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IMPACT OF LACTICASEIBACILLUS PARACASEI SUBSP. TOLERANS, LEVILACTOBACILLUS PARABREVIS AND LATILACTOBACILLUS CURVATUS STRAINS ON TEXTURE, RHEOLOGY AND MICROSTRUCTURE OF DAIRY-BASED FERMENTED PRODUCT

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Keywords

Lacticaseibacillus Paracasei Subsp. Tolerans,
Levilactobacillus Parabrevis,
Latilactobacillus Curvatus,
Texture,
Rheology,
Microstructure.

Abstract

Functional microorganisms and/or their metabolites, as well as the health advantages of foods, their texture, structure and sensory quality are also very important factors that can affect consumer decisions. There are scientific studies on these properties, however, in order to achieve the best function of strain, it firstly need to find the specific strains. This study was aimed to reveal the effect of the *L. paracasei subsp. tolerans*, *L. parabrevis*, and *L. curvatus* isolates, used as co-culture, on the textural property, rheological property, and micro-structure of yogurt. According to the results, it was observed that the *L. paracasei subsp. tolerans* NOC-122 strain increases the water holding capacity and transforms the product into a more fluid form. On the other hand, the *L. parabrevis* NOC-111, and the *L. curvatus* NOC-110 strains caused higher viscosity values. As for texture profiles, the sample coagulated with NOC-122 isolate was softer than those coagulated by other strains. The samples coagulated with the NOC-122 and NOC-110 isolates showed lower chewiness than that coagulated by other strain. Gumminess and springiness showed a similar variable trend to that of chewiness. These results showed the effects of the biotechnological similarities of the strains on the texture, rheology and microstructure.

LACTICASEIBACILLUS PARACASEI SUBSP. TOLERANS, LEVILACTOBACILLUS PARABREVIS VE LATILACTOBACILLUS CURVATUS SUŞLARININ SÜT TEMELLİ FERMENTE ÜRÜNLERDE TEKSTÜR, REOLOJİ VE MİKROYAPI ÜZERİNE ETKİLERİ

Anahtar Kelimeler

Lacticaseibacillus Paracasei Subsp. Tolerans,
Levilactobacillus Parabrevis,
Latilactobacillus Curvatus,
Tekstür,
Reoloji,
Mikroyapı.

Öz

Fonksiyonel mikroorganizmalar ve/veya onların metabolitleri, gıdaların sağlık açısından avantajlarını, tekstürel özelliklerini ve duyusal kalitelerini tüketici açısından etkileyebilecek çok önemli faktörlerdir. Bu özelliklerle ilgili bilimsel çalışmalar vardır, ancak en iyi fonksiyonunu elde etmek için öncelikle spesifik suşların araştırılması gerekir. Bu çalışma, Laticaseibacillus paracasei subsp. tolerans, *Levilactobacillus parabrevis* ve *Latilactobacillus curvatus* türlerine ait izolatların yoğurdun tekstürel ve reolojik özellikleri ve ilaven mikro yapısı üzerine etkilerinin ortaya konulması amaçlanmıştır. Sonuçlara göre *L. paracasei subsp.* toleranslı NOC-122 su tutma kapasitesini arttırmış ve ürünü daha akışkan hale dönüştürmüştür. *L. parabrevis* NOC-111 ve *L. curvatus* NOC-110 suşları ise daha yüksek viskoziteye neden olmuştur. Tekstür özelliklerinde, NOC-122 suşu ile pıhtılaştırılan örnek, diğerlerinden daha yumuşaktı. NOC-122 ve NOC-110 suşları ile pıhtılaştırılan numuneler, diğer suş tarafından pıhtılaştırılandan daha düşük çiğneme gösterdi. Yapışkanlık ve esneklik, çiğneme eğilimine benzer bir değişken eğilim göstermiştir. Bu sonuçlar, söz konusu suşların yoğurttaki biyoteknolojik olarak benzerliklerinin tekstür, reoloji ve mikroyapısı üzerindeki etkilerini göstermiştir.

Alıntı / Cite

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IMPACT OF LACTICASEIBACILLUS PARACASEI SUBSP. TOLERANS, LEVILACTOBACILLUS PARABREVIS AND LATILACTOBACILLUS CURVATUS STRAINS ON TEXTURE, RHEOLOGY AND MICROSTRUCTURE OF DAIRY-BASED FERMENTED PRODUCT

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Highlights

- *L. paracasei subsp. tolerans* NOC-122 strain increases the water holding capacity
- *L. parabrevis* NOC-111, and *L. curvatus* NOC-110 strains causes high viscosity values
- *L. paracasei subsp. tolerans* NOC-122 and *L. curvatus* NOC-110 isolates show low chewiness

Graphical Abstract

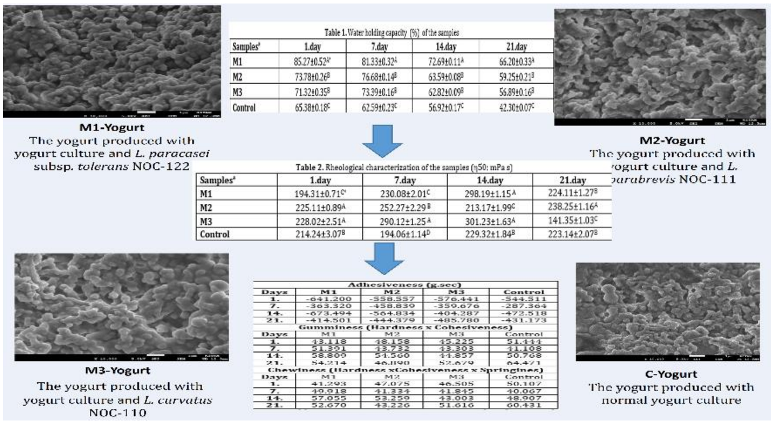

Figure. Graphical Abstract

Purpose and Scope

This study was aimed to reveal the effect of the *L. paracasei subsp. tolerans*, *L. parabrevis*, and *L. curvatus* isolates, used as co-culture, on the textural property, rheological property, and micro-structure of yogurt.

Design/methodology/approach

In this study, the mentioned strains were used individually as co-cultures. The effect of the strains on the texture, microstructure and sensory properties of products such as yoghurt has been demonstrated.

Findings

These results showed the effects of the mentioned strains on the texture, rheology and microstructure of biotechnologically similar samples to yogurt.

Research limitations/implications

Although the individual examination of the strains mentioned in this study on a dairy product has improved the use of the strains, it is quite difficult to relate their biochemical pathways and the primary and secondary metabolite products produced.

Practical implications

This study revealed that functional lactic acid bacteria strains can be used in product development in practice. In addition, it has shown that solutions to sectoral problems can be found with functional strains instead of additives.

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Social Implications
With this study, a natural way to be used instead of additives in dairy products (especially yogurt, etc.) is proposed. This is a safe development in terms of public health.

Originality
There are scientific studies on these properties, however, in order to achieve the best function of a functional strain, it firstly need to find the specific strains. This reveals the originality of the article in co-culture examination of the mentioned strains for these characteristics.

1. Introduction

Functional microorganisms is an important component of many systems such as food products. It is important to lay a solid theoretical and scientific foundation for understanding the behaviors and functions of these microorganisms and utilizating and expansing of microbial resources (Liu et al., 2021). Functional microorganisms belong to the lactic acid bacteria (LAB) group, which is mostly isolated from fermented foods in terms of foods (Florou-Paneri et al., 2013). The ability of LAB to produce various metabolites such as lactic acid, acetic acid, ethanol, aroma compounds, bacteriocins, fungicides, antioxidants, antibiotic agents, vitamins, bioactive peptides, exopolysaccharides or enzymes, and additionally to have probiotic culture and/or starter culture properties, are important factors that add functionality (Raj et al., 2022). There are scientific studies on these properties, however, in order to achieve the best function of strain, it firstly need to find the specific strains.

Recently, the researchers who studying the functional properties of microorganisms, have focused more to the LAB and/or their metabolites that are responsible for maintaining or improving the textural structure, water-holding capacity, rheological properties of the product (Sharma et al., 2021). In a study (Yan et al., 2020), it was determined a significant difference in the textural characteristics of tofu bricks coagulated with different strains. Lactobacillus delbrueckii L1 strain caused tofu hardness. Also, this strain caused the highest level of brittleness, but lowest level of springiness and chewiness. In another study (Olojede et al., 2020), Pediococcus pentosaceus LD7 fermented sourdough had the highest storage modulus (G′) (3.07Pa) and loss modulus (G′′) (6.23Pa). This strain, exhibited the most significant effect on the rheological properties of the sourdough. Besides, in a study, also (Zhang et al., 2014), Streptococcus thermophilus showed significantly higher value than that of *Lactobacillus delbrueckii subsp. bulgaricus* in all level of texture measurements.

The effect of LABs on the rheological properties and textural structures of foods is related to the properties of some LAB species, such as their ability to secrete extracellular polysaccharides (EPS), their lipolytic activities, proteolytic activities, and their ability to degrade gluten and/or starch (Zhang et al., 2014, Walter et al., 2014; Goswami et al. 2019; Ahsan et al., 2021).

In this study, the texture, rheology and micro-structures of the samples produced in the same way, which had been not analyzed in the mentioned study, were examined. It was aimed to reveal the effects of *Lacticaseibacillus paracasei subsp. tolerans* NOC-122, *Levilactobacillus parabrevis* NOC-111, and *Latilactobacillus curvatus* NOC-110 isolates on these properties.

2. Material and Method

In a previous project by us (Ozdemir, 2019), three LAB strains; the *Lacticaseibacillus paracasei subsp. tolerans* NOC-122, *Levilactobacillus parabrevis* NOC-111, and *Latilactobacillus curvatus* NOC-110 strains, isolated from artisanal goat-tulum chees samples, and subsequently identified were used as co-cultures in dairy-based products. In this study, three set type yogurt samples, in which the above-mentioned LAB strains; the NOC-122, NOC-111, and NOC-110 strains, were used as material. The sample produced using only yogurt culture (it contain *Lactobacillus delbrueckii subsp. bulgaricus* ve Streptococcus thermophilus strains and obtained from Chr. Hansen, Charlottenlund, Denmark) was considered as a control. The yogurt sample were produced both using the yogurt culture, and the selected isolates as co-culture. Cow's milk whose dry matter was adjusted to 15% with skim-milk was homogenized for 5 min., and then heat treatment was applied at 95^{o}C for 15 min. The yogurt culture (2%) and active cultures of the aforementioned isolates (10^8 log CFU/mL in product) were added to the milk cooled to inoculation temperature (42^{o}C). After inoculation (at 42^{o}C up to about pH 4.6), they were stored at +4^{o}C. The *Lacticaseibacillus paracasei subsp. tolerans* NOC-122 for M1 sample, the *Levilactobacillus parabrevis* NOC-111 for M2 sample, and the *Latilactobacillus curvatus* NOC-110 isolate for M3 sample were used as co-cultures.

2.1. Water-Holding Capacity Analysis

Briefly, 15 g of fermented sample was centrifuged at 3300 x g, 4 °C for 10 min. The whey expelled (WE) was removed and weighed. All experiments were repeated three times. The WHC expressed in % was defined as:WHC (%) = (a1/a2) x100 .where a1 is the mass of precipitate after centrifugation (g) and a2 is the mass of the sample (g) (Doleyres et al., 2005).

2.2. Rheology and Texture Analysis

Viscosity values of the samples (100 g) will be determined by using the Brookfield Rotational Rheometer (DV-II Pro LV, USA) device at 4 °C 100 rpm with spindle DV-4. Viscosity value will be recorded at 30 seconds. Care will be taken to preserve the torque value in the range of 10%-100% (Ozdemir et al., 2015).

Texture Analyzer (TA-XT plus, Stable Micro Systems) was used for the texture analysis of the samples. The A P/1.5 probe was used to determine the texture profile of the samples. 50 g of sample (+4°C) was taken into 100 mL containers and compressed 20mm with a disc probe. The probe was applied at a speed of 1 $mm.s^{-1}$. Parameters giving information about the textural properties of the samples were obtained from the force-time graphs using Nexygen 2.0 (Lloyd, UK) software (Shiby and Mishra, 2008; Özçelik et al., 2022).

2.3. Scanning Electron Microscopy (SEM) Analysis

Briefly, 10 g of sample was freeze dried. Then, powder samples with a maximum of 1 cm^3 particles were placed on a 1 cm^2 aluminum sample holder. The samples were coated with a 15 nm gold palladium layer with a cathodic coater, followed by observation in a scanning electron microscope (SEM, FEI, Quanta 600 F, USA) operating at 10 kV voltage. Photomicrographs were recorded under magnification of 100 to 5000 X. Structural differences in magnified images will be evaluated (Prasanna et al. 2013; Pang et al., 2008).

2.4. Statistical Analysis

Results are given as mean values±standard error (SE). Variability was determined by whether the means within groups (within-groups variabilty) and between group variables (between- groups variability) were different, and statistical significance was analyzed by Anova using SPSS (Statistics 22 software, IBM, USA) program. The Duncan multiple comparison test was used for comparisons and results with a significance level of $P<0.05$ were indicated as significant.

3. Result and Discussion

3.1. Effect of the L. paracasei subsp. tolerans NOC-122, L. parabrevis NOC-111, and L. curvatus NOC-110 Strains on Water Holding Capacity

Water holding capacity is an important concept for milk-based samples. Observation of phase separation in samples creates a negative effect for consumers. When the water holding capacity of the analyzed samples was examined, it was determined that the highest capacity belonged to the M1 sample (Table 1). This was due to the ability of NOC-122 isolate to produce EPS (not shown in this study). It is considered that it is quite appropriate to use *L. paracasei subsp. tolerans* NOC-122 isolate as an auxiliary culture in the production of milk-based fermented products that best preserve their own structure. In a study, soy protein gel acidified with *Lactobacillus casei* showed the high water holding capacity with 84.58% than glucono-δ-lactone and organic acid (Yang et al., 2021). As given Table 1, The NOC-122 strain showed a value with 85.27% which close to this strain. In other study, it was investigated that effect of EPS, produced by L. delbrueckii ssp. bulgaricus, on the texture and microstructure of buffalo yogurt

Table 1. Water holding capacity (%) of the samples

Samples*	1.day	7.day	14.day	21.day
M1	$85.27±0.52^{A*}$	$81.33±0.32^{A}$	$72.69±0.11^{A}$	$66.20±0.33^{A}$
M2	$73.78±0.26^{B}$	$76.68±0.14^{B}$	$63.59±0.08^{B}$	$59.25±0.21^{B}$
M3	$71.32±0.35^{B}$	$73.39±0.16^{B}$	$62.82±0.09^{B}$	$56.89±0.16^{B}$
Control	$65.38±0.18^{C}$	$62.59±0.23^{C}$	$56.92±0.17^{C}$	$42.30±0.07^{C}$

*M1: It was produced using isolate the *L. paracasei subsp. tolerans* NOC-122 in addition to yogurt culture. M2: It was produced using isolate the *L. parabrevis* NOC-111 in addition to yogurt culture. M3: It was produced using isolate the *L. curvatus* NOC-110 in addition to yogurt culture. A-D: values followed by the different letters (A, B, C, D, E) are significantly different by Duncan's multiple range test in each column for each day (P< 0.05).

3.2. Effect of the L. paracasei subsp. tolerans NOC-122, L. parabrevis NOC-111, and L. curvatus NOC-110 Strains on Rheological Property

Although the microstructural and rheological properties of the yogurt gel are dependent on the protein concentration and the enthalpic/entropic balance of the interprotein bonds, the size and shape of the protein particles and the distribution of the protein attachment points in the gel are also of great importance. By rheological definition, yogurt is a non-Newtonian viscoelastic liquid and shows a flowing property with time. Yogurt gel, which contains viscous and elastic characters together, shows a typical weak viscoelastic gel structure. To characterize the physical properties of a viscoelastic gel, both viscous and elastic characteristics must be measured. According to these results, the effect of the viscosity of the *L. parabrevis* NOC-111 and *L. curvatus* NOC-110 strains were higher than the other (Table 2). Some studies were determined that the viscosity of the yogurts decreased with increasing EPS (Yang et al., 2014). Also, the proteolytic activities of LAB strains is important in their viscosity efficiency. In a study, it was determined that L. delbrueckii subsp. bulgaricus 2501 and 2515 strains which have proteolytic activity decreased the viscosity of yogurt (Shihata and Shah, 2002). In other study, L. delbrueckii SB25 strains with higher proteolytic activities provide more secondary and superficial viscosities (Zhang 2014). Viscosity at shear rate of 50 s^{-1} has been suggested to have a good correlation with perceived thickness, stickiness and sliminess for a wide range of food products from Newtonian fluid to thick emulsion (Nguyen et al., 2017).

Table 2. Rheological characterization of the samples (η50; mPa s)

Samples*	1.day	7.day	14.day	21.day
M1	$194.31±0.71^{C*}$	$230.08±2.01^{C}$	$298.19±1.15^{A}$	$224.11±1.27^{B}$
M2	$225.11±0.89^{A}$	$252.27±2.29^{B}$	$213.17±1.99^{C}$	$238.25±1.16^{A}$
M3	$228.02±2.51^{A}$	$290.12±1.25^{A}$	$301.23±1.63^{A}$	$141.35±1.03^{C}$
Control	$214.24±3.07^{B}$	$194.06±1.14^{D}$	$229.32±1.84^{B}$	$223.14±2.07^{B}$

*M1: It was produced using isolate the *L. paracasei subsp. tolerans* NOC-122 in addition to yogurt culture. M2: It was produced using isolate the *L. parabrevis* NOC-111 in addition to yogurt culture. M3: It was produced using isolate the *L. curvatus* NOC-110 in addition to yogurt culture. A-D: values followed by the different letters (A, B, C, D, E) are significantly different by Duncan's multiple range test in each column for each day (P< 0.05).

3.3. Effect of the L. paracasei subsp. tolerans NOC-122, L. parabrevis NOC-111, and L. curvatus NOC-110 Strains on Textural Property

In the present study, the texture profile analysis of the samples showed a significant difference between the hardness at the first and second compression as well as the area, adhesivenes, chewiness, and gumminess of the samples (P<0.05) (Table 3). Gel hardness is likely associated with the acidification rate: hardness decreased with increasing acidification rate, because less time was available for the formation and arrangement of bonds between the proteins (Yang et al., 2021). This was associated with the NOC-122 strain having a fast milk coagulation rate and having the lowest hardness value. Low firmness (hardness) could result from the poor curd or high proteolysis, of yogurt. Also, the high molecular mass EPS, like the NOC-122 strain, have (in terms of water binding) the ability to decrease syneresis and strengthen the hardness of the casein network by interacting with other milk constituents (Yang et al., 2014).

Cohesiveness, an indicator of the strength of the internal gel bonds, the results suggested that gels were generally weak. In a study (Olojede et al., 2020), Pediococcus pentosaceus SA, Weissella confusa SD, and P. pentosaceus LD starter cultures, increased the cohesiveness, springiness, gumminess and chewiness of the breads.In the present study, the sample coagulated with strain NOC-122 was softer than those coagulated by other strains. The samples coagulated with strains NOC-122 and NOC-110 showed lower chewiness than that coagulated by other strain. Gumminess and springiness showed a similar variable trend to that of chewines.

Table 3. Instrumental texture profile (TPA) of the sample fermented with the *L. paracasei subsp. tolerans*, *L. parabrevis*, and *L. curvatus* strains

Samples*	**Hardness (g)**					**Adhesiveness (g.sec)**				
Days	**M1**	**M2**	**M3**	**Control**		**Days**	**M1**	**M2**	**M3**	**Control**
1.	114.668	122.03	129.947	135.226		**1.**	-641.200	-558.557	-576.441	-544.511
7.	139.463	110.084	117.168	114.182		**7.**	-363.320	-458.839	-359.676	-287.364
14.	149.186	155.298	124.738	133.073		**14.**	-673.494	-564.834	-404.287	-472.518
21.	146.894	134.74	144.185	166.966		**21.**	-414.501	-444.379	-485.780	-431.173
Cohesiveness						**Gumminess (Hardness x Cohesiveness)**				
Days	M1	M2	M3	Control		**Days**	M1	M2	M3	Control
1.	0.376	0.395	0.371	0.380		**1.**	43.118	48.158	45.225	51.444
7.	0.368	0.397	0.370	0.360		**7.**	51.391	43.732	43.303	41.108
14.	0.394	0.351	0.360	0.382		**14.**	58.809	54.560	44.857	50.768
21.	0.369	0.348	0.365	0.386		**21.**	54.214	46.890	52.679	64.471
Springiness						**Chewiness (Hardness xCohesiveness x Springines)**				
Days	M1	M2	M3	Control		**Days**	M1	M2	M3	Control
1.	0.958	0.978	0.964	0.974		**1.**	41.293	47.075	46.505	50.107
7.	0.971	0.945	0.966	0.975		**7.**	49.918	41.334	41.845	40.067
14.	0.970	0.976	0.959	0.963		**14.**	57.055	53.259	43.003	48.907
21.	0.972	0.922	0.980	0.937		**21.**	52.670	43.226	51.616	60.431

*M1: It was produced using isolate the *L. paracasei subsp. tolerans* NOC-122 in addition to yogurt culture. M2: It was produced using isolate the *L. parabrevis* NOC-111 in addition to yogurt culture. M3: It was produced using isolate the *L. curvatus* NOC-110 in addition to yogurt culture

3.4. Effect of the L. paracasei subsp. tolerans NOC-122, L. parabrevis NOC-111, and L. curvatus NOC-110 Strains on Microstructure

SEM micrographs (x 10.000) of pre-prepared yogurt samples are shown in Figure 1. Micrographs show differences in gel structures such as the compactness of the three-dimensional casein micelles and the network of pore sizes. Significant differences are observed between samples. Larger pores and less cross-linking between casein micelles explain the reduced stiffness. Like this; a L. paracasei subsp. tolerance isolate reduces the stress in the yogurt. More densely packed casein networks in micrographs of stored yogurts can be seen in yogurt samples at day 21 compared to day 1, supporting structural rearrangements during storage, resulting in higher depreciation, shear stress, and storage modulus at the end of storage. In a study, it was determined that the average particle size of soy protein gel acidified with a *Lactobacillus casei* isolate was the largest (Yang et al., 2021).

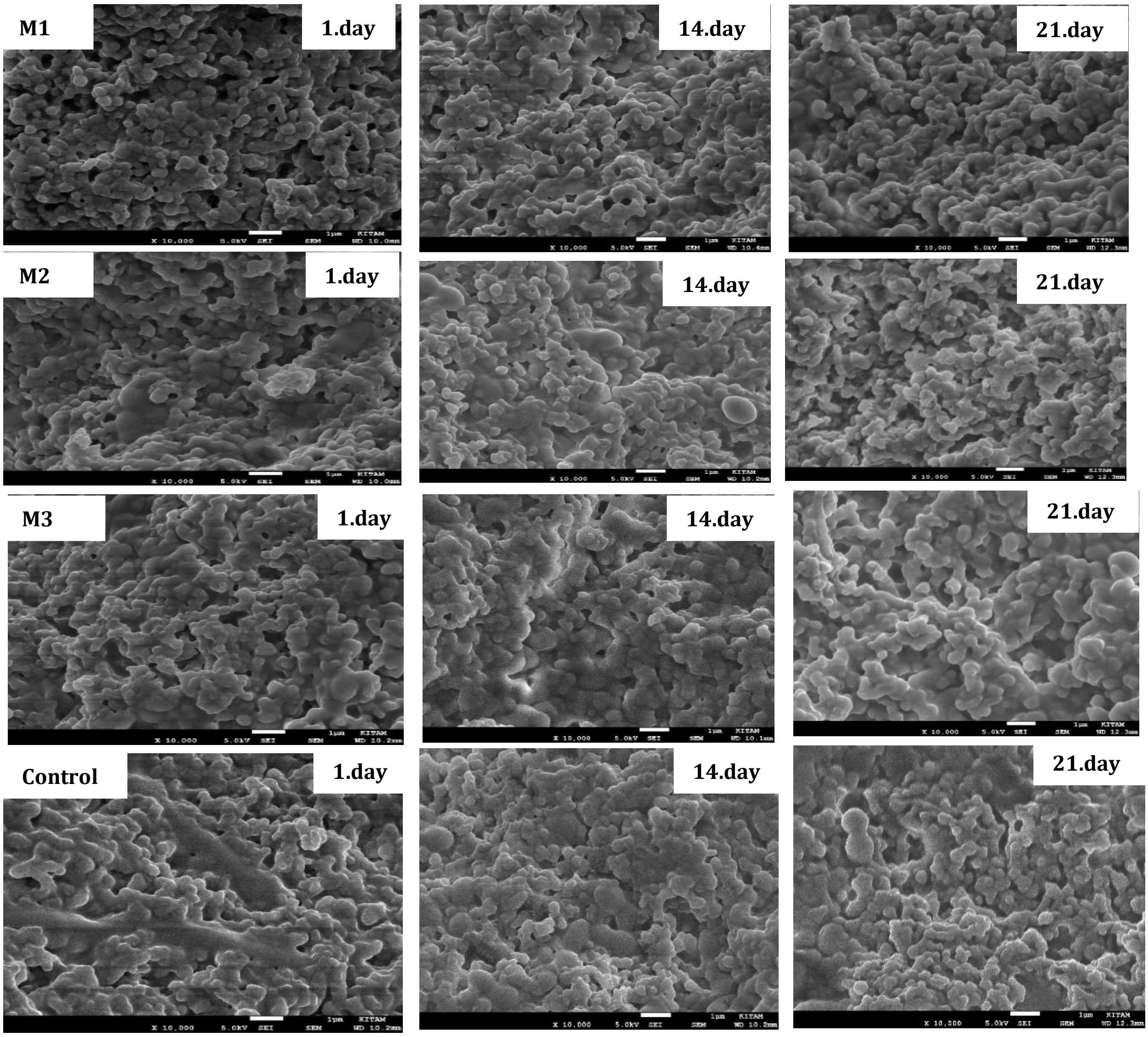

Figure 1. SEM image of samples (10.000X)

*M1: It was produced using isolate the *L. paracasei subsp. tolerans* NOC-122 in addition to yogurt culture. M2: It was produced using isolate the *L. parabrevis* NOC-111 in addition to yogurt culture. M3: It was produced using isolate the *L. curvatus* NOC-110 in addition to yogurt culture

4. Conclusion

In this study, it was observed that the *L. paracasei subsp. tolerans* NOC-122 strain increases the water holding capacity and transforms the product into a more fluid form. On the other hand, the *L. parabrevis* NOC-111, and *L. curvatus* NOC-110 strains caused higher viscosity values. As for texture profiles, the sample coagulated with the NOC-122 strain was softer than those coagulated by other strains. The samples coagulated with the NOC-122 and NOC-110 strains showed lower chewiness than that coagulated by other strain. Gumminess and springiness showed a similar variable trend to that of chewiness. These results showed the effects of the mentioned strains on the texture, rheology and microstructure of biotechnologically similar samples in yogurt.

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Conflict of Interest

No conflict of interest was declared by the authors.

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