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Effect of Different Acids and Salt Application on The Microbiota of Pickled Cabbage

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ABSTRACT

Pickles, which are known to have many nutritional and health benefits, have been used as a nutritional supplement in many cultures in recent years. Lactic acid bacteria, in its natural microbiota of pickle, which have various probiotic properties such as increased natural resistance to infectious diseases in the gastrointestinal system, prevention of various infections, and reduction in cholesterol level are effective in this. In our study, various combinations of salt, vinegar and lemon acid were made to produce cabbage pickled and it was determined how it affected the natural microbiota during fermentation. When the results were evaluated in terms of the viability of lactic acid bacteria, the best results were obtained with 5.35 logarithms in the G2 produced using only 5% lemon juice from eight different groups, one of which was a control. In the G3 which was produced using 5% vinegar, this number was determined as 1.82 logarithms. At the end of fermentation, it was determined that the coliform groups in the experimental groups lost their viability. Yeasts were completed this process with an average of 1 logarithm. When all test groups were examined in terms of all microbiological results was showed that the production of 5% lemon juice G2 has optimally results in terms of growing LAB and inhibition the undesirable microbial groups

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Introduction

It has been determined by many studies and accepted by researchers that foods obtained by fermentation, which dates back to ancient times, mostly have natural microbiota. By the researchers are stated that this microbiota is beneficial for the health of the human digestive system. According to many research results this microbiota predominantly consists of lactic acid bacteria (LAB). Both nutrition elements, various substances of plants contain which are beneficial to health and the quality, taste, aroma of the resulting product are preserved by means of the fermentation (Bamforth 2005; Çetin 2011; Chaiyasut et al., 2018). Pickles are the fermented foods that enable long-term storage without the need for any refrigerant. These fermented foods are preserve foodstuffs under high acid concentration by utilizing microorganisms such as LAB (Chaiyasut et al., 2018; Behera et al., 2020). Pickled cabbage is one of the fermented vegetables that is very common in production and consumption in Türkiye. These are known that fresh/raw cabbage of structure mainly are feature a variety of microorganisms, including aerobic spoilage bacteria, yeasts and molds. The decrease in oxygen concentration during fermentation leads to decrease in dominant aerobic bacterial communities, and these anaerobic conditions help the growth of LAB populations (Penas et al., 2017).

The presence of Lactobacillus strains among lactic acid bacteria is preferred in fermented pickles. This is because some species of lactobacillus are hetero-fermentative and can convert hexoses to lactic acid and then to acetic acid, as well as produce various metabolites. In addition, pickles fermented by LABs may have a distinct taste and positive health effects (Behera et al., 2020).

Besides various inhibitory chemical compounds produced by LAB and released into the environment where food is found, the high salt concentration in fermented foods contributes to suppress the growth of pathogenic or spoilage bacteria (Inatsu et al., 2005). These important features also contribute to ensuring food safety and the technological quality of fermented products (Herreros et al., 2005; Irkın and Songun 2012; Choi et al., 2018). In addition, although the amount of concentration is effective, it is known that the salt in the brine affects the microbial activity in the pickle, and it is a helpful factor in controlling the softening in the product texture and preventing spoilage by limiting the pectinolytic and proteinolytic hydrolysis in the raw material (Tokatlı et al., 2012).

Vinegar is obtained by switch from fermentable sugars to ethanol by yeasts, and then by oxidizing this ethanol by acetic acid bacteria. (Gökırmaklı et al., 2019), it is important in terms of polyphenolic compounds, which is one of its chemical components, therefore, it is estimated that it will increase the bioactive properties by fermenting fresh vegetables (Özkan, 2016). The antimicrobial effect of vinegar is thought to be due to its acetic acid content. The mechanism is thought to be acetic acid is in undecomposed form, and the pH of the conditions is acidic disrupts the osmotic balance of the cell. In addition, the fact that acetic acid disrupts the bacterial cell wall structure and causes ATP loss in the cell is associated with this situation, and due to this feature, it is used as a food additive. The antimicrobial effect of acetic acid may vary depending on the degree of acid ionization constant (Ka), the pH and temperature level of the environment, and the type of target pathogen (Ayhan and Bilici, 2015; Gökırmaklı et al., 2019).

Citric acid, known as tricarboxylic acid is a weak acid with three carboxylic functional groups with three different dissociation (pKa) values. It is a preservative, acidifier, sweetener, emulsifier, sequestrant, and buffering agent widely used in many industries such as food, beverage, pharmaceutical, and cosmetic products, and is found naturally in lemon, raspberry, tomato, pineapple, strawberry, cranberry (Thauer et al. 1988; Erdogan- Eliuz 2020). Information on the mechanism of the antimicrobial action of citric acid remains limited, but it is thought that at low pH, its uncharged, undissociated form can freely cross the microbial membrane. After entering the cytoplasm, it causes acidification of the intracellular environment and dissociates into its anions and protons, causing functional and structural damage in the cell (Burel et al., 2020). Studies have determined that it has antioxidant and antimicrobial properties against food-borne pathogens such as *Escherichia coli*, *Salmonella* spp, *Listeria* spp, and *L. monocytogenes*, thus preventing spoilage and helping to keep the product fresh (Rößle et al., 2009; Ciriminna et al., 2017).

The antioxidant properties, safety characteristics, and flavor of fermented foods are closely linked to the microorganisms present in the food. Pickles, being subject to an open fermentation environment, serve as carriers for numerous microorganisms. Studies have emphasized the close association of LAB with microbial communities in Kimchi (Jung et al., 2012; Zhou et al., 2021). The anticipated positive effects of fermented cabbage, as suggested by studies (Kusznierewicz et al., 2008; Özcelik et al., 2016), include enhanced antioxidant properties with a positive impact on human health and the production of organic acids, which exhibit antimicrobial activities, in comparison to raw cabbage. The higher antioxidant activity in fermented cabbage is linked to the ability of LAB to hydrolyze polyphenols, compounds naturally present in raw cabbage, into simpler and more antioxidant forms (Peñas et al., 2015). Due to a prevalent community belief in the probiotic nature of naturally fermented cabbage pickles, which may include additions such as vinegar, lemon, and salt, the consumption of such pickles is widespread. Nevertheless, the literature reports conflicting results regarding the impact of organic acids and salt, both individually and in combination, on the viability of microorganisms (Entani et al., 1998; Lee and Kang, 2009; Lee et al., 2010; Yoon et al., 2014). To the best of our knowledge, there has been no study on the flora of cabbage pickles produced with the addition of different organic acids and salt, either separately or in combination.

In this study, it was aimed to determine the effects of vinegar and lemon use in pickle production on the natural flora and vitality of cabbage during the fermentation period by creating various acids and salt, both individually and in combination. For this purpose, eight different pickle groups were created, including the control groups, and they titratable acidity and pH properties and microbiological viability analysis were assessed.

Materials and methods

Production of Cabbage Pickles

Small and firm white cabbages were used for pickles. After cleaning the outer leaves of the cabbage, it was divided into at least four with the help of a sterile knife and coarsely chopped. To prevent any microbial contamination bay leaves and chickpeas were exposed to UV light for 5 minutes on the purpose of short-term sterilization. Then bay leaves and some chickpeas were added to the cabbages when they were placed in pre-sterilized jars. Order to increase the protein source in the pickle, chickpeas, in addition to this bay leaf was added low concentration to give only flavor. Afterward the jars which are containing with cabbage were filled with brine in the determined combinations (Table 1).

Since the amount of sugar in the cabbage is sufficient, no added sugar or any preservatives have been added. At the end of the fermentation period was completed, the pickle reached the edible maturity, the white color turned yellow white with the change in taste and smell, and the cabbage leaves lost their breaking feature and gained flexibility. In Figure 1 showed preparation of the cabbage pickle.

Physicochemical Analysis of Pickles

Determination of titratable acidity and pH

Titratable acidity values were determined by the method described by Fernàndez-Diez et al., (1985), and titratable acidity and percent lactic acid (% w/v) were calculated using 0.1 N NaOH. pH measurements were determined with InoLab (pH Level 1, Germany), and measurements were made at room temperature in triplicate and standard deviations were calculated.

Determination of Viability in Pickles of Lactic Acid Bacteria and Other Microorganisms

For the microbiological analysis of cabbage pickles one milliliter of the samples were taken randomly during fermentation then they were transferred into 9 mL of peptone water, decimal dilutions were prepared. Then the number of viable cells was determined by serial plating. For *Enterobacteriaceae* counts, Eosin Methylene Blue (EMB) Agar (Merck, Germany) was used, the plates were incubated at 37˚C for 3 days. To determine the LAB counts, Man Rogosa and Sharpe (MRS) Agar (Merck, Germany) was used, the plates were incubated at 30˚C for 3-5 days (Maragkoudakis et al., 2006). Also, to determine the mold-yeast counts in the pickle samples, Yeast Extract Glucose Chloramphenicol (YGC) Agar (Merck, Germany) was used, and the plates were incubated at 25˚C for 3-5 days. All microbiological analyses were made in triplicate and the mean values and standard deviations were calculated (Beganovic et al., 2011).

Table 1 Pickle combinations and their ingredients

Groups name of pickles	Ingredients			
	Salt	Lemon Juice	Vinegar	Water
G1	%5	-	-	95%
G2	-	5%	-	95%
G3	-	-	5%	95%
G4	5%	5%	-	90%
G5	5%	-	5%	90%
G6	-	5%	5%	90%
G7	5%	5%	5%	85%
Control	-	-	-	100%

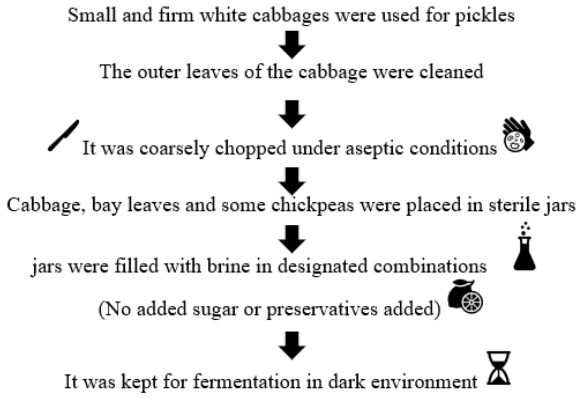

Figure 1. Flowchart of the cabbage pickle

Figure 2. Preparation stage of pickles

Figure 3. Pickles at the end of fermentation

Statistical Analysis

All experiments were carried out in triplicate and obtained data were reported as mean±standard deviation. The Minitab 18 statistical software (Minitab, Inc, State College, PA, USA) was used. Statistical analyses were performed using a one-way analysis of variance (ANOVA) test and the significance between means was determined by Tukey test. Differences were considered significant at $p < 0.05$.

Results and Discussion

Viability of lactic acid bacteria and other microorganisms in white cabbage pickle during fermentation were determined. A total of 8 different experimental groups, one of which was a control, were formed in the study and the microbiological results of these experimental groups are given in Table 2 and Figure 3 showed that the pickles at the end of fermentation.

It is stated that as the osmotic pressure of the medium increases, the tolerance of microorganisms to this high hydrostatic pressure improved, and in various studies conducted on this subject, it has been observed that sodium chloride may have a baro-protective effect (Molina-Höppner et al., 2004). Salt plays a crucial role in sauerkraut fermentation, not only by inhibiting the growth of spoilage microbes and controlling the activity of endogenous pectinolytic enzymes responsible for cabbage softening but also by influencing the population and profile of LAB, as well as the sensory quality of sauerkraut (Peñas et al. 2017; Wieczorek and Drabińska, 2022). The most favorable conditions for sauerkraut production, as indicated by Ray and Montet (2015), include a NaCl content between 2.25% and 2.5% and a temperature of 18˚C. On the other hand, a higher NaCl content, around 6%, is necessary for the fermentation of cabbage heads, as suggested by Niketić-Aleksić (1988). When the results were evaluated, it was seen that the G1 which is containing only 5% salt without any acid addition showed a good result in terms of viability of LAB bacteria (Figure 4). The lack of acid effect has a positive condition, especially in terms of LABs. However, the absence of an acidic effect, resulting from the lack of any acid addition, led to a similar impact on other microorganism groups until the 5th day. Especially between the 5th and 10th day, the count of LAB remained nearly constant. In contrast, the significant decrease in the number of yeast and total bacteria during this period could be attributed to the antimicrobial effect induced by the secondary metabolites produced by LAB. The reduction in pH and the increase in acidity within this timeframe also suggest that these secondary metabolites have an acidic character. The literature clearly shows that using LAB, which have been reported to antagonize pathogenic bacteria through their ability to produce antimicrobial proteins (bacteriocins), diacetyl, organic acids, H_2O_2, ethanol, and acetaldehyde, is one of the hygienic procedures for pickle production (Martinz et al., 2013; Rouhi et al., 2013; Enan et al., 2014b, Enan et al., 2014a, Reda et al., 2018; Almohammadi et al., 2022). It was determined that there was a serious decrease in viability after the 10th day during fermentation.

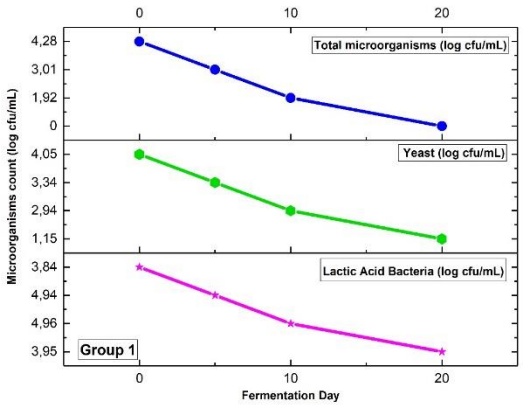

Figure 4. Microbiological results of G1

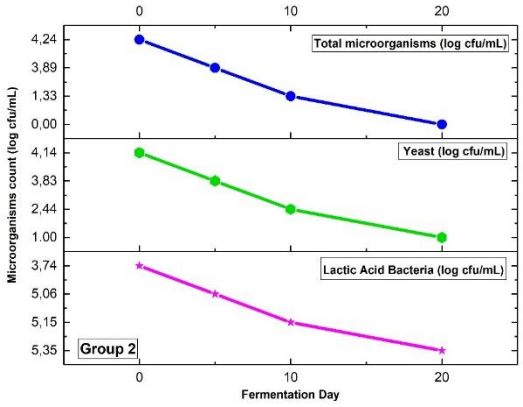

Figure 5. Microbiological results of G2

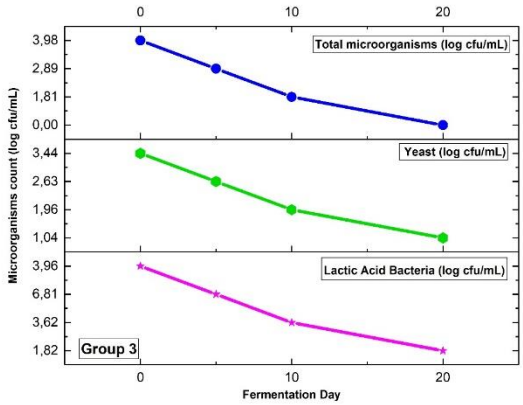

Figure 6. Microbiological results of G3

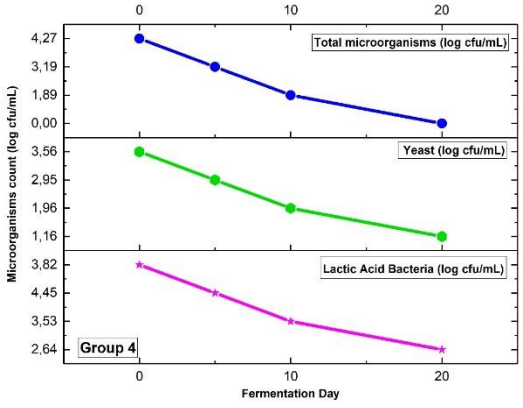

Figure 7. Microbiological results of G4

This decline has been attributed to various factors, including bioactive compounds present in pickles, nutritional deficiencies, or the development of acidity. Berger et al. (2020) determined that the highest organic acid content produced by LAB were achieved with the addition of 60 g/kg NaCl and 0.050 g/kg starter culture at 22°C after 12 days. Subsequently, a slight decrease in antioxidant compounds formed by LAB occurred until the end of the fermentation process, accompanied by a reduction in pH. Yeasts metabolize carbohydrates into ethanol, carbon dioxide, and various secondary products, playing a significant role in alcohol fermentation. However, mold growth is undesirable during a healthy fermentation process. In the present study, it is seen that the yeast loses its viability after a certain period of time. The average yeast count for all groups was 1.30 log cfu/mL at the end of fermentation (Table 2). This situation is attributed to the fermentable sugar remaining in the environment after the primary fermentation ends, yeasts tend to use these sugars to move to the secondary fermentation stage. These findings are generally consistent with the yeast and mold count ranges reported by Şengün et al., (2020).

When G2 (only 5% lemon juice) and G3 (only 5% vinegar) were compared, it was determined that there was a remarkable difference in terms of LAB viability. These viabilities, the G2, completed the fermentation with an average of 5 logarithms with an increase of 2 logarithms (Figure 5), while in the G3 there was a serious decrease in viability, and they completed the fermentation with an average of 2 logarithms (Figure 6).

These results showed that vinegar, which is mainly used in pickle making, especially in industry, has a stronger acidification and antimicrobial effect than lemon. However, it has also showed that this effect does not have a very positive effect on the vitality of LAB, which is the basis of fermentation and has many healthy effects. Organic acids, particularly acetic acid found in vinegar, induce bacterial cell death by affecting the cell membranes of microorganisms (Nascimento et al., 2003; Saqib, 2017 Yangılar et al., 2023). Additionally, the enhancement of antibacterial activity by the presence of phenolic compounds in vinegar has also been reported (Kara et al., 2021; Ousaaid et al., 2022; Yangılar et al., 2023). Acetic acid, identified as a dibasic acid with high dissociative capacity, exerts the strongest inhibitory effect on Gram-negative bacteria at the same concentration compared to other organic acids; furthermore, the inhibitory activity escalates with increasing concentration of organic acid and decreasing pH, aligning with our findings, as reported in the literature (Ji et al., 2023; Tosun, 2021). Several conducted studies have reported that acetic acid exhibits greater antimicrobial activity against various microorganisms compared to other food-grade organic acids. It was expressed that the effectiveness of acetic acid in inactivating studied microorganisms might be attributed to its relatively small molecular weight, as well as its more complex structures and different pKa values (Ryu et al., 1999). Ji et al., (2023) observed that acetic acid (AA) had the most significant destructive impact on the cell membrane, followed by butyric acid (BA), citric acid (CA), and malic acid (MA), with each acid displaying specific destructive effects on the cell wall and intracellular proteins in *Escherichia coli*. Various microorganisms display different antimicrobial responses to distinct types of organic acids (Ahn and Shin, 1999). Citric

acid is a weak organic acid, it has the ability to cross the cell membrane and lower the intracellular pH. This decrease damages DNA, protein, and extracellular membranes and leads to the death of bacteria (Park et al., 2020). On the other hand, citric acid can be used as a carbohydrate source to provide energy to lactic acid bacteria (LAB) and accelerate their growth. Furthermore, citric acid can also effectively reduce the pH and inhibit the growth of yeast and mold (Li et al., 2016; Ke et al., 2017; Ke et al., 2018; He et al., 2020; Lv et al., 2020) which is in harmony with our study. Our data are consistent with those of previous studies showing that the growth of LAB and addition of citric acid can control the other bacteria during the fermentation processes (Li et al., 2020; Lv et al., 2020; Seo et al., 2013)

In G4, 5% salt and 5% lemon juice were used. In this group, there was a linear decrease in the number of LAB over time (Figure 7). Sodium and chloride ions associate with water molecules to decrease water activity, causing microbial cells to undergo loss of water from osmotic shock and inducing cell death or retarded growth (Treesuwan et al., 2023). Results supporting this information were obtained and it was determined that the salt added to lemon juice had a certain inhibitory effect on three groups of microorganisms. Yoon et al. (2014) reported that the addition of salt in treatments with citric acid resulted in rapid reduction the number of Shigella flexnari compared to treatment with acids alone. Bae and Lee (2015) reported that a combination of 3% salt with various acids (citric, malic, tartaric and phosphoric) significantly decreased the survival of *Escherichia coli* O157:H7, *Salmonella Typhimurium* and *Listeria monocytogenes* (synergistic effect). Adding organic acid and salt (NaCl) to food products is a common practice for food preservation as the hurdle effect. When employing hurdle technology, three interaction results are possible: "additive effect", "synergistic effect", and "antagonistic effect" (Lee 2004; Biesta Peters et al., 2010; Bae and Lee, 2015). However, the effectiveness of organic acids individually and combined with salt in killing bacteria has shown conflicting results in the literature. Some studies reported a synergistic effect Entani et al., (1998) with the addition of organic acid and salt, while others determined an antagonistic effect (Lee and Kang 2009; Lee et al., 2010; Yoon et al., 2014). Microorganisms exhibiting various responses under stress could pose challenges to food preservation and may complicate the application of hurdle technology (Lee and Kang, 2009; Bae and Lee, 2015). Therefore, it is crucial to study the effects of these agents on both food-grade and foodborne microorganisms in numerous further research studies.

In G5, 5% salt and 5% vinegar were used (Figure 8). When comparing G4 and G5, the decrease in yeast and total microorganism counts is parallel, while the difference in LAB viability is particularly striking. Entani et al., (1998) demonstrated that acetic acid, even at a concentration as low as 0.1%, inhibited the growth of all 17 food-borne pathogenic bacterial strains, indicating strong bacteriostatic activity. This effect was further enhanced by the addition of sodium chloride. Additionally, they reported that combining diluted vinegar and sodium chloride solutions synergically increased bactericidal activity against *EHEC* 0157:H7, despite reduced concentrations of each component (Entani et al., 1998).

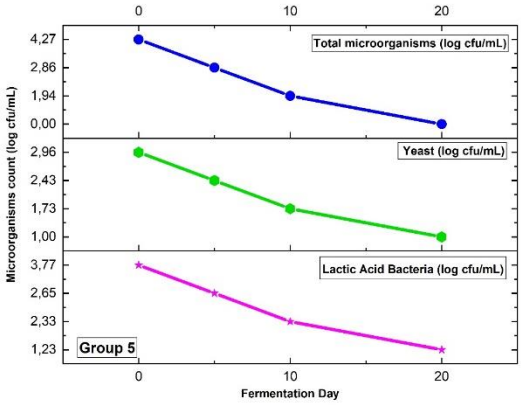

Figure 8. Microbiological results of G5

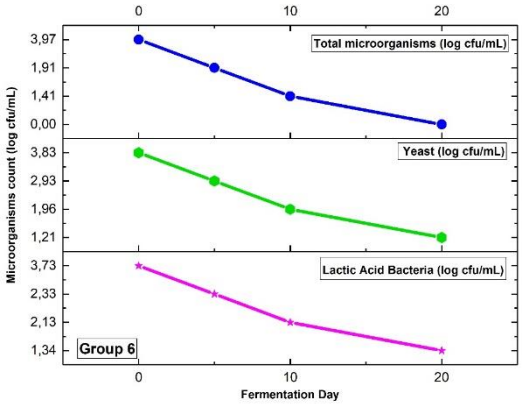

Figure 9. Microbiological results of G6

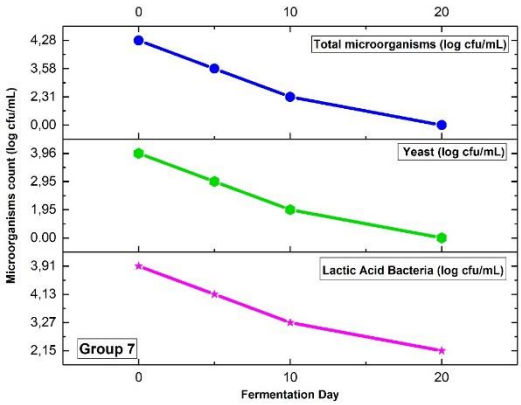

Figure 10. Microbiological results of G7

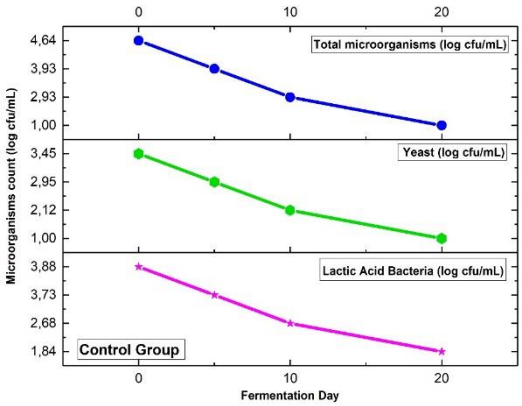

Figure 11. Microbiological results of Control Group

Bae and Lee (2015) reported that a combination of 3% salt with various acids (acetic, propionic, and lactic) significantly decreased the survival of *Listeria monocytogenes* (synergistic effect). Conversely, the same combination of salt and acids significantly increased the survival of *Escherichia coli* O157:H7 and *Salmonella Typhimurium* (antagonistic effect). Yoon et al., (2014) also reported that similar antagonistic effects were observed in *Shigella flexneri* with a treatment of either 0.5 or 1% acetic acid combined with 3% NaCl. Additionally, Entani et al., (1998) determined that temperature also had varying effects on the combined actions of vinegar and sodium chloride, with the time required for inactivation decreasing as the temperature increased. Thus, both antagonistic and synergistic effects might be correlated with the resistance mechanisms of the microorganism, as well as the exposure conditions, including factors such as temperature, time, and acid concentrations.

The antimicrobial activity of organic acids is based on lowering the internal pH of microbial cells and disrupting substrate transport by altering cell membrane permeability. It is known that citric acid is a weak organic acid even so it has some antimicrobial activity, although not as much as acetic acid (Treesuwan et al., 2023).

Using a single organic acid at a specific concentration often results in increased costs, impacts product flavor, and leads to nutrient loss. Hence, combining organic acids through the synergistic activity of target bacteria can enhance bacterial inhibition and reduce the overall usage of organic acids (Ji et al., 2023). In our study, while slightly decrease was observed in the total number of bacteria and yeast during the first 5 days in G2 (5% lemon juice) a notable decrease was observed, particularly after the 5th day, coinciding with an increase in the number of LAB (Figure 9). This suggests that the effect of citric acid on microorganisms other than LAB is minimal, and the inhibitory effect is predominantly due to the antimicrobial agents produced by LAB. However, in G6 (5% lemon juice and 5% vinegar), there was a notable decrease in the total number of bacteria, yeast, and LAB within the initial 5 days. The inhibitory effect on all microorganisms from the beginning to the 20th day is believed to be attributed to the acetic acid used, in contrast to G2. It is known that acetic acid has an inhibitory effect on the growth of various microorganisms. This effect is thought to be due to the penetration of acetic acid into cells and the resulting intracellular acidification (Kusumegi et al., 1998). Citric acid destabilizes bacterial cell membranes and facilitates the membrane translocation of other weak organic acids, thereby enhancing the synergistic antibacterial capability among organic acids (Alakomi et al., 2007). Studies have explored the combination of organic acids for cleaning and disinfecting meat and fresh vegetables (Wang et al., 2019; Gonzalez-Fandos, 2020), demonstrating more effective bacterial inhibition. Ji et al., (2023) found that the combination of acetic acid (AA) and lactic acid (LA) exhibited an additive effect, whereas the combination of AA, LA, and a third organic acid (butyric acid, citric acid, malic acid) demonstrated a synergistic effect in inhibiting the growth of *Escheriachia coli*. They emphasized that, overall, the combination of organic acids can effectively exert antibacterial effects by disrupting the cell barrier and has the potential to demonstrate a synergistic anti-biofilm effect.

In group 7, both types of acid and salt were added in equal percentages (Figure 10). With the use of both acids and the effect of salt, there was a rapid decrease in the number of microorganisms during the fermentation period.

There is no acid or salt additive in the control group (Figure 11). Therefore, there was no significant decrease in the number of microorganisms during the fermentation period. The current decline has been attributed to nutritional deficiency that occurs after a certain period of time.

Generally, yeasts are undesirable in fermentation as they allow oxidative and fermentative activities, CO_2-induced degradation and activities of other degradation factors. However, they can help the flavor of the product by producing flavoring substances such as diacetyl as a result of their metabolism (Ferreira and Mendes-Faia 2020). During the primary fermentation stage during pickle formation, the dominant group in the microbiota is lactic acid bacteria. However, fermentative yeasts can also be found, and their reproduction depends on the complete use of the sugar in the medium or until the acid inhibition occurs. The stage in which fermentative yeasts develop is the secondary fermentation stage. After the growth of lactic acid bacteria slows down due to low pH, yeasts ferment the remaining sugar in the environment (Özkan, 2016). When the study results are examined that the decrease in the number of yeasts slows down with synchronously the decrease in the number of LAB in all groups on the 10th day and after, and after a while, they complete the fermentation with an average of 1 logarithm. In addition, it is noteworthy that there are no live yeast colonies in the G7, which consists of a combination of salt, vinegar, and lemon, when fermentation is completed.

Considering the LAB and yeast viability results, several possibilities were considered. The first is that the fermentable sugar remaining in the medium after the primary fermentation is consumed by the yeasts and takes part in the secondary fermentation stage. Second, there are both positive and negative interactions between yeast and LAB strains. Some LABs can secrete galactose, which can help lactose-negative yeasts grow, and vice versa. For example, yeast growth can be inhibited by compounds such as 4-hydroxy-phenyllactic and phenyl-lactic acid produced by LAB. (Alvarez-Martin et al., 2008).

Microorganisms thought to belong to the coliform group could not survived except the control group at the end of fermentation. Apart from the added acid and salt, it is thought that some organic compounds (lactic, acetic, formic, caproic, propionic, butyric and valeric acid) which are known to have antagonistic effect (Zala´n et al., 2010) may have been effective produced by LAB. In the study, mold growth was not detected in any group.

It is known that when the pH value falls below 4.5 in fermented products, it is a limiting factor for the growth of Enterobacteriaceae (Özer and Kalkan Yıldırım, 2018). When physicochemical analysis results such as pH and titratable acidity of the groups are examined; during the fermentation, the average pH of the pickle groups decreased from approximately 3.87 to 2.55, and the average titratable acidity of them increased from approximately 0.63 to 0.90 %. The results are given in Table 2, and the change graph is shown in Figure 12.

Table 2. Microbiological (log cfu/ml) and physicochemical analysis results of pickle groups during the fermentation period

Day	G1	G2	G3	G4	G5	G6	G7	C
Lactic Acid Bacteria (log cfu/ml)								
0	3.84±0.01^{D}	3.74±0.03^{G}	3.96±0.02^{A}	3.82±0.02^{E}	3.77±0.10^{F}	3.73±0.06^{H}	3.91±0.06^{B}	3.88±0.10^{C}
5	4.94±0.02^{C}	5.06±0.03^{B}	6.81±0.01^{A}	4.45±0.02^{D}	2.65±0.05^{G}	2.33±0.15^{H}	4.13±0.06^{E}	3.73±0.05^{F}
10	4.96±0.01^{B}	5.15±0.04^{A}	3.62±0.02^{C}	3.53±0.02^{D}	2.33±0.06^{G}	2.13±0.06^{H}	3.27±0.06^{E}	2.68±0.10^{F}
20	3.95±0.03^{B}	5.35±0.03^{A}	1.82±0.03^{F}	2.64±0.05^{C}	1.23±0.06^{H}	1.34±0.10^{G}	2.15±0.02^{D}	1.84±0.05^{E}
0-20. Day % Survivability								
	102.86±0.01	143.04±0.03	45.95±0.01	69.10±0.01	32.62±0.03	35.92±0.02	54.90±0.05	47.42±0.02
Yeast (log cfu/ml)								
0	4.05±0.04^{B}	4.14±0.03^{A}	3.44±0.05^{G}	3.56±0.03^{E}	2.96±0.02^{H}	3.83±0.05^{D}	3.96±0.03^{C}	3.45±0.05^{F}
5	3.34±0.04^{B}	3.83±0.05^{A}	2.63±0.04^{G}	2.95±0.05^{E}	2.43±0.05^{H}	2.93±0.05^{F}	2.95±0.05^{D}	2.95±0.05^{C}
10	2.94±0.04^{A}	2.44±0.05^{B}	1.96±0.03^{E}	1.96±0.03^{E}	1.73±0.05^{G}	1.96±0.05^{D}	1.95±0.05^{F}	2.12±0.06^{C}
20	1.15±0.04^{C}	1.00±0.00^{E}	1.04±0.05^{D}	1.16±0.02^{B}	1.00±0.01^{E}	1.21±0.01^{A}	0.00±0.00^{F}	1.00±0.00^{E}
0-20. Day % Survivability								
	28.39±0.03	24.15±0.00	30.23±0.02	32.58±0.01	33.78±0.01	31.59±0.01	0.00±0.00	28.98±0.01
Total microorganism(log cfu/ml)								
0	4.28±0.01^{B}	4.24±0.06^{E}	3.98±0.01^{F}	4.27±0.06^{D}	4.27±0.10^{C}	3.97±0.02^{G}	4.28±0.15^{B}	4.64±0.15^{A}
5	3.01±0.02^{E}	3.89±0.09^{B}	2.89±0.09^{F}	3.19±0.03^{D}	2.86±0.05^{G}	1.91±0.12^{H}	3.58±0.02^{C}	3.93±0.06^{A}
10	1.92±0.06^{D}	1.33±0.05^{H}	1.81±0.05^{F}	1.89±0.06^{E}	1.94±0.06^{C}	1.41±0.03^{G}	2.31±0.02^{B}	2.93±0.06^{A}
20	0.00±0.00	0.00±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.00±0.00
0-20. Day % Survivability								
	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	21.55±0.06
pH Change								
0	3.90±0.06^{E}	4.08±0.02^{C}	4.34±0.04^{A}	3.96±0.02^{D}	4.22±0.01^{B}	3.20±0.02^{H}	3.84±0.04^{F}	3.43±0.03^{G}
5	3.75±0.04^{D}	3.91±0.03^{B}	3.83±0.04^{C}	3.04±0.04^{G}	3.98±0.02^{A}	2.98±0.01^{H}	3.33±0.03^{E}	3.11±0.01^{F}
10	2.91±0.05^{G}	3.42±0.03^{B}	3.07±0.06^{E}	2.94±0.03^{F}	3.43±0.03^{A}	2.74±0.04^{H}	3.07±0.06^{D}	3.07±0.06^{C}
20	2.83±0.02^{F}	2.99±0.01^{A}	2.86±0.02^{E}	2.77±0.02^{G}	2.94±0.04^{B}	2.54±0.03^{H}	2.86±0.05^{D}	2.87±0.01^{C}
Titration Acidty %								
0	0.76±0.01^{A}	0.69±0.01^{C}	0.59±0.01^{F}	0.54±0.02^{H}	0.60±0.01^{E}	0.69±0.03^{B}	0.56±0.02^{G}	0.62±0.03^{D}
5	0.82±0.02^{A}	0.73±0.03^{C}	0.64±0.02^{E}	0.59±0.01^{G}	0.66±0.03^{D}	0.75±0.01^{B}	0.59±0.01^{F}	0.66±0.02^{D}
10	0.83±0.01^{C}	0.82±0.01^{D}	0.65±0.02^{H}	0.80±0.03^{G}	0.88±0.01^{A}	0.81±0.04^{E}	0.80±0.02^{F}	0.87±0.01^{B}
20	0.88±0.03^{D}	0.96±0.02^{E}	0.78±0.01^{H}	0.91±0.01^{G}	0.90±0.02^{B}	0.92±0.02^{C}	0.94±0.01^{F}	0.89±0.01^{A}

G1: (5 % Salt); G2: (5 % Lemon Juice); G3: (5 % Vinegar); G4: (5 % Salt and 5 % Lemon Juice); G5: (5 % Salt and 5 % Vinegar); G6: (5 % Lemon Juice and 5 % Vinegar); G7: (5 % Salt, 5 % Lemon Juice and 5 % Vinegar); C: Control; (No Salts or Acids); Values are means of three independent determinations and standard errors. In the same column, the letters from A to H represent the largest to smallest order of numeric values, respectively ($P<0.05$)

Cabbage fermentation is deemed complete when the pH value of the final product falls within the range of 3.4–3.6 (Hutkins, 2006). In this regard, fermented cabbage is characterized by its appealing aroma, flavor, color, and texture (Jevšnik et al., 2009; Terefe, 2016). All pickle groups achieved a pH between 3.4 and 3.6 before the 10th day, with a significant decrease in pH value ($P<0.05$) observed during the fermentation process. It is thought that LAB affects the decrease of pH during fermentation, and this is very important for the success of the fermentation process. By this means this rapid decrease, most Gram (-) bacteria and spore-forming bacteria are inhibited, and the deterioration of the pickles is delayed/prevented. Berger et al 2020, reported that results of pH on 5th, 12th, 27th, and 62nd fermentation day were in range 5.31–4.67, 4.37–3.51, 3.25–3.04, and 3.06–2.93, respectively for fermented cabbage. In contrast to Berger's study, our research suggests that the initial pH levels are lower. This could be related to the suppression of non-lactic acid bacteria (non-LAB) microorganisms, and thus, due to the rapid growth of LAB, the pH may have decreased more rapidly over a shorter period, influenced by the secondary metabolites of LAB. It is noted that the fermentation process can be halted at any time after the pH value falls below 4.1 and, furthermore, 3.8 for a mild acid taste (Holzapfel et al., 2008) and distributed as a pasteurized product. Based on the pH values, fermentation should be stopped before the 10th day in all pickle samples. Considering the most suitable alternative for both pH levels between 3.4 and 3.6 and higher LAB viability, G2 fermentation should be stopped between the 5th and 10th days. The titration acidity of the samples on the first day of fermentation was found to be between 0.65% on average, these results were thought to be based on citric and acetic acid added to the brine. Acid development in the control sample was slower, as was the pH change. In addition, it was observed that the development of acidity was slower in G3. The highest titration acidity (0.96%) was determined in G2 at the end of fermentation. The results are given in Table 2, and the change graph is shown in Figure 13. As observed in our study, the lower abundance of LAB, the development of slow acidity, and additionally, the exact opposite situation can be explained consistently with the findings of Tomita et al., (2024). They reported that in pickles with low salt content, the metabolism of lactic acid bacteria can suppress environmental acidity. In the production of sunki (unsalted pickles), lactic fermentation may be inhibited for various reasons, including a lack of nutrients and infection with bacteriophages. Consequently, this leads to a low *Lactobacillus* count and an insufficient decrease in pH, significantly compromising the quality of the sunki product.

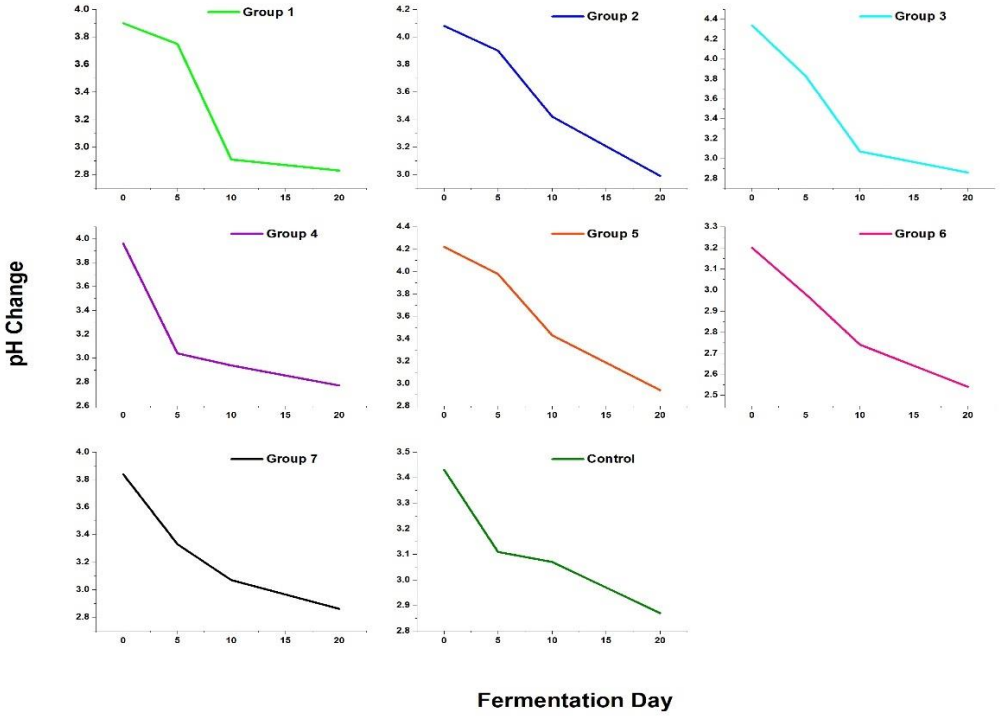

Figure 12. pH changes of pickle groups during fermentation

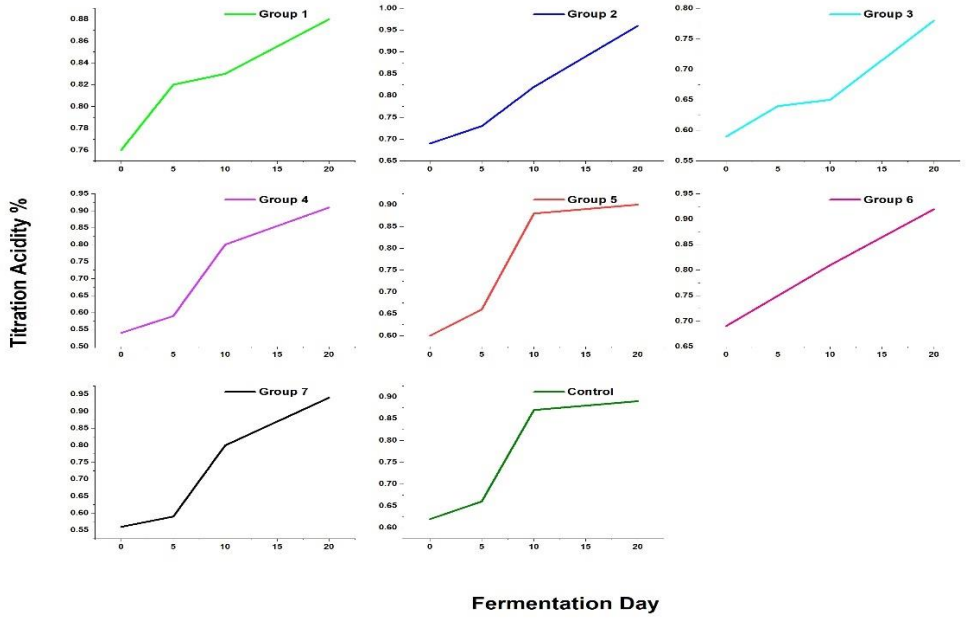

Figure 13. Titration acidity of pickle groups during fermentation

Conclusion

The positive effects of fermented foods on our digestive system are associated with beneficial bacteria and their various properties. These bacteria and some yeasts use various fermentation pathways to improve the taste and nutritional quality of vitamin-rich vegetables (Al-Shawi et al., 2019). In addition, lactic acid bacteria in the microbiota of pickles, have been associated with various probiotic properties such as increased natural resistance to infectious diseases in the gastrointestinal tract, prevention of various infections, and reduction in cholesterol level (Liong and Shah, 2005). In our study, it was investigated how acid diversity and salt affect the viability of LAB, which has the potential to have these properties, in cabbage pickle obtained by self-production without starter culture. The

results proved that acetic acid exhibits a stronger inhibitory effect than citric acid on foodborne microorganisms and also LAB, and the inhibitory effects of both acids are further enhanced by the addition of salt and/or when used organic acids in combination rather than individually. On the other hand, citric acid had a protective effect on LAB and accelerated their growth, likely acting as a carbohydrate source to provide energy for LAB. Consequently, based on our results, especially lemon juice (G2) could be preferred in homemade pickles production for promoting the growth of LAB and inhibiting undesirable microbial groups. Moreover, the LAB present in the natural microbiota can be effective in terms of obtaining high viability rate at the end of fermentation.

Conflict of interest

The authors declare that there is no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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