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RESEARCH ARTICLE

Optimization of lactic acid bacteria viability using fuzzy soft set modelling

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ABSTRACT

Lactic acid fermented vegetables are important sources of vitamins and minerals. In recent years consumers demand for non-dairy based functional products has increased. Cabbage pickle has high enough concentrations of fiber and also it may show health effect with the containing high numbers of lactic acid bacteria. The aim of this study is to optimize mathematically cabbage-carrot pickle fermentation for the viability of *Lactobacillus acidophilus*, *Lactobacillus casei* cultures and the sensory scores in brine with 5% and 7% (w/v) salt concentrations. Viability optimization of lactic acid bacteria is done via the notion of "*fuzzy soft set*" method. *Lb. casei*, *Lb. acidophilus*, total lactic acid bacteria, *Enterobacteriaceae* sp., yeast-mould counts and pH values have been reported during the 30 days of storage. The results are compared with the control traditional fermented cabbage-carrot pickle. Organoleptic properties are evaluated. We conclude that the fermented pickle samples contain a significant number of beneficial lactic acid bacteria and high sensory marks at the end of the storage.

1. Introduction

Nowadays consumers pay a lot of attention to the relation between food and health. The market for foods with health promoting properties called functional foods has shown remarkable growth over the last years. In this context, functional foods have received considerable attention in recent years [1].

Functional lactic acid bacteria can reduce the number of undesired microorganisms in vegetable products [2]. It has some functions on antibiotic-associated diarrhea and immune system functions [3]. *Lb. acidophilus* and *Lb. casei* produce lactic acid as the main end product of fermentation. In addition, lactic acid bacteria produce hydrogen peroxide, diacetyl and bacteriocin as antimicrobial substances. *Lb. acidophilus* strains can exert anti-listerial bactericidal activity which could be of great technological importance (see [3] and [4] for more details). Also, *Lb. casei* decreased the severity of infection with *Salmonella enterica* serovar Typhimurium, a daily supplementation of *Lb. acidophilus* during post antibiotic therapy reduced the extent of disruption to the intestinal microbiota (see [3] and [5] for more details). These strains can prevent allergic disease, reducing lactose intolerance, enhancing bioavailability of nutrients. Researchers reported that *Lb. acidophilus* (acidolin , acidophilin producer) into the diet lowers the incidence of chemically induces colon tumors in rats (see [3] and [6] for more details).

Vegetables are good sources of natural antioxidants such as carotenoids, vitamins, minerals and dietary fibers. Vegetables may be preserved by fermentation, direct acidification, or a combination of other processing conditions and additives

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to yield products that are referred to as pickles. Cucumbers, cabbages, olives, peppers account for the largest volume and lesser quantities of onions, tomatoes, cauliflowers, carrots, melon rinds, okra, artichokes, beans etc. are also pickled. This method of food preservation has been used for many centuries [7]. Fermented vegetables are good sources of lactic acid bacteria (LAB). Representatives of some important genera such as *Leuconostoc*, *Lactobacillus*, *Lactococcus* and *Pediococcus* are found in fermented vegetables. It was reported that *Leuconostoc* sp. was the main species in the early stages of fermentation, while *Lactobacillus* sp. became predominant with the pH value gradually falling to 4.0 [8]. Plant fibers provide a promising alternative to suppress systemic inflammation, to reduce the risk of developing other chronic diseases and to considerably improve quality of life. Treatment with specific lactic acid bacteria and plant fibers has shown a unique ability to suppress inflammation in animal models and to prevent destruction of tissues [9].

The usage of starter culture (Lactic acid bacteria - LAB) performs food safety and causes rapid decrease of the pH. The controlled fermentation of pickles reduces economic losses and leads to get uniform quality product over a short period time. In the last years, *Lb. casei* and *Lb. acidophilus* were began to use in vegetable and fruit juice based products such as tomato, cabbage, beet, orange, pineapple, carrot, grape juices (see [10], [11] and [12] for more details). The high salt levels are used to select for naturally occurring, heterofermentative and homofermentative lactic acid bacteria (LAB) to carry out the fermentation and then to protect against spoilage after the active fermentation period. However, health authorities recommend a reduction of the salt content in food and nowadays consumers of industrialised countries demand low-salt foods for health reasons (see [6], [13], [14] and [15] for more details).

In this study our aim is to establish the growth prediction models under two different salinity conditions (5% and 7% w/v) for two critical microorganisms of *Lb. casei* and *Lb. acidophilus* in functional pickled cabbage-carrot processing involved. We analyze the obtained results using the notion of a fuzzy soft set. There exist some applications of the notions of "*soft set*" and "*fuzzy soft set*" in many areas of science (see [16], [17], [18], [19] and [20] for more details). This study is the first application of the fuzzy soft set theory in food engineering. By this approach, it can be easily optimize the results by using appropriate parameters and degree of membership functions. We see that this decision making method gives accurate and adequate predicts.

2. Materials and methods

In this section we explain our approach and methods.

2.1. Pickle production

Cabbage and carrots were bought from a local market in Balıkesir, Turkey. Cabbage outer leaves were separated and after cut the halves, all pieces shredded small pieces (about $2 \times 2 \times 0.02$ cm^3 dimensions), they washed with tap water before treatment rinsed with a disinfectant (Surfcera-Vegisafe, Japan) solution. Carrots were trimmed, washed and cut into slices. After that cabbage and carrot pieces were mixed and divided into the groups and filled into the glass-jars.

5% and 7% of (w/v) NaCl were added in to the tap water. Brine solutions were sterilized at 100°C/20 min and cooled to 20° C. Garlic 10 g/lt, Saccharose 5 g/lt, Grape vinegar 10 ml/lt were added into the per jar and brine solution were filled completely in pickle jars.

2.2. Bacterial cultures

Lactobacillus casei (NRRL B-1922) and *Lactobacillus acidophilus* (NRRL B-4495) freeze-dried strains were obtained from United States Department of Agriculture, Illinois, US. They activated in sterilized Liver Infusion Broth (Difco, US) and then activated young culture (10^{10} cfu/ml) of them added (5 ml/lt) into the pickle jars and mixed homogeneously. Lactic acid bacteria were not added into the control groups. *Lb. casei* and *Lb. acidophilus* as starter cultures were inoculated about 10^8/ml into the samples and the number of total lactic acid bacteria was 10^6/ml on raw vegetables before the fermentation process. *Lb. casei* is one of the normal microflora bacterium in pickles and it was determined as < 2 log cfu/g in control pickle groups. *Lb. acidophilus* was not found in control pickles.

At first, pickles were incubated in 35°C/8 h growing conditions for *Lb. acidophilus* and *Lb.casei* (see [21] and [22] for more details) for developing acidity. Then they were incubated at 20 °C/15 d [23] after that they stored in refrigerator at 4°C. Acidity, viable numbers of *Lb. casei*, *Lb. acidophilus*, total lactic acid bacteria and yeast and mould counts were determined in 1., 4., 10., 17. and 30. days.

2.3. Microbiological analysis

Samples were diluted in (1:10) in Buffered Peptone Water (BPW) and homogenized for 20 s in a stomacher (Bag mixer, Interscience, FR). Subsequently, a decimal dilution series made in BPW and enumeration was performed by pour plate or spread plating techniques (see Table 1).

Table 1. Media and incubation conditions used for determining microorganism numbers.

Microorganism	**Media**	**Incubation conditions**
Total lactic acid bacteria numbers	Man Rogosa Sharpe agar	30°C/2-3 d
Lb. casei number	MRS-Vancomycine agar 2 ml/l vancomycine (0.05 g vancomycine/100 ml) in MRS.	30° C/2-3 d
Lb. acidophilus number	MRS-Sorbitol agar (10 ml, 10% (w/v), D-Sorbitol/90 ml agar)	35 °C/2-3 d
Enterobacteriaceae sp.	Violet Red Bile Dextrose Agar	37°C/ 24 h
Yeast-Mold numbers	Rose Bengal Agar	30 °C/3-5 d

2.4. Sensory analysis

After the 30 days of storage, the products were sensorially evaluated for taste, odor, color, texture, and overall acceptability by a panel consisting of 7 sensory experts. The panelists, who have considerable background knowledge in sensory evaluation, were selected from the staff, researchers. The score given by the panel varied from 1 (dislike extremely) to 5 (like extremely). The sensory characteristics of odor, texture, and taste were assessed on each test sample. Sensory analysis of the pickle sample was evaluated explaining the following descriptors – salty, acidic sourness, sweet acidic, smelly, bitter, kraut sulfur flavor, raw cabbage flavor, discoloration, pleasant aroma and overall acceptability. A sample was considered as unacceptable for a sensory characteristic if the score was less than 2.5 (see [24] and [25] for more details).

2.5. Decision making via "Fuzzy Soft Sets"

We optimize the lactic acid bacteria viability and sensory acceptability in the production of white cabbage-carrot pickle fermentation in different salinity conditions by means of "*fuzzy soft sets* " [26] modelling. At first, the membership functions which through all of the results using MATLAB (Matlab R 2015 a and Curve Fitting Toolbox -Version 8.5, The Mathworks, Inc., Natick, Massachusetts, United States) for pH, *Lb. casei*, *Lb. acidophilus*, lactic acid bacteria and yeast–mold numbers were constructed. It was used the notion of "*fuzzy soft set*" to optimize the results. To do this it was defined a universal set and a parameter set. Fuzzy soft sets were obtained by use of the membership functions, the universal set and the parameter set. Finally, Table 3 was constructed using the values of membership functions and matrix theory [18]. Using this table it can be decided the most appropriate salt concentration in pickles and compared with the sensory scores.

3. Results

In this section we present the obtained results.

3.1. Acidity results

The usage of the starter culture performs food safety and causes rapid decrease of the pH. Decline of the pH resulted in all pickle samples with 5% and 7% salt (w/v) brine conditions. Rapid increases of active culture in the pickles caused optimum fermentation conditions and decreasing of the pH.

3.2. Microbiological analysis results

In our study, *Lb. casei* in 7% (w/v) salt concentration was affected from low pH (3.32) and high salt concentration conditions and the numbers were low (7.85 log cfu/gr) than the others at the end of the storage (see Figures 1-4). At the first day of fermentation *Lb. casei* numbers were 7.74 and 9.02 log cfu/g in pickles with 5% (w/v) and 7% (w/v) brine solution, respectively. However, at the end of the storage *Lb. casei* numbers were reached to 8.7 log cfu/g in pickles with 5% (w/v) brine solution and 7.85 log cfu/g with 7% (w/v) brine solution.

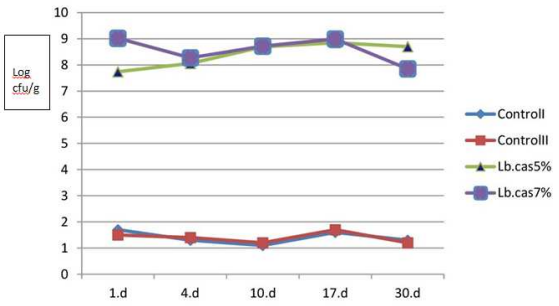

Figure 1. *Lb. casei* numbers (log CFU/gr).

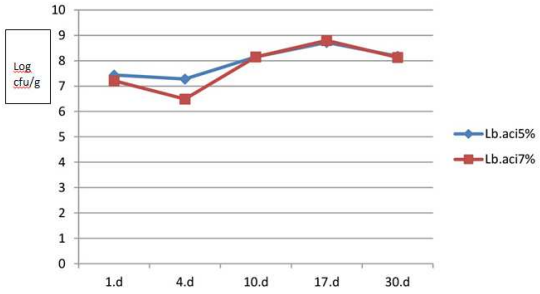

Figure 2. *Lb. acidophilus* numbers (log CFU/gr).

Total lactic acid bacteria numbers were determined as max. 9.5 and 8.88 log cfu/g for *Lb. casei* and *Lb. acidophilus*, respectively on the 17^{th} day of storage of 7% (w/v) brine solutions. However, it was found that 8.66 and 8,72 log cfu/g for *Lb. casei* and *Lb. acidophilus* numbers in 5% (w/v) brine solutions in the same day. After the 30 days of storage *Lb. casei* numbers decreased to 7.77 and 7.6 log cfu/g in 7% and 5% (w/v) salt concentration brine, respectively (see Figure 3).

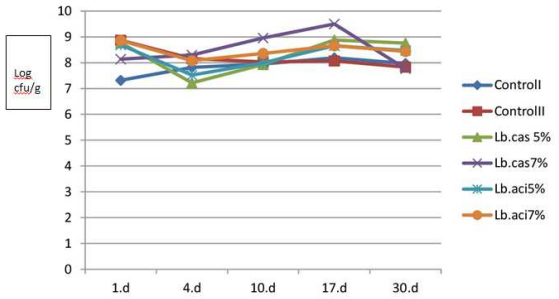

Figure 3. Total lactic acid bacteria (LAB) numbers (log CFU/gr).

Moulds didn't grow in any pickles. Only some yeasts were observed. Yeast numbers showed a sharply decline during the 30 days (see Figure 4). Maximum yeast numbers were 5.16 log cfu/g in *Lb. casei* containing pickles with 5% (w/v) salt concentrations brine on the first day. Then, minimum yeast numbers were determined as 0.28 log cfu/g in *Lb. acidophilus* containing pickles with 7% (w/v) salt concentrations brine on the 30^{th} day.

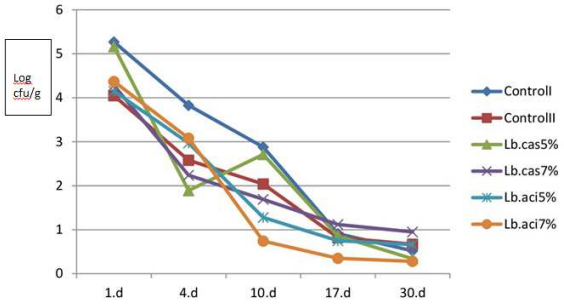

Figure 4. Total yeast numbers (log CFU/gr) during the storage of pickles.

In research, *Enterobacteriacea* spp. were not determined in pickles and it can be thought that because of washing vegetables with a disinfectant solution before treatments cause inhibition effects on these groups of bacteria.

3.3. Sensory analysis results

From the Figure 5, we can see that the sensory analysis results. Using these results we deduce that the pickles with *Lb. casei* culture in 5% salt concentration were preferred according to the overall, colour, taste and odour quality properties. But the texture of the pickles with 7% salt concentrations have higher scores than the others.

Figure 5. Sensory profile of pickle samples during the storage of 30 days.

3.4. Microbial analysis via fuzzy soft sets

Now we analyze the obtained results using the notion of "*fuzzy soft set*". The notion of a fuzzy soft set was introduced in [26] as follows:

Let U be a universal set, E be a set of parameters and $A \subset E$. Let $P(U)$ denotes the set of all fuzzy subsets of U. Then a pair (F, A) is called a fuzzy soft set over U, where F is mapping from A to $P(U)$.

Following algorithm can be presented for optimization of the salt concentration :

Step 1. Defining a universal set X and a parameter set E.

Step 2. Using MATLAB (Curve Fitting Toolbox- 2015), choose appropriate functions through all of the results obtained for *ph*, *lc*, *la*, *lab* and *ym*, respectively.

Step 3. Using the functions chosen in Step 2, define the membership functions for *ph*, *lc*, *la*, *lab* and *ym*, respectively.

Step 4. Constructing the fuzzy soft sets $(F_{x,i}, E_i)$, $i = 1$ to 6 where $x \in \{1, 4, 10, 17, 30\}$.

Step 5. Computing the fuzzy soft sets $(F_{30,i}, E_i)$, $i = 1$ to 6.

Step 6. Constructing a table of the fuzzy soft sets $(F_{30,i}, E_i)$ and insert "0" or "1" for each E_i, $i = 1$ to 6.

Step 7. Adding sensory scores column obtained by a panel consisting of 7 sensory experts to the above table.

Step 8. Making decision is the E_i, $(i = 1$ to $6)$ which has the top score "1".

Then, data can be established by fuzzy soft modeling to optimize the results as follows:

It can be defined the following notations:

ph : *pH value,*
lc : *Lb. casei number,*
la : *Lb. acidophilus number,*
lab : *Lactic acid bacteria numbers,*
ym : *Yeast - mold number,*

and

E_1 : Control %5,
E_2 : Control %7,
E_3 : *Lb. casei* %5,
E_4 : *Lb. casei* %7,
E_5 : *Lb. acido* %5,
E_6 : *Lb. acido* %7.

A universal set and a parameter set were defined as follows, respectively:

$$X = \{ph, lc, la, lab, ym\} ,$$

and

$$E = \{E_1, E_2, E_3, E_4, E_5, E_6\}.$$

Table 2. pH ± standard deviations (S.D.) of pickles during the storage at (1., 4., 7., 10., 17. and 30. days) +4 °C.

Products	**1**	**4**	**10**	**17**	**30**
Control I 5%	3.44±0.4	3.47±0.5	3.40±0.4	3.35±0.2	3.34±0.8
Control II 7%	3.58±0.6	3.61±0.9	3.55±0.5	3.41±0.5	3.39±0.4
Lb.casei 5%	3.83±0.3	3.70±0.7	3.52±0.7	3.45±0.5	3.39±0.5
Lb. casei 7%	3.73±0.5	3.59±0.6	3.44±0.9	3.33±0.6	3.32±0.7
Lb. acidophilus 5%	3.62±0.7	3.55±0.3	3.48±0.6	3.40±0.4	3.36±0.2
Lb.acidophilus 7%	3.63±0.4	3.55±0.8	3.46±0.3	3.42±0.9	3.39±0.5

Appropriate functions such as ph_i, lc_i, la_i, lab_i, ym_i were chosen using MATLAB (Curve Fitting Toolbox- 2015) where $i \in \{1, 2, 3, 4, 5, 6\}$. Then it was given membership functions for pH, *Lb. casei* numbers, *Lb. acidophilus* numbers, lactic acid bacteria numbers and yeast – mold numbers as the following cases:

Case1: Using MATLAB – Curve Fitting Toolbox and the results given in Table 2, following functions for pH value (see Figure 6) were chosen:

$$ph_1(x) = 0.0004x^3 - 0.0068x^2 + 0.0371x + 3.4094,$$
$$ph_2(x) = 0.0001x^3 - 0.0030x^2 + 0.0223x + 3.5619,$$
$$ph_3(x) = 0.0026x^2 - 0.0580x + 3.8882,$$
$$ph_4(x) = 0.0017x^2 - 0.0491x + 3.7719,$$
$$ph_5(x) = 0.0004x^2 - 0.0205x + 3.6352,$$
$$ph_6(x) = 0.0015x^2 - 0.0329x + 3.6606,$$

where $x \in \{1, 4, 10, 17, 30\}$.

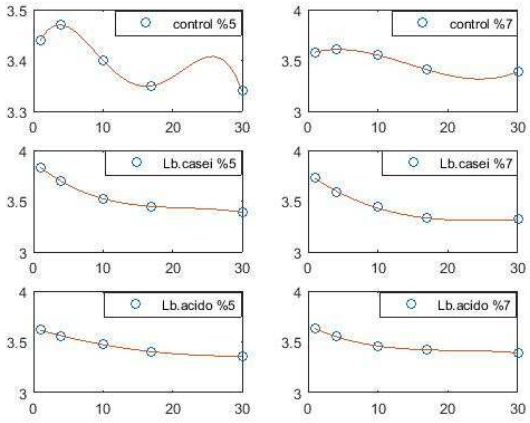

Figure 6. The graphics of the membership functions for pH value (the x-axis represent the days and the y-axis represent the pH values).

The following membership function was defined

$$\mu_i^{ph}(x) = \frac{ph_i(x)}{10^n},$$

for all $i \in \{1,2,3,4,5,6\}$, where n is the number of digits of the integer part of $ph_i(x)$.

Case 2: By a similar way, using the results given in Figure 1, following functions for *Lb. casei* number (see Figure 7) were chosen:

$$lc_3(x) = -0.0010x^3 + 0.0123x^2 + 0.0656x + 7.6632,$$
$$lc_4(x) = 0.0001x^4 - 0.0049x^3 + 0.0963x^2 - 0.6350x + 9.5735,$$

where $x \in \{1,4,10,17,30\}$.

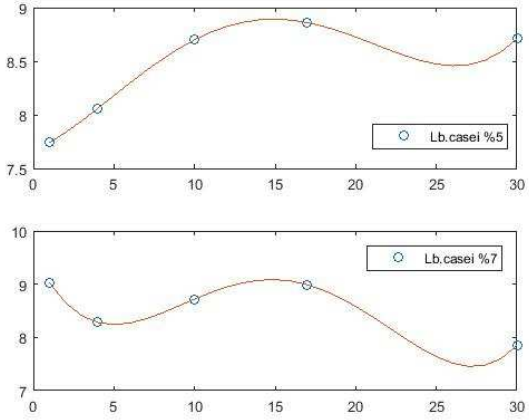

Figure 7. The graphics of the membership functions for *Lb. casei* number (the x-axis represent the days and the y-axis represent the *Lb. casei* numbers).

The following membership function was defined

$$\mu_i^{lc}(x) = \begin{cases} \frac{lc_i(x)}{10^n} \text{ if } i \in \{3,4\} \\ 0 \text{ if otherwise} \end{cases},$$

for all $i \in \{1,2,3,4,5,6\}$, where n is the number of digits of the integer part of $lc_i(x)$.

Case 3: Using the results given in Figure 2, following functions for *Lb. acidophilus* number were chosen (see Figure 8):

$$la_5(x) = 0.0001x^4 - 0.0035x^3 + 0.0657x^2 - 0.3156x + 7.7034,$$
$$la_6(x) = 0.0002x^4 - 0.0098x^3 + 0.1760x^2 - 0.9286x + 7.9723,$$

where $x \in \{1,4,10,17,30\}$.

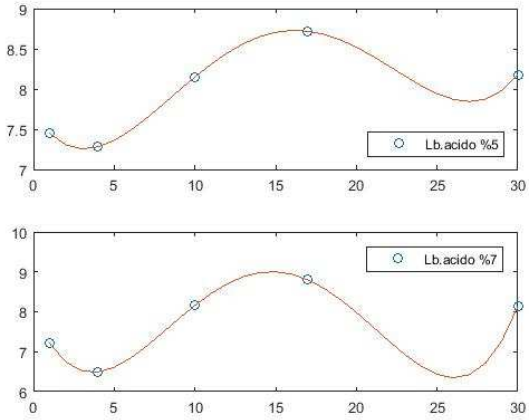

Figure 8. The graphics of the membership functions for *Lb. acidophilus* number (the x-axis represent the days and the y-axis represent the *Lb. acidophilus* numbers).

The following membership function was defined

$$\mu_i^{la}(x) = \begin{cases} \frac{la_i(x)}{10^n} \text{ if } i \in \{5,6\} \\ 0 \text{ if otherwise} \end{cases},$$

for all $i \in \{1,2,3,4,5,6\}$, where n is the number of digits of the integer part of $la_i(x)$.

Case 4: Using the results given in Figure 3, it can be chosen the following functions for lactic acid bacteria numbers (see Figure 9):

$$lab_1(x) = 0.0023x^3 - 0.0430x^2 + 0.3338x + 7.0369,$$
$$lab_2(x) = -0.0028x^3 + 0.0592x^2 - 0.4768x + 9.2904,$$
$$lab_3(x) = 0.0001x^4 - 0.0087x^3 + 0.1778x^2 - 1.2342x + 9.8350,$$
$$lab_4(x) = -0.0008x^3 + 0.0172x^2 - 0.0160x + 8.1396,$$
$$lab_5(x) = 0.0001x^4 - 0.0064x^3 + 0.1315x^2 - 0.9209x + 9.4857,$$
$$lab_6(x) = 0.0001x^4 - 0.0045x^3 + 0.0891x^2 - 0.6211x + 9.4065,$$

where $x \in \{1,4,10,17,30\}$.

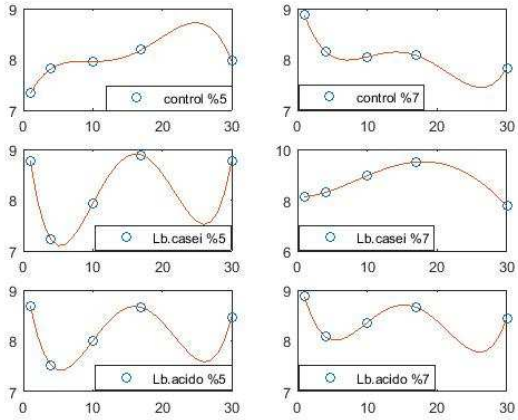

Figure 9. The graphics of the membership functions for lactic acid bacteria numbers (the x-axis represent the days and the y-axis represent the lactic acid bacteria numbers).

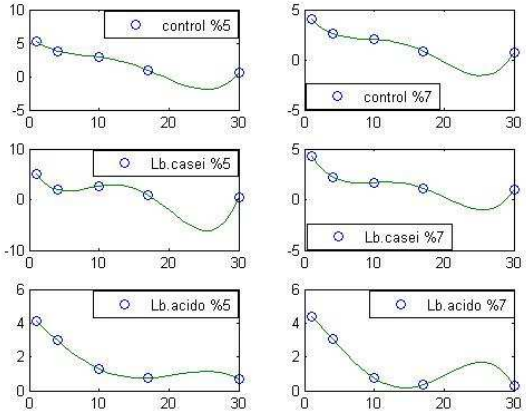

Figure 10. The graphics of the membership functions for yeast and mold numbers (the x-axis represent the days and the y-axis represent the yeast and mold numbers).

The following membership function was defined

$$\mu_i^{lab}(x) = \frac{lab_i(x)}{10^n},$$

for all $i \in \{1,2,3,4,5,6\}$, where n is the number of digits of the integer part of $lab_i(x)$.

Case 5: Using the results given in Figure 4, the following functions for yeast – mold numbers were chosen (see Figure 10):

$$ym_1(x) = 0.0001x^4 - 0.0069x^3 + 0.1173x^2 - 0.9326x + 6.0921,$$
$$ym_2(x) = 0.0001x^4 - 0.0073x^3 + 0.1321x^2 - 1.0078x + 4.9329,$$
$$ym_3(x) = 0.0004x^4 - 0.0238x^3 + 0.4215x^2 - 2.7341x + 7.4959,$$
$$ym_4(x) = 0.0001x^4 - 0.0086x^3 + 0.1700x^2 - 1.3615x + 5.4800,$$
$$ym_5(x) = 0.0010x^3 + 0.0018x^2 - 0.4209x + 4.5682,$$
$$ym_6(x) = -0.0001x^4 + 0.0038x^3 - 0.0392x^2 - 0.3070x + 4.7125,$$

where $x \in \{1,4,10,17,30\}$.

The following membership function was defined

$$\mu_i^{ym}(x) = \frac{ym_i(x)}{10^n},$$

for all $i \in \{1,2,3,4,5,6\}$, where n is the number of digits of the integer part of $ym_i(x)$.
The fuzzy soft sets $(F_{x,i}, E_i)$, $i = 1$ to 6 were constructed by considering the membership values $\mu_i^{ph}(x)$, $\mu_i^{lb}(x)$, $\mu_i^{la}(x)$, $\mu_i^{lab}(x)$ and $\mu_i^{ym}(x)$. It can be defined

$$(F_{x,i}, E_i) = F_i = \left\{ \frac{ph}{\mu_i^{ph}(x)}, \frac{lc}{\mu_i^{lc}(x)}, \frac{la}{\mu_i^{la}(x)}, \frac{lab}{\mu_i^{lab}(x)}, \frac{ym}{\mu_i^{ym}(x)} \right\},$$

where $i \in \{1,2,3,4,5,6\}$ and $x \in \{1,4,10,17,30\}$.
Now it can be investigated fuzzy soft sets for $x = 30$ and $i = 1$ to 6.

$$(F_{30,1}, E_1) = F_1(\text{control } \%5) = \left\{ \frac{ph}{0.334}, \frac{lc}{0}, \frac{la}{0}, \frac{lab}{0.797}, \frac{ym}{0.52} \right\}.$$

$$(F_{30,2}, E_2) = F_2(\text{control } \%7) = \left\{ \frac{ph}{0.339}, \frac{lc}{0}, \frac{la}{0}, \frac{lab}{0.783}, \frac{ym}{0.67} \right\}.$$

$$(F_{30,3}, E_3) = F_3(\text{Lb.casei } \%5) = \left\{ \frac{ph}{0.339}, \frac{lc}{0.871}, \frac{la}{0}, \frac{lab}{0.876}, \frac{ym}{0.34} \right\}.$$

$$(F_{30,4}, E_4) = F_4(\text{Lb.casei \%7})$$
$$= \left\{ \frac{ph}{0.332}, \frac{lc}{0.785}, \frac{la}{0}, \frac{lab}{0.777}, \frac{ym}{0.95} \right\}.$$

$$(F_{30,5}, E_5) = F_5(\text{Lb.acido \%5})$$
$$= \left\{ \frac{ph}{0.336}, \frac{lc}{0}, \frac{la}{0.818}, \frac{lab}{0.847}, \frac{ym}{0.66} \right\}.$$

$$(F_{30,6}, E_6) = F_6(\text{Lb.acido \%7})$$
$$= \left\{ \frac{ph}{0.339}, \frac{lc}{0}, \frac{la}{0.813}, \frac{lab}{0.843}, \frac{ym}{0.28} \right\}.$$

By a similar way, other fuzzy soft sets can be defined for $x \in \{1, 4, 10, 17\}$ and $i = 1$ to 6.

A results' table can be constructed to see the most accurate and adequate predictions in the research. It can be written "1" for the largest value of the membership function for each parameter E_i, $i = 1$ to 6 and "0" for other values. Then the row which has the top "1" score is chosen for the prediction. It can be seen from the Table 3 parameter E_3 is the optimum salt concentration for our research.

As it is seen from the Table 3, this model could accurately and adequately predict the growths of *Lb. casei*, *Lb. acidophilus*, total lactic acid bacteria and yeasts in pickles. And it can be shown that pickles with 5% (w/v) salt concentrations and *Lb. casei* culture gave the highest scores in this study.

Table 3. The results for $x = 30$ and $i = 1$ to 6.

	ph	*lc*	*la*	*lab*	*ym*	*sensory scores*
E_1	0	0	0	0	0	0
E_2	1	0	0	0	0	0
E_3	1	1	0	1	0	1
E_4	0	0	0	0	0	0
E_5	0	0	1	0	0	0
E_6	1	0	0	0	1	0

4. Discussions

Heterofermentative LAB are more sensitive to high salt concentrations than homofermentatives. Therefore high salt levels favor the growth of homofermentative LAB and resulting in an accelerated production of lactic acid. Maintaining the viability (minimum numbers of probiotic cultures present in the final product recommended to be 10^6 cfu/ml or higher) and the activity of lactic acid bacteria in foods to the end of shelf life are two important criteria [27].

Salt concentration can affect the growth of the naturally present microorganisms and the sensory properties of the pickles [28]. In the following studies they have shown potential benefits of using starter cultures in low-salt pickle fermentations. In Xiong et al. [29] study unfavorable conditions resulting from low pH contributed to the rapid decline of the lactococci as fermentation progressed. Similarly, Weng et al. [30] found that NaCl concentration affects the growth of *L. citreum* L-33 in pickle and the specific growth rate decreases with the increase of NaCl concentration. It also demonstrated that less salt addition can lead to a rapid growth for the important lactic acid bacteria in pickling production. Beganovic et al. [28] showed that the application of the probiotic strain *Lb. plantarum* L4 together with *Lc. mesenteroides* LMG 7954, positively influenced the fermentations by improving the quality of the final product with added probiotic properties, considerably shortening the fermentation time and offering the possibility of low salt fermentations (2.5 w/v). The rapid increase in acidity minimizes the influence of spoilage bacteria. Reducing the influence of spoilage bacteria would most probably improve the microbiological and sensory quality of the fermented end product significantly (see [31] and [32] for more details).

Yoon et al. [33] researched red beet juice fermentation with *Lb. acidophilus*, *Lb. casei*, *Lb. delbrueckii* and *Lb. plantarum* in their study. They found that *Lb. acidophilus* in fermented beet juice could be remained at $10^6 - 10^8$ cfu/ml after 4 weeks of cold storage and the others lost their viability. In this study, all viable numbers after the storage were $> 10^6$ cfu/g and it can be considered pickles have probiotic meaning health of view (Figures 1 and 2). *Lb. acidophilus* numbers reached max. 8.8 log cfu/g on the 17^{th} day of storage in pickles with 5% (w/v) and 7% (w/v) salt concentration brine. On the 30^{th} day, bacteria numbers were very close to each other and declined to 8.13 log cfu/g.

LAB produce several antimicrobials, including organic acids (lactic, acetic, formic, phenyllactic, caproic acids) carbondioxide, hydrogen peroxide, diacetyl, ethanol, bacteriocins, reuterin and reutericyclin and they can prevent mould spoilage and growth of some pathogenic bacteria (see [8], [34] and [35] for more details). It can be concluded that these specific lactic acid bacteria in pickles were able to produce bacteriocins that inhibit yeasts at the last of storage.

Using bacterial cultures must not only aim at expressing functional properties upon microbial growth, but also at the impact on other quality

changes in the pickles. Among these changes, the sensory or organoleptic property is the most important [13]. In Weon and Lee [36] study, perception and preference of low salt Korean pickle "Jangachi" were evaluated. Low sodium Jangachi was found safe, sanity, safekeeping and the most preferred by the consumers.

5. Conclusions

Researches' results provided a useful basis for further studies of the development of optimization sodium chloride concentration level in brine during fermentation of pickles. It was demonstrated that concentration of sodium chloride in brine solution have significant effects on the growths of *Lb. acidophilus*, *Lb. casei*, total lactic acid bacteria and yeast counts during fermentation. The population dynamics during cabbage-carrot pickle fermentation can be predicted by the use of the notion of fuzzy soft set at the same production conditions. Application of "*fuzzy soft set*" in optimization of pickle fermentation helps and allows better understanding of the interaction between the variables.

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