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Effect of Activated Carbon in Yogurt Production

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Abstract

Yogurt has been one of the leading consumption products of fermented milk products for centuries and has many positive effects in terms of human health. However, yogurt consumption is sometimes a problem for individuals with lactose intolerance. It is known that activated carbon ensures the removal of heavy metals from the body by adsorbing, and slows down the growth of Escherichia coli and Staphylococcus aureus. In this study, the effects of activated carbon on the formation of yogurt were investigated. In this study, before investigating the effects of activated carbon on milk fermentation, its physical properties were determined by electron microscopy. Lactose and calcium interactions were determined in silico studies of activated carbon on yogurt. Yogurt with added activated carbon was created and protein, fat, lactic acid, pH, calcium, sensory analyzes, and microbiological parameters were determined in groups on different days. In the study, it was determined that the use of activated carbon during milk fermentation did not impair the physical, chemical, sensory and microbiological structural properties of yogurt.

Keywords:
Yogurt, activated carbon, microbiological study, in silico
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Introduction

People need food as long as they live, and food is an integral part of human life. With the increasing population in the world, including global climate changes, the demand for a sufficient amount of clean and safe food is increasing day by day. In the meta-analyses, it is expected that the global food demand will increase by 30-50% by 2050 (van Dijk et al., 2021). In addition to these, the variety of foods consumed is increasing and increasing food intolerance and allergies present a big problem (Peters et al., 2021).

One of the most common causes of food intolerance is lactose intolerance, which occurs after the use of milk and dairy products. Lactose found in milk and dairy products is digested in the small intestine and converted into glucose and galactose. However, if no lactase catalyzes this conversion, or if it is in small amounts, lactose that passes into the large intestine creates a food source for microorganisms and leads to the formation of fatty acids and gases (such as hydrogen, carbon dioxide, and methane) by microorganisms (Delacour et al., 2017). These fatty acids and gases cause abdominal bloating, pain, and eventually diarrhea in people with lactose intolerance. While most newborns have high concentrations of lactase, its concentrations decrease after weaning (Casellas et al., 2009). This occurs to varying degrees in different populations and can cause lactose intolerance.

The consumption of milk and dairy products in the world is quite high. The use of yogurt in dairy products is increasing day by day. Scientific studies on yogurt are also increasing each day. Today, yogurt has found its rightful place in healthy nutrition recommendations due to its positive contributions to human health (Aryana & Olson, 2017). In the formation of yogurt, after milk pasteurization, inoculation with *Lactobacillus bulgaricus* and *Streptococcus thermofilus* bacteria is performed at a certain temperature. Then, it is incubated at a certain temperature until it reaches the appropriate pH (Kosikowski & Mistry, 1999). The desired pH is also taken to the +4 °C storage warehouse. Yogurt is consumed plain, as well as yogurts with additives. While many fruits such as strawberries, bananas, and peaches are used as additives, sweeteners such as honey are also used (Janiaski et al., 2016).

Activated carbon has been known and widely used since the early 1900s. Activated carbon is the most widely used adsorbent due to its exceptionally high porosity and high adsorption capacity (Fierro et al., 2008; Shahraki et al., 2014). Activated carbon attracts a lot of attention for its use in many branches of science with this potential adsorbent feature (Jaroniec & Choma, 1986; Morgan & Fink, 1989). From the moment it was first discovered, it has become indispensable, especially for treatment processes. For this purpose, it is widely included in our lives in many areas including water treatment plants, the food, and the pharmaceutical industry. In the health field, it is mostly used to benefit from the adsorbent feature in poisoning (Neuvonen & Olkkola, 1988; Bonilla-Velez & Marin-Cuero, 2017). For these purposes, it is used orally at a dose of 10-25 g in children and 1 g/kg in adults, depending on age. Today, activated carbon particles are injected into the tissue in cancer surgery. These applications further increase the confidence in activated carbon (Huang et al., 2004; Du et al., 2016).

When determining the quality and properties of activated carbons, it is not sufficient to classify only by looking at the surface characteristics (Marsh & Rodriguez-Reinonso, 2006; Menéndez-Díaz & Martín-Gullón, 2006; Akyıldız, 2007).

As the size of the adsorbed molecule changes according to the usage area, the surface area also changes. The pore diameter is divided into three groups by IUPAC (International Union of Fundamental and Applied Chemistry) as micropores smaller than 2 nm, mesopores between 2-50 nm, and macropores larger than 50 nm (Menéndez-Díaz & Martín-Gullón, 2006). In practice, micropores are preferred for high gas and vapor adsorption, while meso and macropores are preferred for the solution and liquid adsorption.

The porosity and surface structures of activated carbons, especially the micro-mesoporosity and their distribution on the surface of the activated carbon, can be visualized by scanning electron microscopy (SEM). An idea about the surface structure of an adsorbent obtained in this way can be obtained (Marsh & Rodriguez-Reinonso, 2006).

In this study, our aim is to examine the effect of adsorbent property of activated carbon in milk fermentation on physical, chemical, microbiological and sensory factors on yogurt.

In this study, we reported dusky grouper *E. marginatus* from the southwestern Black Sea coast as a first occurrence in the Black Sea and present a new Mediterranean species in the Black Sea fish fauna.

Materials and Method

SEM Imaging

Surface structures of activated carbons, pore sizes, the structure of micropores, properties of their distribution on the surface were visualized by SEM. The study was carried out in Cukurova University's central laboratory SEM unit. The pore sizes of the activated carbons were measured on the image(FEI/Quanta 650 Field Emission SEM).

Determination of Activated Carbon Molecular Structure

In our searches, we could not find the activated carbon structure to be used in silico studies. That's why we created the drawings of the activated carbon structure in the Gaussian program. Computational analyzes were performed with GaussView 6.0.16 and Gaussian 09 (Version: AS64L-G09RevD.01) (Gaussian 09, 2009;GaussView, 2009). In terms of quantum chemical calculations, it is reported that a carbonaceous type such as AC is usually represented by a single sheet of graphene. The unsaturated edge can be activated sites for adsorption because these carbon atoms have unpaired electrons that are easy to transfer. Therefore, a zigzag-shaped graphene model with 6 fused benzene rings ($C_{24}H_8$) as the basic framework was applied in this study. After the model was drawn in Gaussview software, it was subjected to DFT calculations. In the Gaussian software, the B3LYP hybrid function, which is more regulatory and careful than the Hartree-Fock method, was chosen. The three parameters defining B3LYP are taken without modification from Becke's original fit of the B3PW91 functional, which is similar to a set of atomization energies, ionization potential, proton affinity, and total atomic energy. Also, 6-311G (d, p) was used as the base set.

Receptor/Ligand Retrieval and Preparation

Small molecule and ion (lactose and calcium) used as ligands in the molecular docking study were downloaded from PubChem National Library of Medicine database in structure data file (SDF) format and then converted into the 3D structure (PDB format) using the Avogadro program (The lactose molecule was optimized by applying MMFF94 force field using the steepest descent algorithm in the Avogadro software and saved in PDB format. Activated carbon used as a receptor in docking experiments was drawn manually in GaussView 6.0.16 program and then optimized with the UFF (united-atom force field) force field using the steepest descent algorithm in the Avogadro program and saved in PDB format. The ionic charges of calcium were added using the 'Structure Editing' module implemented in the Chimera program (version 1.14) before docking and then saved in mol2 format.

Molecular Docking with Autodock Vina 1.2.0

In our study, AutoDock Vina version 1.2.0 was used to perform docking simulations of activated carbon against lactose molecule and calcium ions. Along with AutoDock4, AutoDock Vina is one of the docking programs in the AutoDock Suite and is among the most widely used and successful docking programs in the world. The reasons for this success are its ease of use, speed (up to 100 times faster than AD4), and open-source code, both in the suite and when compared to other docking programs. The AutoDock Vina 1.2.0 program, which we used in our rigid receptor-flexible ligand (only for lactose) docking simulations, ensured that the rotatable bonds of the lactose molecule move freely during the docking simulation, thus providing a certain level of flexibility in its interaction with activated carbon.

AutoDockTools-1.5.6 was used in the preparation of target (activated carbon) and ligands (lactose and calcium) and parameters before initiating the docking study by AutoDock Vina 1.2.0. In molecular docking studies with activated carbon, polar hydrogen atoms in receptor and ligand molecule (in this case only lactose) were retained; however, non-polar hydrogens were merged. Kollman charges were assigned to the receptor molecule, while Gasteiger charges were assigned for the ligands, and then the molecules were saved in pdbqt format. During the docking calculations, rotatable bonds of lactose were allowed for free rotation. A grid box size of $40\times20\times20$ Å points (x: 0.0; y: -0.09; z: 0.0) with 0.375 Å grid spacing was set for activated carbon and ligands. The grid box size covered the entire surface of the activated carbon to ensure that ligands could easily interact with the receptor.

After five separate docking runs (20 dockings for each ligand; exhaustiveness: 200) of the ligands against activated carbon, all potential binding modes of the ligands were clustered through AutoDock Vina 1.2.0 and were ranked based on free energy of binding (ΔG^{o}; kcal/mol) of the ligand conformation that showed the lowest binding energy against the receptor. The best docking solutions of all the ligands calculated by AutoDock Vina 1.2.0 against the receptor structure was visualized and analyzed using Discovery Studio Visualizer v16.

Production of Trial Yogurt

Trial yogurts were made by considering previous methods (Mann, 1976; Çakmakçı et al., 1997). Milk (pasteurized, semi-skimmed, homogenized cow's milk) milked on the same day was used in the study (Milk was obtained from Çukurova University Faculty of Agriculture Research and Application Farm livestock branch). A total of 20 L of milk was put into 2 separate boilers with 10 liters each. An additional 10 g of activated carbon (Medical Charcoal, CAS No:7440-44-0, Production date:01/2021, BATCH NO:2102H0060, GALENİK) was added into a boiler and mixed for 4 minutes.

Milk in both groups was pasteurized at 90 °C. Plain milk was left to cool to ferment. After the milk with activated carbon was taken from the stove, it was filtered through a sterile milk filtration filter a total of 3 times, once at 15 and 30 minutes. A starter (Danisco, Yo Mix 505 LYO 200 DCU-France) was used in both groups at 47 °C at a rate of 3% (0.26 g per 10 L). After the starter was added, the milk was mixed gently for 4 minutes and then left for 4 minutes. Then the milk was placed in standard yogurt cups (200mL standard yogurt cups). After this process, the milk is placed in ovens (47±2 °C) to be incubated.

The products that had the consistency of yogurt were checked and removed from the oven after 4 hours and 10 minutes. The mouths of the containers were closed and removed to +4 °C cold storage without waiting. All operations were carried out in the Çukurova University Faculty of Agriculture Research and Application Farm Food branch dairy business.

Protein Measurement

The protein content was estimated according to the Kjeldahl method. The yogurt sample (1 g) was digested in concentrated sulfuric acid and the total nitrogen content was multiplied by 6.25. Values were calculated as percent (Duchaufour, 1970).

Oil Measurement

The fat content was determined while using the Gerber Method and the percent crude fat was determined by directly reading calibrated butyrometer (Badertscher et al., 2007). The resulting value was evaluated as the % fat ratio.

pH Measurement

The pH value of the samples was determined at 20±1 ºC by immersing the probe of the InoLab brand pH meter in yogurt. The fixed value in the measurement was saved as the result value.

Acid (Lactic Acid) Measurement by Titration

After the sample is diluted with water at a certain weight, it is titrated with NaOH adjusted against the phenolphthalein indicator. The total milliequivalent number of acids in the 100 g sample is calculated, and the result is given in lactic acid (Kebede, 2005). Values were evaluated as % acidity.

Calcium Measurement

The concentration of Ca elements in the yogurt was determined by atomic absorption spectroscopy. Results were set in ppm (Perkin Elmer- PinAAcle 900T model).

Aroma Test

The yogurt groups used in the study were evaluated sensorially with their color, general likability, texture, flavor, salinity, bitterness, and sourness properties. Sensory characteristics were recorded verbally with a sensory type scale, in line with the principles of previous studies. The intensity of the senses was described as numerical values from 1 to 5. Sensory evaluation was performed by a laboratory-type panel group of 8 people (Akyüz, 1980; Çakmakcı et al., 1997). In the aroma tests, it was stated to the participants that the gray color formed in the activated carbon yogurt was a color change caused by carbon.

Microbiological Measurements

Total coliform bacteria, total lactic acid bacteria, *S. thermophilus* bacteria and total yeast-mold counts were determined in the 0th, 5th, and 10th-day samples of yogurt containing activated carbon (3 cups of 200 g) and plain yogurt (3 cups of 200 g) were used in the study. 1 g was taken from each of the samples and diluted to a ratio of 10-8 in 9 mL of 0.9% sterile saline solution. To determine the total number of coliform bacteria, dilutions of 10-2, 10-3, 10-4 yogurt samples were planted on Eosin Methylene Blue (EMB) Agar and incubated at 37 ºC for 24 hours (Andersen, 1993). For total Lactic Acid Bacteria (LAB) count, 10-8 dilution of yogurt samples were made on Man Ragosa Sharpe (MRS) Agar and incubated at 30°C for 24-48 hours. At the end of the incubation, petri plates containing colonies were counted and averaged (Çelikyurt, 2008).

Samples for S. thermophilus were incubated at 4 °C for 24 hours. Then, dilutions up to 10-8 concentrations were prepared with 4.5 ml of peptone water solution and inoculated into appropriate media. Sowing was done on M17 agar at appropriate dilutions and incubated at 370 °C for 48 hours under aerobic conditions (Davis et al., 1971). For total yeast-mold count, dilutions of 10-3, 10-4, 10-5 ratios of yogurt samples were made on Potato dextrose agar (PDA) medium and the petri dishes were incubated for 48-72 hours at 30°C (Halkman, 2005). All microbiological analyzes were performed in 3 parallel and 3 replicates.

Statistical Assessment

Statistics were done with SPSS V.26. The T-paired sample analysis method was chosen and plain and activated carbon mixed yogurts were inserted as variables. After that TT population plots were used as descriptive statistics. $p < 0.05$ was considered statistically significant.

Results and Discussion

SEM Images

In the SEM images obtained, the activated carbon had mesopores with pore diameters between 2-50 nm and a macropore structure over 50 nm (Figure 1).

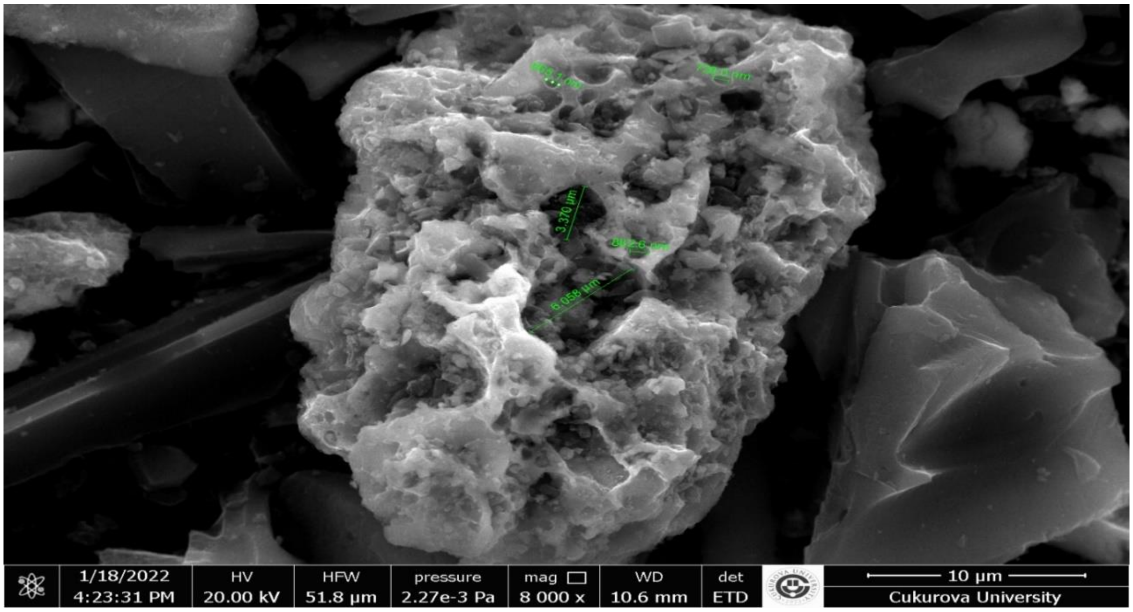

Figure 1. SEM images of activated carbon particles.

Activated Carbon Molecular Structure

Activated carbon was found to be a durable and good bonding structure. The first, as a result of the simulation, it was determined that there was no virtual frequency and the structure was stable. The second dipole moment increased to 4.945 Deb, indicating that the structure is polar. The third, enthalpy of formation was -919.011971 Hartree, which showed that the formation of the structure was favorable in terms of thermodynamics (1 Hartree = 627,503 kcal/mol). The fourth, Heat Capacity (Cv) was 66.052 cal/mol-kelvin. The fifth, entropy being 123.807 cal/mol-kelvin and having a positive sign is an indication of thermodynamically enabling the formation of the structure (Figure 2).

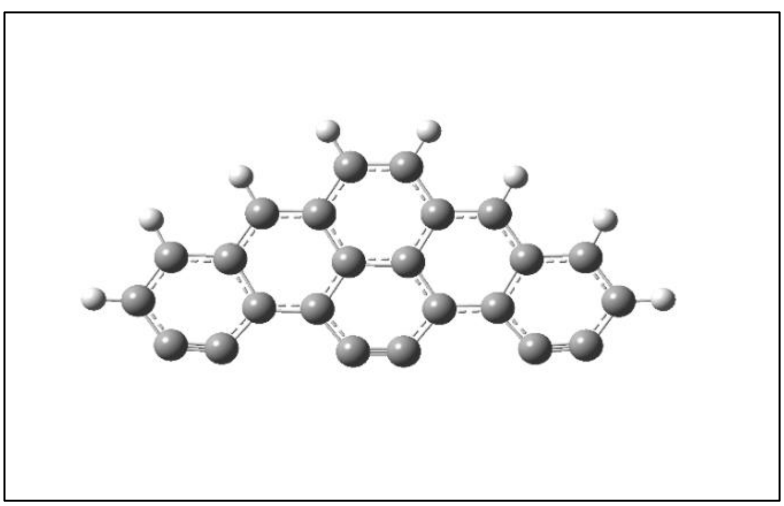

Figure 2. Optimized activated carbon

In the load distribution map of the structure, it is seen that the bindings will take place from the lower part of the structure (red region) (Figure 3).

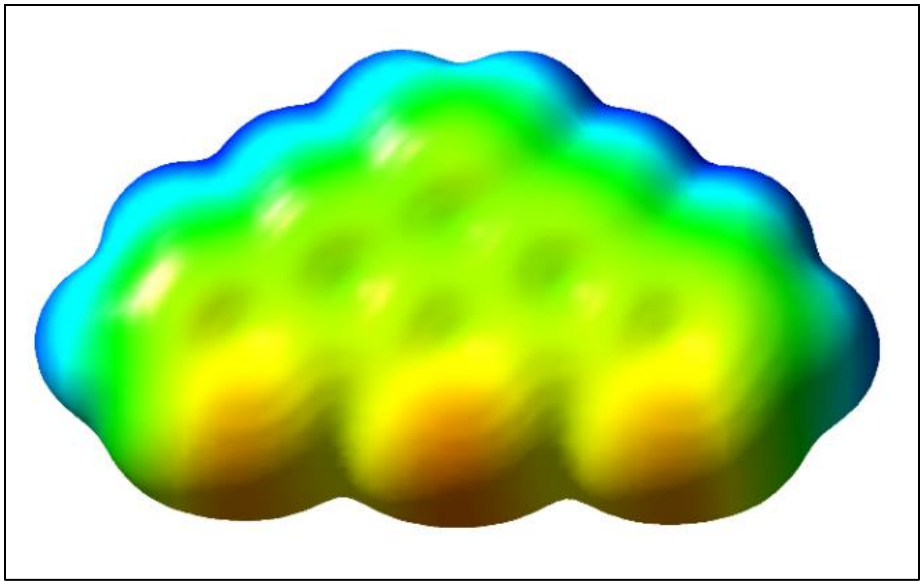

Figure 3. Activated carbon molecular electrostatic potential (MEP) map

The structure of lowest unoccupied molecular orbital (LUMO) orbitals are given in figure 4. When we look at this structure, the activated carbon structure has a very strong structure in establishing return bonds.

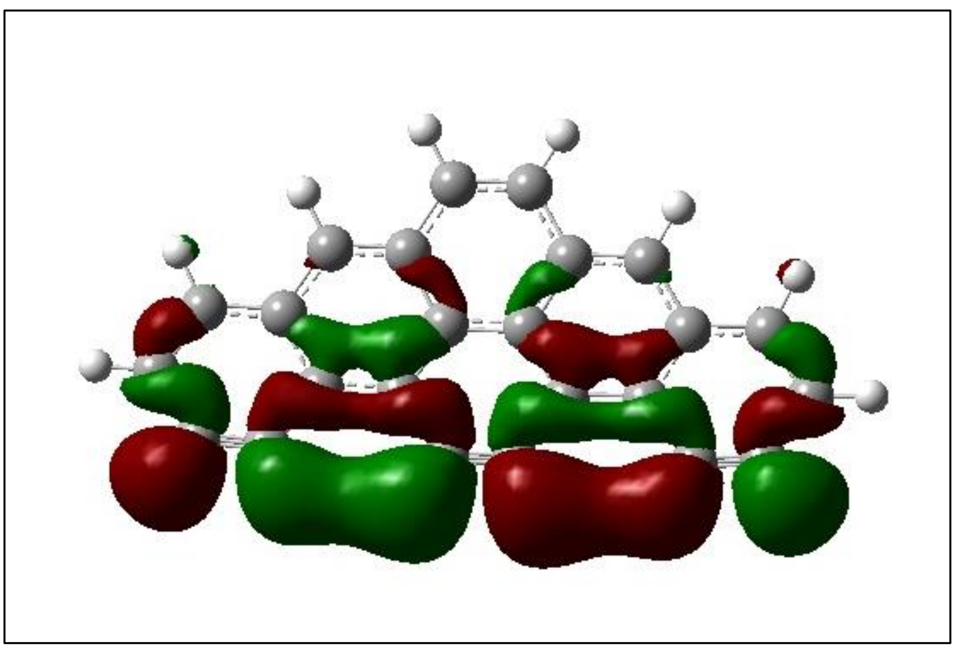

Figure 4. LUMO orbital structure of activated carbon

In Silico Study

In this study, molecular docking was carried out to examine the interatomic interactions of activated carbon with lactose as well as the ion: calcium. As seen in Figure 5a, the lactose molecule formed one hydrophobic pi-sigma contact with a hexagonal carbon ring of activated carbon over the third carbon atom of the galactopyranosyl ring in its structure. While the length of this non-bonded contact is 3.40 Å, the binding free energy of lactose with the activated carbon is favorable (ΔG° = - 4.01 kcal/mol) (Table 1). Calcium (Ca +2) ion formed three pi-sigma interactions (bond lengths between 3.67 Å – 3.68 Å) with three neighboring hexagonal carbon rings of activated carbon (Figure 5b)(Table 1).

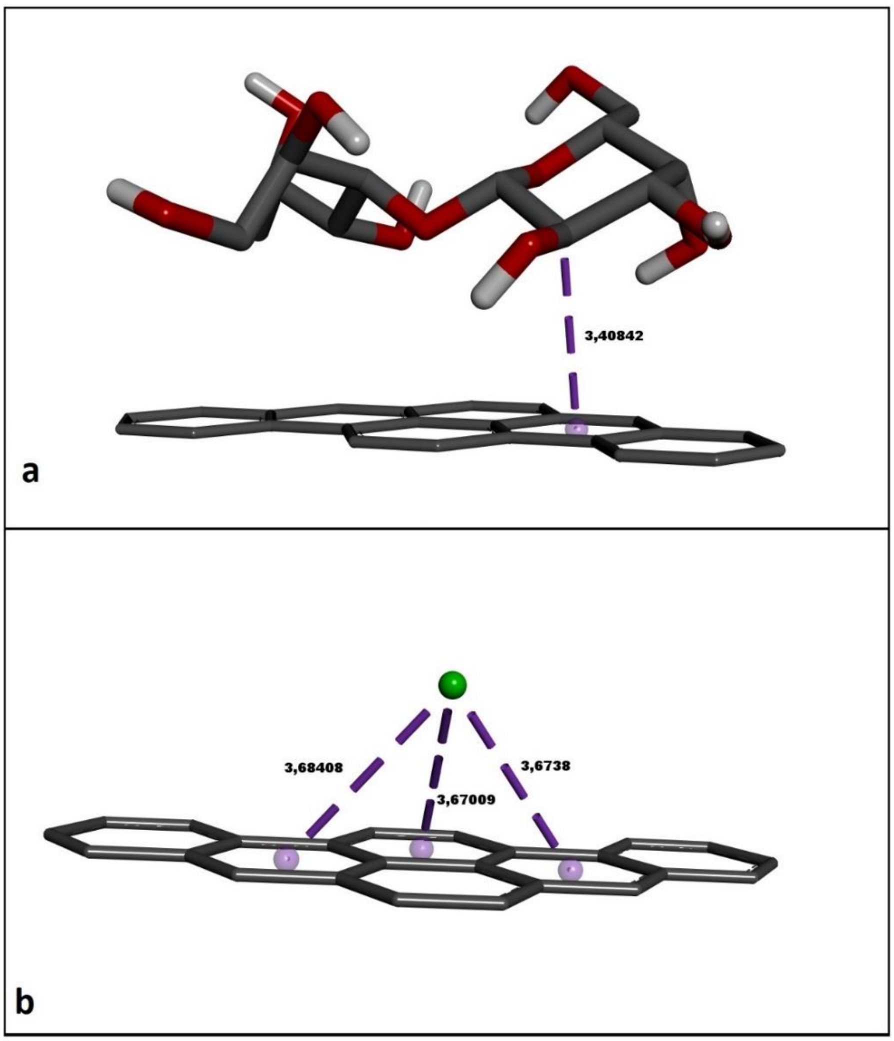

Figure 5. Top-ranked molecular docking conformations of activated carbon in complex with lactose and calcium ion. a) Activated carbon-lactose complex; b) Activated carbon-calcium complex.

In conclusion, the data we obtained from the molecular docking study indicate that the intermolecular interaction between activated carbon and lactose is favorable, however, since there are no electronegative groups in the structure of activated carbon (lack of partial negative charges), the interaction with cation (Ca^{+2}) is much weaker than it could be ignored.

Table 1. Binding free energy (kcal/mol), non-bond interactions and bond distances (Å) formed as a result of molecular docking experiments between the activated carbon and lactose and calcium ions

Complex	**Binding Free Energy (Δ G ° =kcal/mol)**	**Non-bond interactions**	**Bond Distance**
Activated Carbon-Lactose	-4.01	1 pi-sigma (hydrophobic)	3.40 Å
Activated Carbon-Calcium	-0.28	3 pi-sigma (hydrophobic)	3.67 Å , 3.67 Å , 3.68 Å

Protein Levels

The protein level of the milk made from yogurt was 3.40 %. This value was observed more on the first, fifth and tenth days in yogurt with activated carbon addition compared to plain yogurt (1st-day $p < 0.05$, 5th-day $p<0.05$, 10th-day $p<0.05$) (Figure 6).

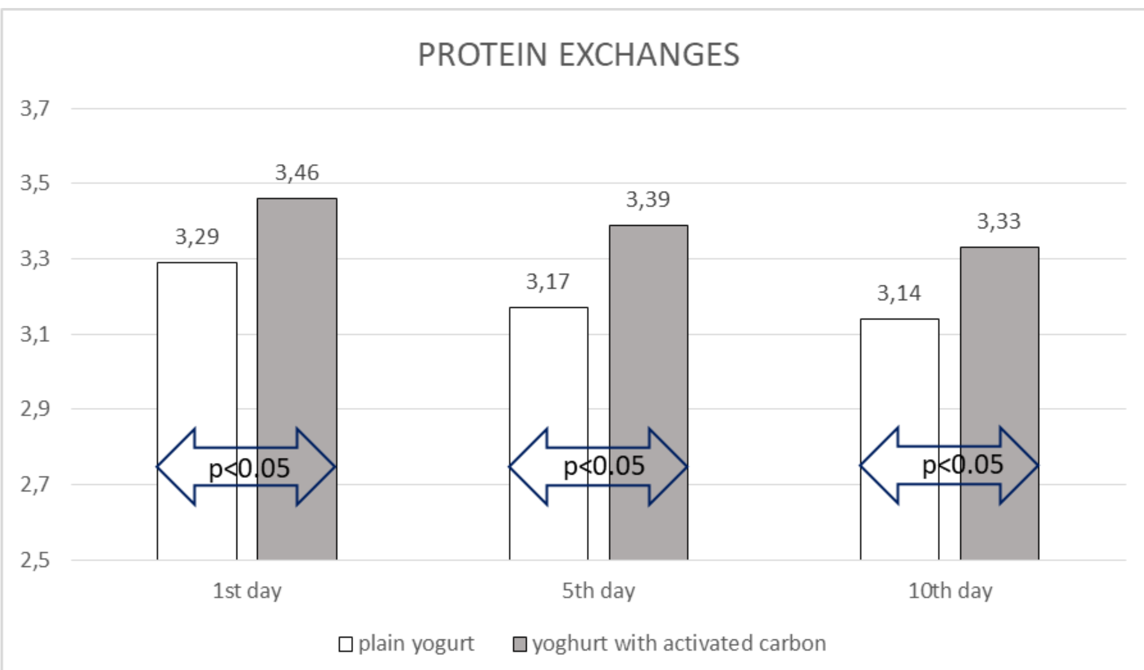

Figure 6. Protein levels by days

Fat Levels

The fat content of the milk made from yogurt was 4% (fat ratio in whole milk). It was observed that this value did not differ in the 1st, 5th and 10th days of yogurt with activated carbon addition compared to plain yogurt (1st day p=0.685, 5th day p=0.598 and 10th day p=1) (Figure 7).

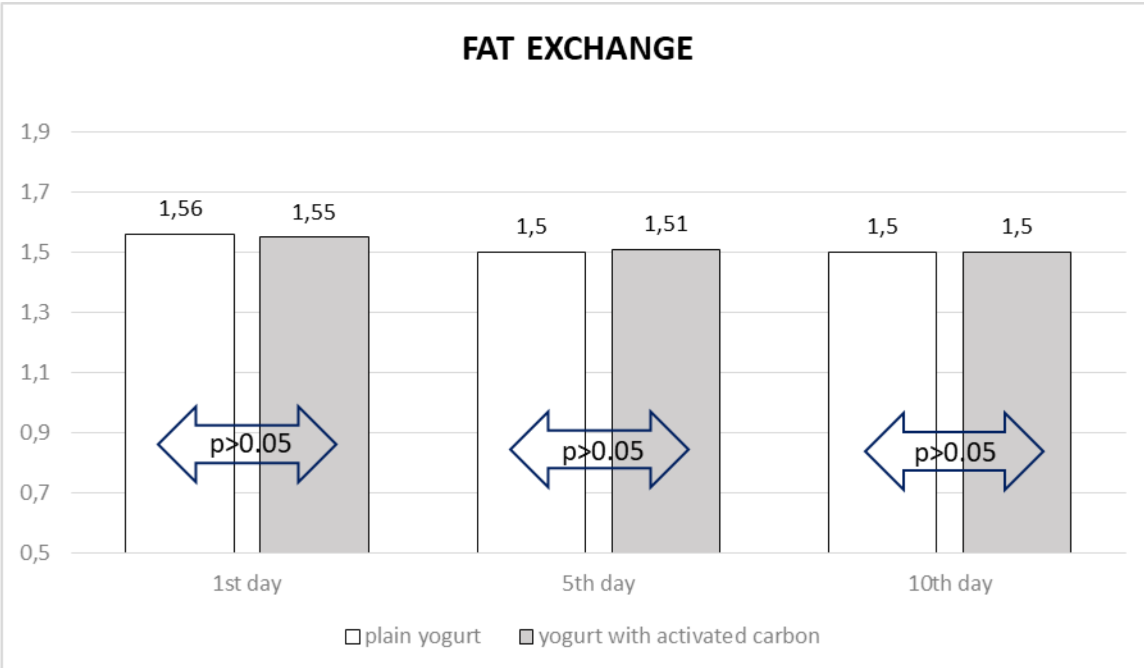

Figure 7. Fat levels by day

pH Changes

This change was found to be more alkaline on the 1st, 5th, and 10th days in the yogurt with activated carbon addition compared to the plain yogurt. These differences were statistically significant on the 1st and 10th days. On the 5th day, although the difference was not statistically significant, the pH levels were more alkaline in the yogurt to which activated carbon was added (day 1 $p<0.05$, day 5 $p>0.05$, and 10. day $p<0.05$) (Figure 8).

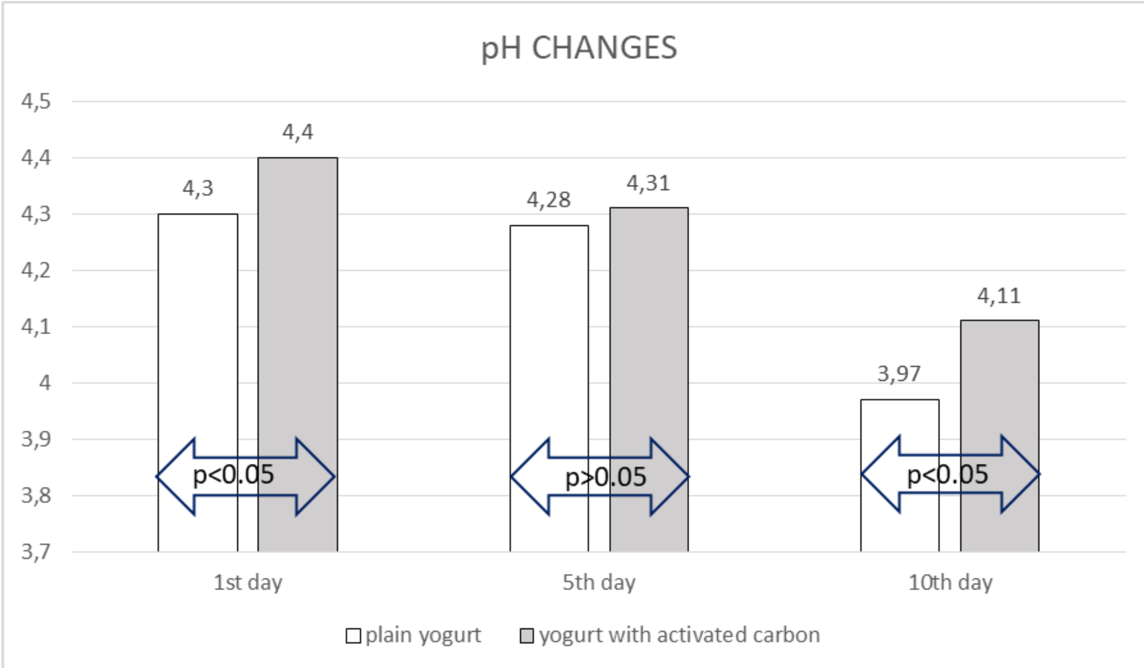

Figure 8. pH levels by days

Acid (Lactic Acid) Measurement by Titration

It was found to be lower in the 1st, 5th, and 10th days of yogurt with activated carbon addition compared to plain yogurt (Figure 9). These differences were statistically significant on day 1 and day 10. Although it was not statistically significant on the 5th day, the lactic acid levels were lower in the yogurt to which activated carbon was added ($p<0.05$ on the 1st day, $p=0.239$ on the 5th day, and $p<0.001$ on the 10th day).

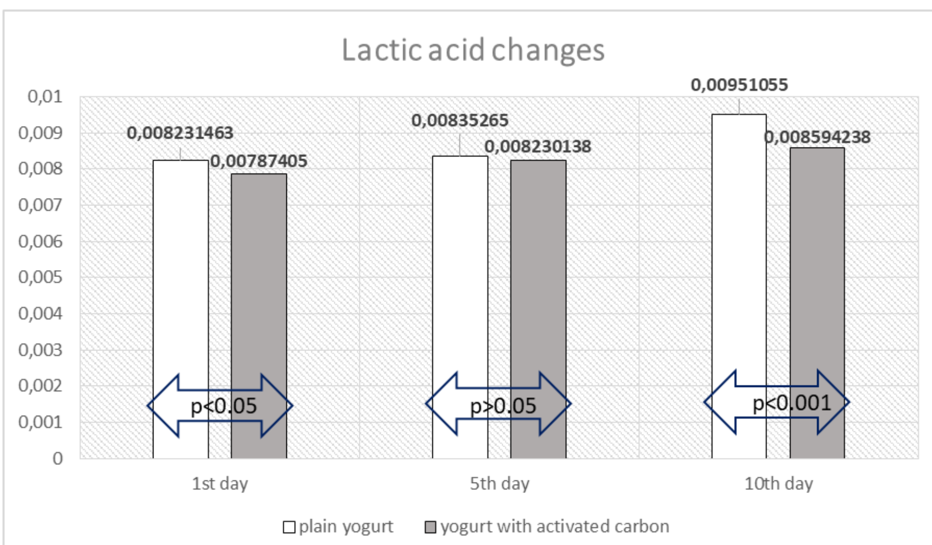

Figure 9. Lactic acid levels by day

Calcium Levels

There was no difference between plain yogurt and yogurt with added carbon on the 1st and 5th days ($p>0.05$). On the 10th day, this difference was more significant in carbonated yogurt ($p<0.05$) (Figure 10).

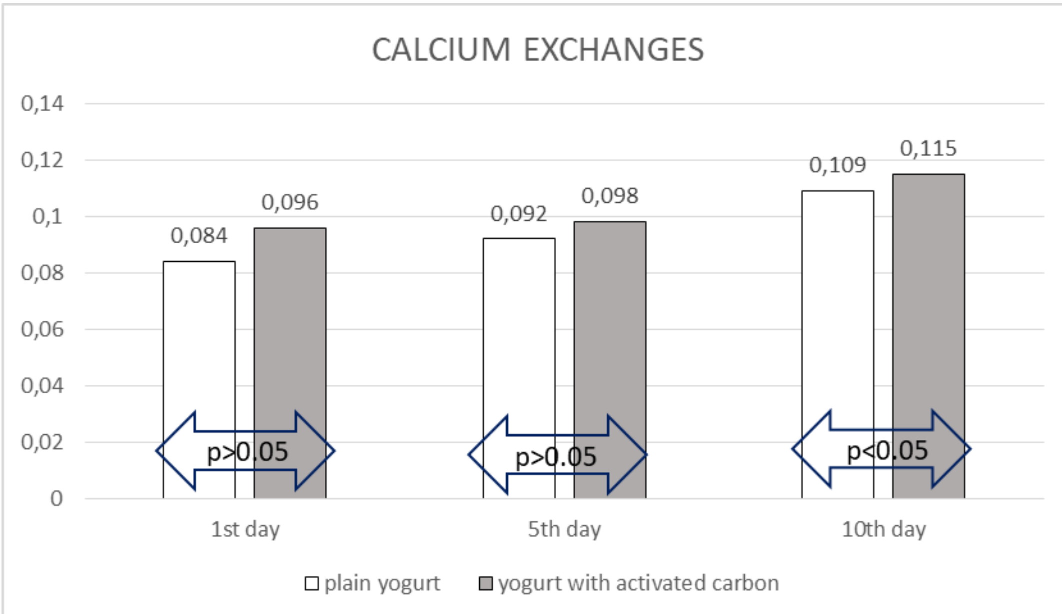

Figure 10. Calcium values by day

Aroma Test

Texture (1st, 5th and 10th days) and flavor (5th and 10th days) of yogurt obtained from the study resulted in higher sensory measurement in yogurt with activated carbon. However, these values were not statistically significant ($p>0.05$). General desirability was statistically significant in favor of activated carbon at the 5th and 10th days. Sour, salty, and bitter flavors could not be detected in plain yogurt and yogurt with activated carbon, and no difference was found ($p>0.05$) (Table 2). The gray color of yogurt with activated carbon was observed by all of the panelists who performed the aroma test.

Table 2. Aroma sensory evaluation by days

Yogurt Type and Day	Texture	Flavor	General Like	Sour	Salty	Spicy
Plain yogurt day 1	4.125	4.125	3.625	0	0	0
Yogurt with activated carbon day 1	4.375	4.125	4.25	0	0	0
p value	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
Plain yogurt day 5	4.25	2.75	3.25	0	0	0
Yogurt with activated carbon day 5	4.75	3.625	4.25	0	0	0
p value	$p > 0.05$	$p > 0.05$	**$p = 0.05$**	$p > 0.05$	$p > 0.05$	$p > 0.05$
Plain yogurt day 10	3.375	2.75	2,5	0	0	0
Yogurt with activated carbon day 10	3.375	3.75	3.75	0	0	0
p value	$p > 0.05$	$p > 0.05$	**$p < 0.05$**	$p > 0.05$	$p > 0.05$	$p > 0.05$

Microbiological Results

It was determined that there was no total coliform bacteria (EMB Agar) and total yeast mold (PDA) growth on the 0., 5th, and 10th days of the yogurt containing activated carbon and plain yogurt samples. Total LAB (MRS Agar) was determined (Figure 11).

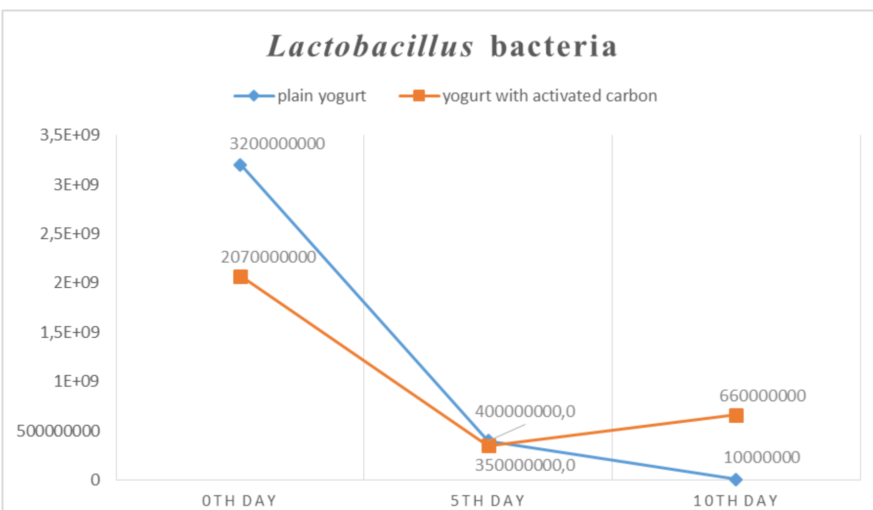

Figure 11. Total LAB (cfu/gr: Colony Forming Unit) number of plain and carbon-containing yogurt by days

According to the results obtained; The total LAB count was higher in plain yogurt on day 0 than in yogurt containing activated carbon ($p<0.05$). On the 5th day, although it was higher in plain yogurt, this difference was not significant ($p>0.05$). On the 10th day, it was determined that the total number of LABs in yogurt containing activated carbon was higher than in plain yogurt, but the difference was not significant ($p>0.05$).

S. thermophilus bacteria in yogurt samples was the highest on the 0th day, a gradual decrease was detected in the 5th and 10th-day values. As a result of the statistical analysis, no difference was found between the growth of *S. thermophilus* in plain and carbonated yogurt ($p>0.05$) (Figure 12).

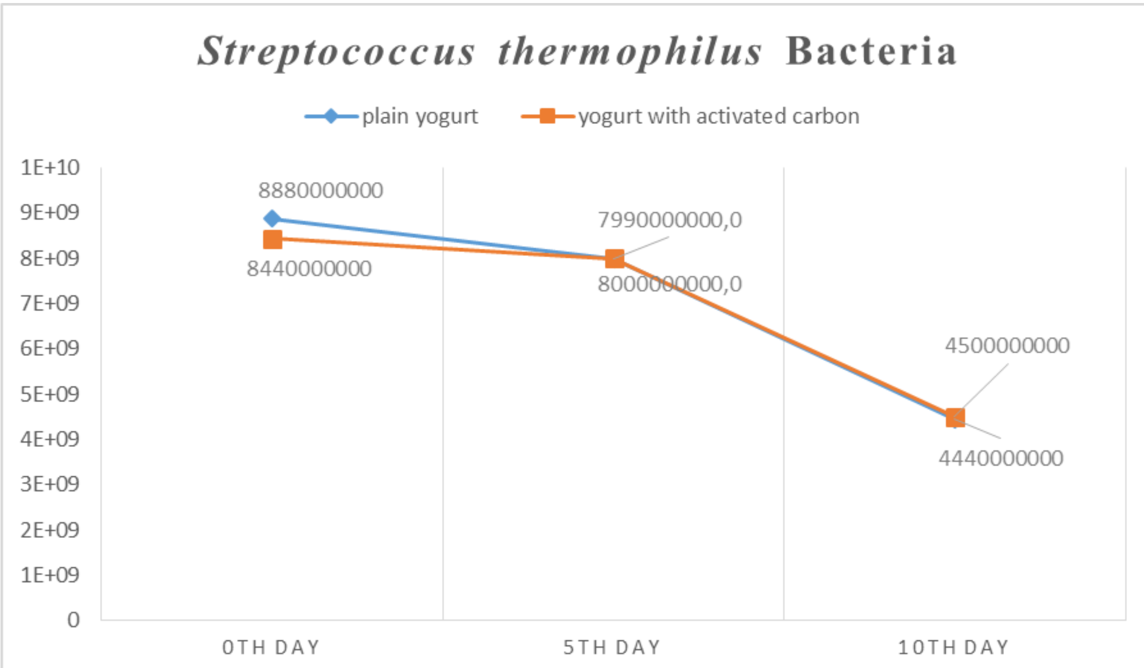

Figure 12. The total number of *S. thermophilus* (cfu/gr: Colony Forming Unit) of plain and carbon-containing yogurt by days

In conclusion, people's use of milk and dairy products as nutrients, BC. It is believed to have started between 10,000-5,000 years ago (Fisberg & Machado, 2015). The use of milk and dairy products has also brought lactose intolerance. Studies have shown that approximately 2/3 of the world population is lactose intolerant (Casellas et al., 2009).

It is reported that the lactose ratio in milk after fermentation during the formation of yogurt does not change at the rate of 50-65% (Lourens-Hattingh & Viljoen, 2001). It is known that this level of lactose in yogurt can cause dyspeptic complaints in people with lactose intolerance, although not as much as in milk.

Storage conditions are followed until yogurt is consumed. In this process, it is important for health to prevent the growth of pathogenic microorganisms in yogurt.

It is known that the increase in the world population and the spread of agricultural products and drugs that are produced quickly, long-lasting, but away from natural methods, pose the risk of heavy metal poisoning. Heavy metals taken from the outside can cause a wide range of diseases from psychological disorders to physiological problems, depending on the organ they accumulate and the system they affect.

The use of activated carbon appears in a wide variety of fields, including food, textiles, and water treatment technologies. One of these areas is human health. In drug intoxications, activated carbon is used at a dose of 10-25 g/day for children under 5 years of age in emergency services, while it is used at a dose of 1 g/kg for adults. The fact that activated carbon is mixed with yogurt to increase the adsorption of the intoxicating agent reinforces the safety of use in our study (Groth Hoegberg et al., 2005).

The ability to adsorb heavy metals, as it is not absorbed, increases the confidence in the use of activated carbon in human health (Larsen & Cummings, 1998; Olson et al., 2012).

Studies have shown that activated carbon reduces the growth of E.coli and S.aureus and adsorbs heavy metals (Shi et al., 2007; Anirudhan & Sreekumari, 2011; Bohli et al., 2015; Burchacka et al., 2021).

The use of this adsorbent feature of activated carbon in the consumption of an important food such as yogurt may contribute to the prevention of lactose intolerance, as well as the protection of yogurt from pathogenic microorganisms and the absorption of heavy metals taken with yogurt by the body.

The activated carbon used in our study was compatible with the activated carbon structure with meso and macropore structure suggested for the absorbance of liquids and solids (Neuvonen & Olkkola, 1988). This fit was detected by SEM images in our study. (Figure 2).

In the simulation studies, the high affinity of activated carbon to lactose with -4.01 kcal/mol shows us the adsorbent property of activated carbon to lactose (Table 1). The fact that lactic acid, which is a lactose breakdown product, is lower in the yogurt with activated carbon, and the associated pH is higher, shows us that lactose use is less in activated carbon yogurt (Figure 8, Figure 9). The lower detection of Lactobacillus bacteria that produce lactic acid using lactose in yogurt with carbon on the 0th and 5th days (Figure 11) supports these findings.

In microbiological studies, the growth of Lactobacillus bacteria increased on the 10th day ($p<0.05$), but the lower lactic acid level ($p<0.05$) and higher pH in yogurt with activated carbon added ($p<0.05$) created an adaptation to the environment for the growth of Lactobacillus bacteria makes us think. It is thought that this adaptation is due to the use of activated carbon, which is the only difference between the groups of Lactobacillus bacteria as a positive food source.

As a result of microbiological analysis, coliform bacteria and yeast -mold were not detected in yogurts of both groups. From this point of view, both groups show the reliability of yogurt in terms of food hygiene and sanitation. Coliform bacteria mix with various foodstuffs such as milk and yogurt as a contaminant. Therefore, the absence of this group of microorganisms indicates that the production line where the sterilization steps are carried out is suitable in terms of hygiene and sanitation.

Coliform bacteria, which are pathogenic to humans, are abundant in the feces of warm-blooded animals and are transmitted to milk during milking. For this reason, milk sterilization during milk processing is important for human health. Another factor that can be harmful to human health is mold. Molds formed in milk and dairy products and other foods can cause poisoning due to the toxins they secrete.

The reason for the presence of Lactic Acid Bacteria and *S. thermophilus* bacteria in yogurt is that the microorganisms used as yeast are in this group. Bacterial counts were found to be more than 1x108 cfu/g on days 0, 5, and 10 in both groups.

The presence of LAB in fermented foods such as milk and dairy products does not pose any adverse effects. In addition, this group of bacteria is known to have antagonistic activity against foodborne pathogens.

The protein required in yogurt should be at least 3%, the fat content should be at least 1.5%, the titration acidity should be 0.6-1.5%, the pH should be below 4.6, the texture and aroma values should be within acceptable limits, the total specific microorganism (cfu/g) should be at least 107, there should be no *E. Coli* reproduction. It was observed that yogurt texture, fat content, protein level, titration acidity, pH, calcium level, and microbiological properties of milk fermentation were preserved in the formation of yogurt using activated carbon. *E. coli* growth was not observed in the culture medium in any of the yogurts made.

It was observed that the fat ratios did not differ in carbonated and plain yogurt, but protein and calcium levels were higher in yoghurt with activated carbon. During protein and calcium measurements, yogurt is burned at high temperature and the remaining material is measured. It was thought that the activated carbon formed in carbon added (1g/L) yogurt at 900 °C would not change during these measurements and would affect protein and calcium measurements with an increase of 0.1%. Therefore, it was interpreted that the difference in the increase in protein and calcium results in yoghurt with activated carbon may be due to this reason.

In the aroma tests, general likability, flavor, and texture were found to be higher in activated carbon yogurt.

In the study, it was seen that the properties of activated carbon yogurt were compatible with physical, chemical, microbiological, and aroma tests within the limits of desired dimensions in yogurt. It was observed that the addition of activated carbon to milk before milk fermentation for the formation of yogurt did not spoil the structure of yogurt and made a positive difference.

The importance of yogurt consumption for a healthy person has been scientifically demonstrated by many system studies. This study shows that individuals with lactose intolerance can prevent yogurt consumption restricted with activated carbon yogurt.

In addition, activated carbon is a useful mechanism that reduces the growth of pathogenic microorganisms, and its contribution to the immune system by reducing the existing toxins in the body with its adsorption feature to heavy metals.

In our world where yogurt consumption is so common, activated carbon yogurt can be a healthy alternative yogurt option.

Author Contributions

All author contributions are equal for the preparation research in the manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

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