International Journal of Agriculture, Environment and Food Sciences

JAEFS

e-ISSN : 2618-5946

DOI: 10.31015/jaefs.2021.2.15

www.jaefs.com

Research Article

Int J Agric Environ Food Sci 5 (2):243-249 (2021)

# Effects of capsanthin on surface hydrophobicity and auto-aggregation properties of *Lactobacillus acidophilus* and *Lactobacillus rhamnosus*

**Esra Çelik[1]** **Hasan Ufuk Çelebioğlu[1,*]**

[1]Bartın University, Faculty of Science, Department of Biotechnology, Bartın, Turkey

[*]Corresponding Author: ufukcelebioglu@gmail.com

**Abstract**

Paprika is a one-year culture plant that grows in temperate climates and derives its color from the carotenoid compounds. The basic red color in paprika originates from capsanthin and capsorubin. People must have a healthy gastrointestinal system to maintain a healthy life. Lactic acid bacteria, which constitute the most important group of probiotic microorganisms, are natural members of a healthy intestinal microflora. Main lactic acid bacteria are *Lactobacillus acidophilus* and *Lactobacillus rhamnosus,* which can inhibit pathogenic microorganisms, strengthen immune system, and improve the microbial balance of the gastrointestinal tract. Such bacteria can be modulated by diet constituents, thusw the present study aims to investigate the effects of capsanthin on probiotic bacteria *Lactobacillus acidophilus* LA-5 and *Lactobacillus rhamnosus* GG. For this, different concentrations of capsanthin were added to growth media of probiotic bacteria, and their effects on bacterial growth kinetics, bacterial surface hydrophobicity (Microbial Adhesion to Solvents - MATS Test) and bacterial auto-aggregation were examined. According to the results, capsanthin did not show any negative effects on the growth, while decreased the hydrophobicity of *Lactobacillus rhamnosus* GG dose-dependent manner but increasing the hydrophobicity of *Lactobacillus acidophilus* LA-5. In auto-agregation, changes were observed depending on the dose and time. This study shows carotenoids taken together with the diet can affect beneficial bacteria.

**Keywords:** Auto-aggregation, Capsanthin, Hydrophobicity, Paprika, Probiotics

## Introduction

The intestinal microbiota begins to resemble the gastrointestinal tract microbiota of a young person after the first year of life and is considered to have reached the adult microbiota composition for an average of 3 years. This bacterial group, which is synbiotic with the human body, is defined as a microbiota with the “Human Microbiome Project”. Probiotics are living microorganisms that show beneficial effects on a person’s health and physiology when taken in sufficient quantities, and the most important advantage of probiotics in microbiota is their effect in maintaining the proper balance between the pathogens and bacteria required in the organism (İsmailoğlu and Öngün Yılmaz, 2019).

Probiotics are microorganisms that have positive effects on human health when taken into the body in the required amounts (Hill et al., 2014). Probiotic microorganisms have beneficial effects such as vitamin production, reducing lactose tolerance and calcium absorption, by strengthening the immune system, it has benefits such as reducing the risk of intestinal cancer, regulating the digestive system, inhibiting tumor formation, inhibiting diarrhea formation (Gill and Prasad, 2008). Probiotics are also described as health-promoting bacteria.

Lactic acid bacteria (LAB), which constitute the most important group of probiotic bacteria, are gram-positive, catalase-negative, non-spore, cocci or cocobacilli, aerotolerant microorganisms (Vandenbergh, 1993). *Lactobacillus* species

**Cite this article as:**
Çelik, E., Çelebioğlu, H.U. (2021). Effects of capsanthin on surface hydrophobicity and auto-aggregation properties of Lactobacillus acidophilus and Lactobacillus rhamnosus. Int. J. Agric. Environ. Food Sci., 5(2), 243-249
**Doi:** https://doi.org/10.31015/jaefs.2021.2.15
**Orcid:** Esra Çelik: https://orcid.org/0000-0003-1671-617X and Hasan Ufuk Çelebioğlu: https://orcid.org/0000-0001-7207-2730
**Received:** 24 September 2020 **Accepted:** 16 April 2021 **Published Online:** 28 June 2021
**Year:** 2021 **Volume:** 5 **Issue:** 2 (June) **Pages:** 243-249
**Available online at :** http://www.jaefs.com - http://dergipark.gov.tr/jaefs

such as *Lactobacillus casei*, *L. paracei*, *L. rhamnosus,* and *L. plantarum* are among the most widely used probiotic bacteria used in the production of many dairy and non-fermented food products (O'Toole et al., 2017). *Lactobacillus acidophilus* produce lactic acid, hydrogen peroxide and various bacteriosins (acidolin, acidophilin, lactosidine), which can have antibacterial effects against intestinal pathogens (Sanders and Klaenhammer, 2001). Lactic acid lowers the pH of the environment and creates an unsuitable condition for other bacteria, while hydrogen peroxide plays a role in the antagonistic effect against intestinal pathogens (Sezen, 2013). The use of foods for increasing nutritional value, extending shelf life and controlling intestinal infections has made lactic acid bacteria important in recent years (Soomro et al., 2002).

The importance of nutrition on human health has been understood and accordingly, the concentration on natural antioxidant consumption has increased in developed countries. The main color substance of plants that have therapeutic effect against oxidative pressures, diabetes, Alzheimer, neurological diseases and some types of cancer in the last years as well as attracting the attention of many researchers is the carotenoid pigment (Kadakal et al., 2001). Especially, the relationship between cancer and carotenoid is a factor preventing tumor formation (Dai and Mumper, 2010). The antioxidant and anti-inflammatory effect of carotenods is of great importance in terms of cancer diagnosis and treatment methods (Dai and Mumper, 2010). Pigmentation-providing carotenoids cannot be synthesized by animal tissues, and therefore this molecule is derived from food (Fernández-García et al., 2012). The identified carotenoid substance is over 600 and it has potential health benefits as well as its coloring feature (Fernández-García et al., 2012).

Capsanthin is one of carotenoids from red pepper (Figure 1). 70% of carotenoids in all intense varieties of red intense constitute of capsanthin and capsorubin, while green stuffed peppers do not contain them. The ratio of the capsule in different varieties of red-colored species is between 34-60%. More than 60% of the color is red, and 53.5% of this is due to the capsanthin (Kadakal et al., 2001). The coloring agents in red pepper are rich in provitamin A and C, B1, B2, E vitamins (Demiray and Tülek, 2012).

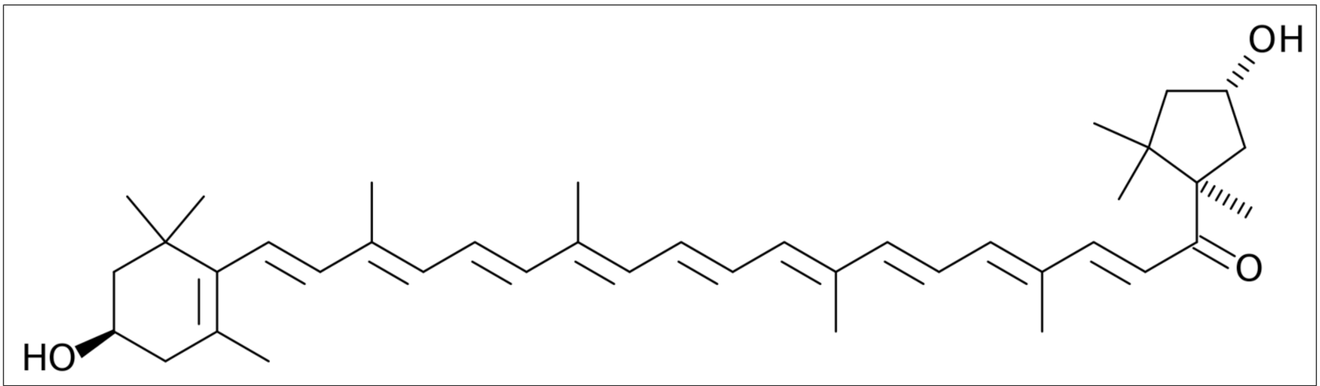


**Figure 1**. Chemical structure of capsanthin.

Previous researches have found that capsanthin is well absorbed by humans, and has antitumor and anticancer effects, as well this compound plays an important role in the prevention of many diseases (Akdoğan et al., 2008). Capsanthin, which has a beneficial effect, was used in this project and we hypothesized that using it together with probiotic bacteria can have a positive effect on health and prevent gastrointestinal diseases.

The aim of the present study is to determine the effects of capsanthin on probiotic properties, surface hydrophobicity and auto-aggregation of lactic acid bacteria, *Lactobacillus acidophilus* LA-5 and *Lactobacillus rhamnosus* GG.

## Materials And Methods

### Growth of probiotic bacteria in the presence of capsanthin

The lactic acid bacteria, *Lactobacillus acidophilus* LA-5 and *Lactobacillus rhamnosus* GG, which are kind gift of Chr. Hansen, Turkey, were grown in de Man, Rogosa, and Sharpe (MRS) medium at 37°C in an aerobic environment without shaking (Celebioglu et al., 2018).

Different cultures from the same bacterial stock were grown at different concentrations of the capsanthin (0, 25, 50, 100, 250, and 500 µg $mL^{-1}$). The control groups were divided into two, Control A and Control B, which have without or with ethanol, respectively. Freeze-dried bacteria were suspended in MRS medium and added to the groups. Capsanthin was added to the treatment groups as 25, 50, 100, 250 and 500 µg $mL^{-1}$. Bacterial growth was measured by allowing the bacteria to grow at 37°C and their densities were recorded every 4 hours for up to 24 hours as McFarland values (Celebi et al., 2020).

### Bacterial Hydrophobicity Test (Microbial Adhesion to Solvents – MATS Test)

Bacterial surface hydrophobicity was measured by microbial adhesion to solvents (MATS) test (Kos et al., 2003; Köroğlu et al., 2019). Bacteria (control and treatment groups) were harvested during the stationary phase (3200xg, 15 minutes), washed with Phosphate-buffered saline (PBS) and resuspended in 0.1 M $KNO_3$ (pH 6.2). $OD_{600}$ of the suspensions were set to 0.5. One mL of xylene (apolar solvent) was added to 3 mL of bacterial suspension and incubated for 10 minutes



Hour

at room temperature. The biphasic system was vortexed for 2 minutes, the aqueous phase was separated and incubated for an additional 20 minutes at room temperature. Bacterial adhesion test to solvents was calculated by measuring absorbance values at 600 nm according to the equation (1).

$$\% \, Adhesion = (1 - A1 \, A0^{-1}) \times 100 \quad (1)$$

where, A1 is the absorbance value measured after incubation, and A0 is the value measured before incubation.

**Bacterial Auto-Aggregation**

Bacterial cells were harvested during the stationary phase (3200xg, 15 minutes), washed and resuspended with PBS to $OD_{600}$ 0.5. Then, 4 mL of bacterial suspensions in test tubes were vortexed for 10 seconds and incubated for 4 hours at room temperature.

Absorbances were measured every hour at the wavelength of 600 nm by adding 0.1 mL of suspension and 0.9 mL of PBS to the tube (Kos et al., 2003; Köroğlu et al., 2019). The results were calculated according to the equation (2).

$$\% \, Auto\text{-}Aggregation = (1 - At \, A0^{-1}) \times 100 \quad (2)$$

where, At is the absorbance value measured after incubation, and A0 is the absorbance value measured at $0^{th}$ hour.

**Statistical Analysis**

All experiments were performed with at least 3 replicates. The results were represented with the mean ± standard deviation and the comparison between the control group and treatment groups was performed with Student's *t*-test. Statistical values of $p<0.05$ were considered as significant.

**Results And Discussion**

**Bacterial Growth Kinetics**

In this study, capsanthin was used at concentrations of 0, 10, 25, 50, and 100 µg mL$^{-1}$. As seen in Figure 2, capsanthin did not show any negative effects on the growth of probiotic bacteria. This shows that capsanthin does not have inhibitory effects against probiotic bacteria.

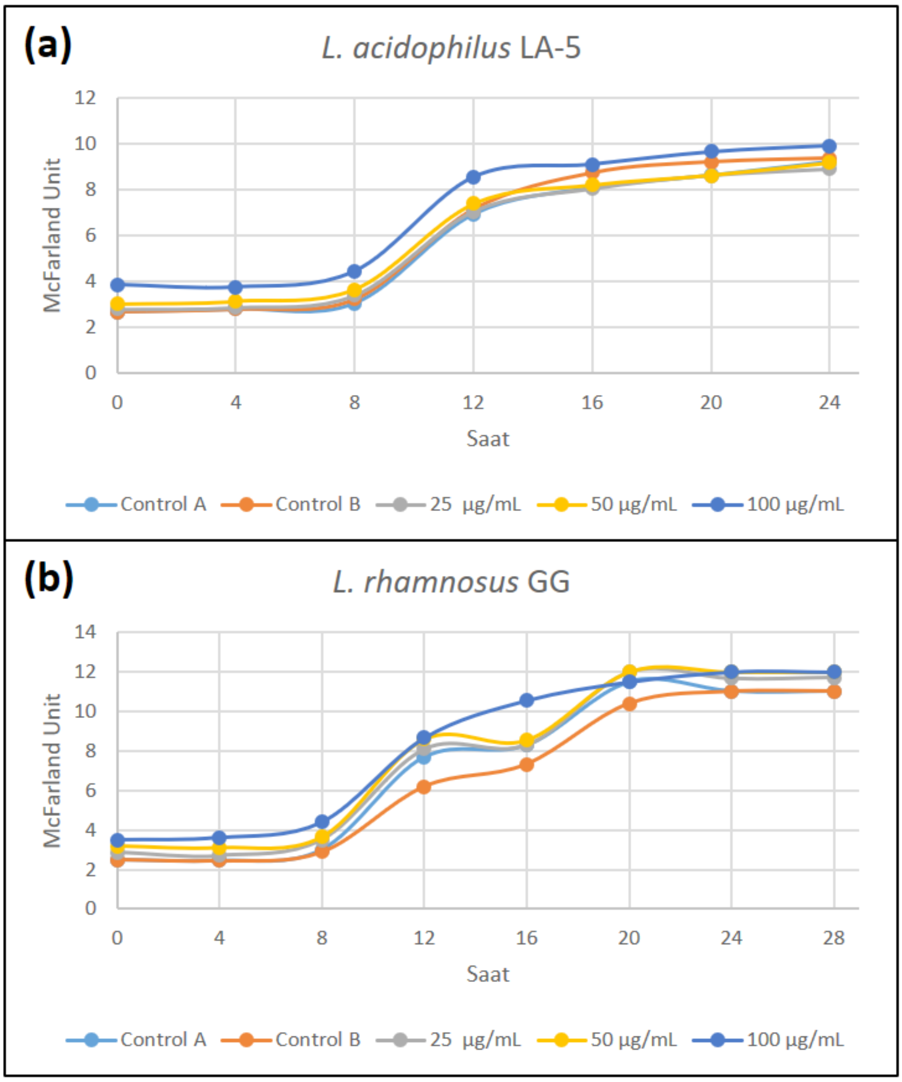


**Figure 2.** Growth kinetics of probiotic bacteria. **(a)** Growth kinetics of *L. acidophilus* LA-5, grown in the presence of 0, 25, 50 and 100 µg mL$^{-1}$capsanthin. **(b)** Growth kinetics of *L. rhamnosus* GG, grown at 0, 25, 50 and 100 µg mL$^{-1}$capsanthin.

**Bacterial Surface Hydrophobicity**

Bacterial surface hydrophobicity is an important feature for keeping bacteria in the gastrointestinal tract (Vadillo-Rodríguez et al., 2005; van Loosdrecht et al., 1987). This feature is one of the factors that can enable probiotic bacteria to better adhere to the mucosa (Krasowska and Sigler, 2014). In the present study, the Control A group does not contain ethanol, while the Control B group contains ethanol as ethanol was used to dissolve capsanthin. As a result of the MATS test, the hydrophobicity of *L. acidophilus* LA-5 was significantly ($p<0.05$) increased by 500 µg $mL^{-1}$of capsanthin, as compared to the control groups (Figure 3a). Depending on the dose, the capsanthin can increase the surface hydrophobicity of *L. acidophilus* LA-5 bacteria, which can better adhere to these mucous membranes in the gastrointestinal tract. In addition, increased cell surface hydrophobicity may allow *L. acidophilus* LA-5 bacteria to better colonize the intestinal tract to demonstrate their probiotic activity. Therefore, as the surface hydrophobicity increases, it also increases the possibility of *L. acidophilus* LA-5, a beneficial bacterium, to be attached to the mucosa.

The hydrophobicity of *L. rhamnosus* GG showed a dose-dependent decrease in the concentrations of 25, 50, 100, 250 and 500 µg $mL^{-1}$ of capsanthin compared to the two control groups (Figure 3b). Surface hydrophobicity of *L. rhamnosus* GG decreased depending on the dose. This means that no change has been made and was unable to change the adhesion of the *L. rhamnosus* GG bacteria treated with the capsanthin to the mucus layer and major components.

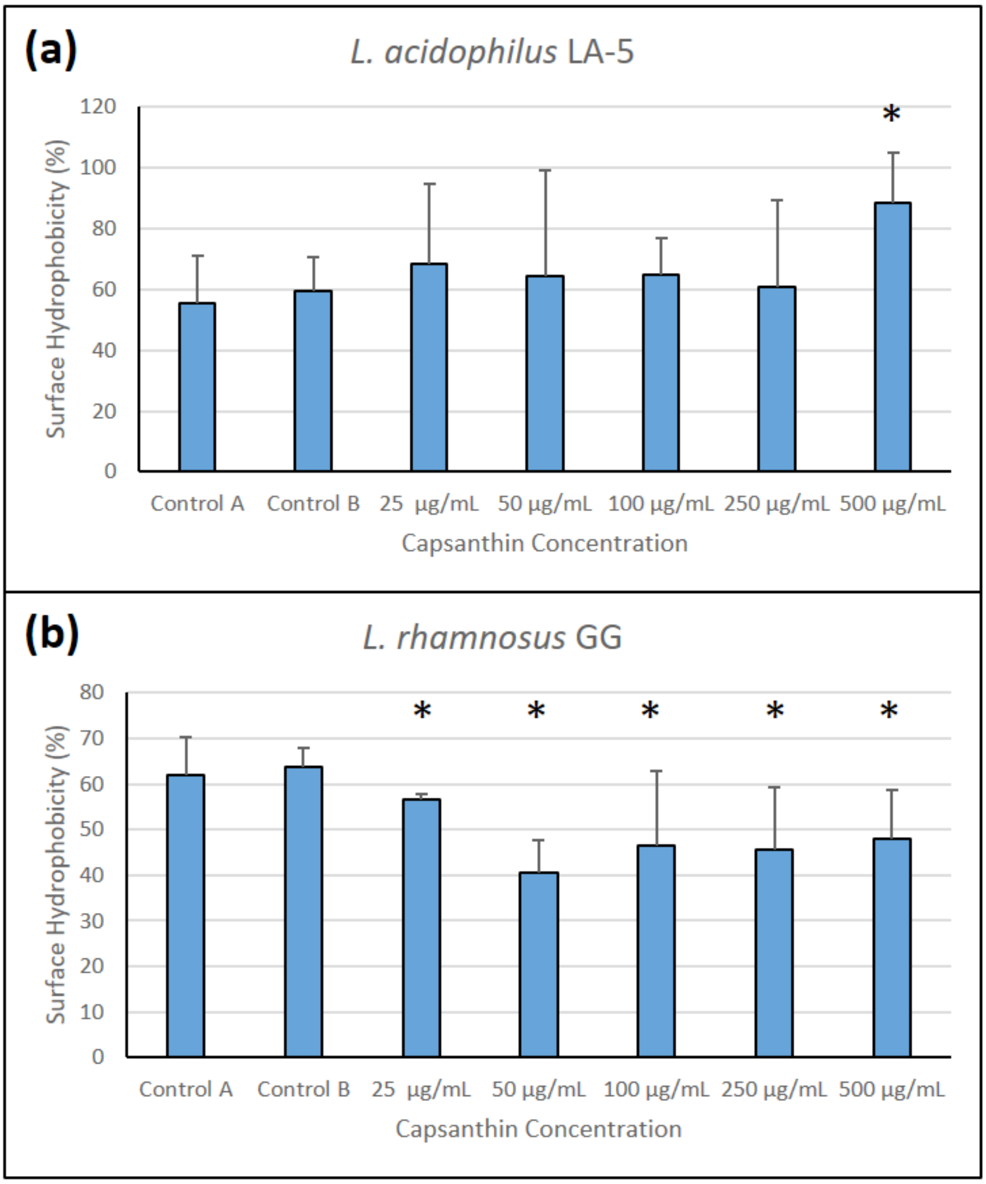


**Figure 3.** Surface hydrophobicity of probiotic bacteria. **(a)** MATS test result of *L. acidophilus* LA-5 treated at 25, 50, 100, 250 and 500 µg $mL^{-1}$ concentrations. **(b)** MATS test result of *L. rhamnosus* GG treated at 25, 50, 100, 250 and 500 µg $mL^{-1}$ concentrations. Asterisks (*) indicate that the difference is statistically significant ($p<0.05$) as compared to Control B (containing ethanol).

With the use of capsanthin, according to the MATS test result, colonization of *Lactobacillus acihophilus* LA-5 bacteria can lead to better adhesion to the intestinal mucosal structure, which plays important roles in the probiotic properties.

The use of capsanthin with other bacterial strain *Lactobacillus rhamnosus* GG did not cause any change in surface hydrophobicity, and this could not affect the adherence of beneficial microorganisms to the intestinal mucosal structure. However, this effect of capsanthin on *Lactobacillus rhamnosus* GG can be investigated by further studies for the intestinal mucosal system. With different studies, the effect of capsanthin *on Lactobacillus rhamnosus* GG at different doses and time can be examined.

**Bacterial auto-aggregation**

Bacterial auto-aggregation is defined as clustering of bacteria by sticking together. In addition, high aggregation can cause better adhesion, however, other factors involved in adhesion to the mucosa are proteins in the surface layer of bacteria, particularly S-layer proteins, extracellular polysaccharides, and lipoteichoic acid (Alp and Kuleaşan, 2019). Bacterial aggregation could be important for the binding of probiotic bacteria to the intestinal mucosa (Kos et al., 2003). High aggregation status can be a better adhesion indicator (Del Re B et al., 2000).

**Table.1.** Auto-aggregation of *Lactobacillus acidophilus* LA-5 and *Lactobacillus rhamnosus* GG. The results are represented as mean value of three independent experiments with standard deviations.

| | 1st hour | 2nd hour | 3rd hour | 4th hour |
|---|---|---|---|---|
| *L. acidophilus* LA-5 | | | | |
| Control A | 47.5 ± 6.9 | 76.5 ± 14.3 | N.D. | N.D. |
| Control B | 44.3 ±19.0 | 93.2 ± 5.4 | N.D. | N.D. |
| 25 µg mL$^{-1}$ | 56.0 ± 13.6 | 93.8 ± 1.6 | N.D. | N.D. |
| 50 µg mL$^{-1}$ | 57.5 ± 27.3 | 92.9 ± 5.5 | N.D. | N.D. |
| 100 µg mL$^{-1}$ | 54.7 ± 20.2 | 91.9 ± 4.4 | N.D. | N.D. |
| 250 µg mL$^{-1}$ | 45.6 ± 23.9 | 87.9 ± 7.8 | N.D. | N.D. |
| 500 µg mL$^{-1}$ | 36.5 ± 27.0 | 78.8 ± 3.1 | N.D. | N.D. |
| *L. rhamnosus* GG | | | | |
| Control A | N.D. | 4.9 ± 0.4 | 46.1 ± 35.6 | 21.9 ± 10.9 |
| Control B | N.D. | 22.3 ± 7.2 | 50.7 ± 20.6 | 44.5 ± 8.7 |
| 25 µg mL$^{-1}$ | N.D. | 12.8 ± 5.0 | 13.2 ± 11.0 | 24.2 ± 9.8 |
| 50 µg mL$^{-1}$ | N.D. | 14.9 ± 3.4 | 24.0 ± 13.8 | 27.5 ± 10.6 |
| 100 µg mL$^{-1}$ | N.D. | 26.5 ±1.1 | 34.9 ± 15.2 | 22.9 ± 7.9 |
| 250 µg mL$^{-1}$ | N.D. | 16.9 ± 9.6 | 26.7 ± 9.8 | 20.9 ± 6.7 |
| 500 µg mL$^{-1}$ | N.D. | 22.9 ± 6.4 | 25.3 ± 14.0 | 46.7 ± 9.3 |

N.D.: Not determined

Bacterial auto-aggregation test results of *L. acidophilus* LA-5 and *L. rhamnosus* GG are given in Table 1. The results showed that bacterial auto-aggregation of *L. acidophilus* LA-5 showed in 25, 50 and 100 µg mL$^{-1}$ capsanthin concentrations are higher than Control A group at 1st hour. In the 2nd hour, concentrations of 25, 50, 100 and 250 µg mL$^{-1}$ of capsanthin concentrations increased the auto-aggregation compared to the Control groups. Changes in auto-aggregation of *L. rhamnosus* GG bacteria depending on time and dose were observed.

The results showed that the auto-aggregation values were increased in 3 different concentrations in growth of *L. acidophilus* LA-5. However, high concentrations of capsanthin showed a reduction in auto-aggregation of *Lactobacillus acidophilus* LA-5. These bacteria, treated with capsanthin, can aggregate and this can provide better binding to the intestinal mucosa. *Lactobacillus acidophilus* LA-5, which adhere better to the intestinal mucosal structure, can have a health-promoting effect when used with capsanthin. On the other hand, when capsanthin was added to the growth of *Lactobacillus rhamnosus* GG, the other probiotic bacterial strain, auto-aggregation values were not significantly altered. However, detailed research can be performed on the adhesion of *Lactobacillus rhamnosus* GG bacteria by treatment with capsanthin.

Adhesion seems very crucial for especially probiotic bacteria, as high attachment of the bacteria to the mucosa could mean higher biological activities, such as higher colonization, inhibition of pathogen binding, and better modulation of immune system (Alp and Kuleaşan, 2019; Bermúdez-Brito et al., 2012). Even though S-layer proteins are very important in adhesion of probiotic bacteria, non-selective interactions on the surface such as hydrophobicity and also auto-aggregation could play roles in adhesion (Alp et al., 2020; Kos et al., 2003). Thus, in the present study, we showed that these properties of the probiotic bacteria could be modulated by a dietary carotenoid, capsanthin.

**Conclusion**

The present study investigates the effects of capsanthin, one of the major carotenoids present in paprika, on some probiotic properties of lactic acid bacteria *lactobacillus acidophilus*

LA-5 and *lactobacillus rhamnosus* GG. The results indicate that this carotenoid may alter the properties of probiotic bacteria so that they can prolong their resistance in the gastrointestinal system. Further studies can give insight into more details how capsanthin can alter the adhesion properties of probiotic bacteria to the mucus, as well as to the intestinal cells; furthermore, how capsanthin can be metabolized by these bacteria.

Capsanthin, a natural antioxidant compound found in red pepper, will shed light on many research and studies as a result of this project. Today, due to the spread of diseases, healthy nutrition and strengthening the immune system have gained importance. Thus, the red pepper component capsanthin, which is taken in the diet, can be combined with beneficial microorganisms in the intestines and have more effects on the immune system. It can inhibit the growth of pathogenic microorganisms by strengthening the gastrointestinal system. Capsanthin and probiotic bacterial strains together can exert more beneficial effects on the host. This study can assist in more extensive studies to investigate the health effects of taking probiotic bacteria with carotenoid compounds.

**Compliance with Ethical Standards**

**Conflict of interest**

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

**Author contribution**

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

**Ethical approval**

Not applicable.

**Funding**

This study was supported by TÜBİTAK 2209-A University Students Research Projects Support Program (1919B011900220).

**Data availability**

Not applicable.

**Consent for publication**

Not applicable.

**Acknowledgement**

The authors thank to Chr. Hansen, Turkey for supplying the probiotic strains.

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