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 (25°C) 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.

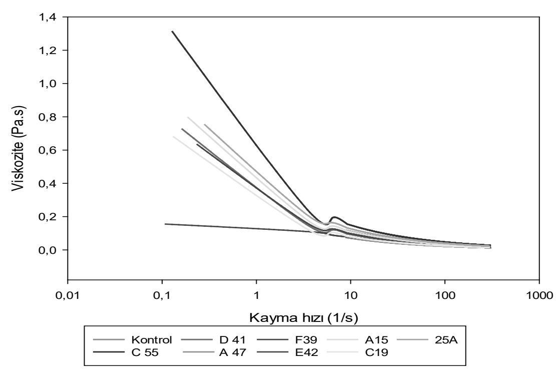

Figure 1: Viscosities of all wheat water samples through different shear rates.

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 heterefomentetive. 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.

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