest viscosity is *L. coryniformis* C55 and it is seen that the EPS produced by it increases the viscosity at constant pH. Various specifications of lactic acid bacteria species used in the study are given below.

Lactobacillus coryniformis is a catalase negative, oxidase-negative, Gram (+) and rod-shaped bacterium **[7].** Although it is generally facultative heterofermentative, it also has homofermentative subspecies **[8]**. *L. coryniformis* is one of the less studied genes among the bacteria *Lactobacillus* genus and is generally associated with fermented vegetable products **[9].** *L. coryniformis* has subspecies that produce 99% and more of total lactic acid as D-lactate. With this property, it functions as a good biocatalyser **[8].** In addition, antimicrobial activities are important because it has been observed in studies that *L. coryniformis* strains can produce antifugal components with broad spectrum protein **[7].**

Lactobacillus paracasei is generally insulated from foods such as fermented vegetables, milk, dairy products. It is also found in the flora of the human intestinal tract. While only a few strains cause infections, the majority are GRAS and are considered probiotics. By virtue of its technological features, its use in the development of innovative and patented probiotic vegetable products has been commercially approved by the Italian Ministry of Health. In addition, when probiotics strains are combined with starter cultures in fermented products, they contribute positively to the probiotic functions of these foods **[10].** *L. paracasei* is facultative heterofermentative. Hexoses such as galactose, glucose and fructose are converted into lactic acid through the Embden-Meyerhof cycle. In addition to lactic acid, they are mostly metabolized to acetic acid and ethanol, while they form a small amount of butyric acid, diacetyl and formic acid. In general, only the L (+) isomer of lactic acid is synthesized, while some strains of *L. paracasei* can synthesize equal amounts of L (+) and D (-) isomers. *L. paracasei* strains generally develop at 4oC [11].

Lactococcus lactis species are bacteria that are Gram (+), catalase negative, facultative anaerobic, asporogenic , round-shaped and capable of forming chain of various lengths **[12, 13].** *Lactococcus lactis* is a mesophilic species of bacteria that constitute one of the main components of starter cultures on an industrial scale **[13].** It is generally homofermentative and produces L-lactic acid. It can develop between 12 and 40^{o}C, and optimum growth temperature is around 30^{o}C. Some strains can survive at temperatures lower than 10^{o}C and some strains at temperatures above 50^{o}C. This species is not tolerant to high salt concentration and does not develop at a salt concentration above 6.5%. It is generally isolated from fresh vegetables, fruits and cereals, but also from animal skins. *Lactococcus lactis* is used as a starter in many fermented milk products, especially cheese, in the production of fermented foods **[12].**

Located in lactic acid bacteria, *Pediococcus parvulus* is often found in dairy products and is used in cheese making. The importance of *Pediococci* species in fermentation and cheese ripening is still unknown, but some strains have been reported to have the potential to heal tissue due to EPS production. It has an antimicrobial effect because they produce bacteriocin **[14].** *Pediococcus parvulus* develops at 35^{o}C and pH 4.0, but not at 45^{o}C and pH 9.0 **[14].** *Pediococcus parvulus* is an anaerobic bacteria that requires folinic acid for food. *Pediococci* species are used in the food industry as biopreservative and probiotics [15].

Streptococcus macedonicus is a lactic acid bacteria that is often found in cheese and can produce EPS, form acid from N-acetylglycosamine, arbutin, lactose, maltose and raffinose, and produce acetoin. It can develop at 45^{o}C, but its development in the presence of ballast has not been detected. *Streptococcus* species are not widely used in food fermentation, but since *S. macedonicus* is a multifunctional bacterium, it gives hope for its use as a starter culture. Since strains can produce bacteriocin, they can be used as bioprotectors in fermented and non-fermented foods **[16].**

Weissella confusa is a bacterium isolated from peppers, tomatoes, blackberries and papaya, and found mostly in fermented vegetable juices **[17].** *W. confusa* has strains that can produce EPS, but are currently used directly in animal feeding *[18].* Weissella species are a good dextran producer, especially in leavened bread. It is used in bread production due to its properties such as extending the shelf life of leavened bread, increasing volume and providing softness **[19].**

Lactobacillus brevis has been isolated from dairy products, green plants and animals. They are available in single or short chain form. They can develop at 15°C, they do not develop at 45°C. *Lactobacillus brevis* strains are obligate hetereformentetive. Strains can fermantate esculin, galactose, maltose, lactose, raffinose, sucrose and xylose. It produces superoxides in the presence of oxygen. It has catalase activity that can eliminate hydrogen peroxide **[20].** In general, they use as flavorant or texture improver in milk products. It has probiotic and antimicrobial potential **[20].** *Lactobacillus brevis* has been frequently detected during beer fermentation and ripening stages **[21].** It is used as a probiotic culture and support culture in some cheese types **[20].**

Polysaccharide and its derivatives are used in many areas such as food and cosmetics with many properties such as gelling agent, thickener, stabilizer **[22].** EPSs are also frequently used in areas such as biomedical, biopharmacy and cosmetics because of their anti-tumor, stimulating immune system, anticoagulation, hypoglycemic, and antioxidant effects outside of the food industry [2]. Bacterial EPSs are one of the substances that play a role in the attachment of probiotic bacteria to the surfaces. EPS biosynthesis has been performed in many *Bifidobacterium* species species and these molecules are thought to play a role in the immune modulating capacity of strain. It was observed that the physicochemical properties of EPS such as monomer composition and molecular weight were also effective in these positive developments. Neutral feature and high molecular weight EPS shows a suppressor profile, while negatively charged bifido-bacterial EPS can activate different immune cells. These properties apply not only to bifido-bacterial EPS molecules, but also to other microbially sourced polymers **[23].** Bacterial EPSs are used as an additive to prevent syneresis and improve texture and viscosity in dairy products. It is also recommended to consumers in terms of health benefits.

CONCLUSION

Wheat is one of the primary food sources, which ranks second after rice, and 35% of the world population regularly consumes wheat-based food. The protein content of wheat is higher than other grains. It was demonstrated with this study that EPSs of lactic acid bacteria can be used as a stabilizer in wheat juice. In this study, the highest increase in viscosity in wheat juice was achieved with EPS obtained from *Lactobacillus coryniformis* C55 species. Results obtained in the study showed that *Lactobacillus coryniformis* C55 has the potential to use as a thickener in the development of new cereal-based products. It is thought that determining the structural and molecular properties of EPS with future studies will be beneficial in terms of contributing to new areas of use.

REFERENCES

[1] Heperkan, D., Daşkaya-Dikmen, C. & Bayram, B. (2014). Evaluation of lactic acid bacterial strains of boza for their exopolysaccharide and enzyme production as a potential adjunct culture. Process Biochemistry, 10, 1587-1594.

[2] Maalej, H., Boisset, C., Hmidet, N., Buon, L., Heyraud, A., & Nasri, M. (2014). Purification and structural data of a highly substituted exopolysaccharide from *Pseudomonas stutzeri* AS22. Carbohydrate Polymers, 112, 404–411.

[3] Malik, A., Sheilla, S., Firdausi, W., Handayani, T., & Saepudin, E. (2015). Sucrase Activity and Exopolysaccharide Partial Characterization From Three *Weissella confusa* Strains. Journal of Biosciences, 22(3), 130–135.

[4] Miao, M., Huang, C., Jia, X., Cui, S. W., Jiang, B., & Zhang, T. (2015). Physicochemical characteristics of a high molecular weight bioengineered α-D-glucan from *Leuconostoc citreum* SK24.002. Food Hydrocolloids, 50, 37–43.

[5] Gorska-Fraczek, S., Sandström C, Kenne L, Paściak M, Brzozowska E, Strus M, Heczko P. & Gamian A. (2013). The structure and immunoreactivity of exopolysaccharide isolated from *Lactobacillus johnsonii* strain 151. Carbohydrate Research, 378, 148-153.

[6] Russo, P., de Chiara, M. L. V., Capozzi, V., Arena, M. P., Amodio, M. L., Rascón, A., Dueñas, M. T., López, P., & Spano, G. (2016). *Lactobacillus plantarum* strains for multifunctional oat-based foods. LWT- Food Science and Technology, 68, 288–294.

[7] Martin, R., Olivares, M., Marín, M. L., Xaus, J., Fernández, L., & Rodríguez, J. M. (2005). Characterization of a reuterin-producing *Lactobacillus coryniformis* strain isolated from a goat's milk cheese. International Journal of Food Microbiology, 104(3), 267–277.

[8] Slavica, A., Trontel, A., Jelovac, N., Kosovec, Ž., Šantek, B., & Novak, S. (2015). Production of lactate and acetate by *Lactobacillus coryniformis* subsp. *torquens* DSM 20004 T in comparison with Lactobacillus amylovorus DSM 20531 T. Journal of Biotechnology, 202, 50–59.

[9] Yi, L. Dang, J., Zhang, L., Wu, Y., Liu, B. & Lü, X. (2016). Purification, characterization and bactericidal mechanism of a broad spectrum bacteriocin with antimicrobial activity against multidrug-resistant strains produced by Lactobacillus coryniformis XN8. Food Control, 67, 53-62.

[10] Lavermicocca, P., Dekker, M., Russo, F., Valerio, F., Di Venere, D., & Sisto, A. (2016). Lactobacillus paracasei- Enriched Vegetables Containing Health Promoting Molecules. In Probiotics, Prebiotics, and Synbiotics, 4, 361–370.

[11] Gobbetti, M. & Minervini, F. (2014). *Lactobacillus*: *Lactobacillus casei*. Encyclopedia of Food Microbiology, 2, 432-438.

[12] Demarigny, Y. (2014). *Lactococcus*: *Lactococcus lactis* subspecies *lactis* and *cremoris.* Encyclopedia of Food Microbiology, 2, 442-446.

[13] Mills, S., Ross, R.P. & Coffey, A. (2011). Lactic acid bacteria: Lactococcus lactis. Encyclopedia of Dairy Sciences, 2, 132-137.

[14] Holland, R., Crow, V., & Curry, B. (2011). Lactic Acid Bacteria| Pediococcus spp. In: Encyclopedia of Dairy Sciences, 2, 149-152.

[15] Raccach, M. (2014). *Pediococcus.* Encyclopedia of Food Microbiology, 2, 1-5

[16] Gobbetti, M. & Calasso, M. (2014). Streptococcus| Introduction. Encyclopedia of Food Microbiology, 2, 535-553.

[17] Cagno Di, R. & Coda, R. (2014). Fermented foods | Fermented Vegetable Products. Encyclopedia of Food Microbiology, 2, 875-883

[18] Pérez-Ramos, A., Nácher-Vázquez, M., Notararigo, S., López, P., & Mohedano, M. L. (2015). Current and future applications of bacterial extracellular polysaccharides. Probiotics, Prebiotics, and Synbiotics. Eds. Preedy, VR, Watson, RR ISBN: 9780128021897.

[19] Shi, Q., Juvonen, M., Hou, Y., Kajala, I., Nyyssölä, A., Maina, N. H., Maaheimo, H., Virkki, L. & Tenkanen, M. (2016). Lactose- and cellobiose-derived branched trisaccharides and a sucrose-containing trisaccharide produced by acceptor reactions of Weissella confusa dextransucrase. Food Chemistry, 190, 226–236.

[20] Calasso, M. & Gobbetti, M. (2011). Lactobacillus spp.: Other Species. Encyclopedia of Dairy Sciences, 2, 125-131. doi:10.1016/B978-0-12-374407- 4.00265-X.

[21] Suzuki, K. (2015). Gram-positive spoilage bacteria in brewing. Brewing Microbiology, 141-173.

[22] Raposo, M., Morais, A. & Morais, R. (2014). Influence of sulphate on the composition and antibacterial and antiviral properties of the exopolysaccharide from Porphyridium cruentum. Life Sciences, 101, 56-63.

[23] Hidalgo-Cantabrana, C., Nikolic M, López P, Suárez A, Miljkovic M, Kojic M, Margolles A, Golic N. & Ruas-Madiedo P. (2014). Exopolysaccharide-producing *Bifidobacterium animalis* subsp. lactis strains and their polymers elicit different responses on immune cells from blood and gut associated lymphoid tissue. Anaerobe, 26, 24-30.

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