The impact of the cucumber fruit preparation on their mechanical properties and microbial status of the souring brine during souring procedure

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Abstract. Mechanical damage occurring during harvest, transportation or on various stages of processing of fruit and vegetables (in particular ground cucumbers) may lead to spoiling and disqualification of final products. Therefore investigations have been conducted to determine the cucumber's resistance to mechanical damage and possible water loss in the raw material, as well as to enable the selection of best cucumber varieties suitable for processing. The goal of the conducted research was the determination of the impact of the cucumber variety, ozonation and the composition of the brine with the addition of probiotic bacteria on the mechanical properties and the microbial status of the final product. Mechanical properties were determined using a Zwick/ Roell Z010 machine and the data on the microorganisms were collected using Trypicase soy agar and MALDI TOF MS Biotyper. Other bacteria were detected using Man, Rogosa, and Sharpe agar media. The results indicate that a pre-ozonation or the addition of the probiotic both have a positive impact on the mechanical properties of the investigated product after the souring procedure. It was concluded the impact of both procedures on the puncture strength of final product was dependent on fruit variety.

Key words*:* lactic acid bacteria, mechanical properties, ozonation souring.

INTRODUCTION

Cucumber (*Cucumis sativus L.*) is an annual plant. The place of origin of cucumbers are India, so it is sensitive to frosts, winds and chill, moreover it requires fertile soils [1,2]. The optimum germination temperature is about 30 °C, but under field conditions it can grow at slightly lower temperatures, above 18 °C [3].

The monitoring of the mechanical properties of fresh cucumbers intended for processing is important due to the potential damage to the cucumber fruit that may lead to spoiling and disqualification of final products. Therefore, cyclic investigations have to be conducted that allowed the determination of the cucumber fruit quality e.g., its firmness and hardness, which both are properties evaluated by the producers and consumers of fruit products [4,5,6].

Fruit and vegetable fermentation is one of the basic methods of food processing, it allows an increase in the product shelf-life but also it results in generation of processed food products, differing in organoleptic characteristics from the raw material used in production. Most of the plant raw materials are susceptible to souring; the only condition is the appropriate content of sacharides [6]. The final product consists of metabolites and living cultures of lactic bacteria that have a positive impact on human health, but also vitamins - C, B2 i PP [7]. Fermentation allows preservation of plant raw materials characterized by high water content and chemical composition suitable for growth of the desired fermenting microorganisms acting as natural preservatives [8,9,10,11].

A major factor affecting the course of the fermentation process and therefore the changes of the mechanical properties of cucumber fruit is the endemic microflora found on the fruit. Changes in this microflora can potentially affect the course of the souring process.

One of the factors that can be successfully utilized for the modification of the microflora composition may be gaseous ozone, which is an allotropic form of oxygen characterized by a high reactivity. This compound has

been successfully used to reduce pesticide residues or microbial contamination of plant material [12,13,14,15]. Furthermore Gorzelany at al.,[16] showed that the pre-ozonation process had impact on the mechanical properties of the cucumber fruit after souring. Other researchers, e.g., Xiaoyi et al. [17] reported, that the cucumbers soured in the brine with the addition of lactic acid bacteria were also characterized by higher values of the puncture strength. Therefore in this work, the impact of the cucumber variety, ozonation and the composition of the brine with the addition of probiotic bacteria on the mechanical properties and the microbial status of the final product have been investigated.

MATERIALS AND METHODS

Characterization of research material

The research material was the fruit of three varieties of ground cucumbers: Polan F1, Partner F1 and Izyd F1. They were obtained from experimental plots located on a farm at the edge of the city of Rzeszow. The fruit of the analyzed varieties were collected, packed in properly labeled bags and transported to the Laboratory of Mechanical Analysis of the Department of Agro and Food Production Engineering (University of Rzeszow) where they were pre-selected, washed and divided into size fractions (in length): fraction I- 3.5-5.5 cm, fraction II- 5.6-7.5 cm. Subsequently, the test material was divided into a control samples and a test samples on which the ozonation procedures were performed.

Measurement of the water content

The measurement of the water content in fresh fruit of chosen cucumber varieties was conducted using a moisture analyzer. From the research material slices (a few mm trick) were taken. Than they were pre dried for 6h at 70ºC in the laboratory incubator. After that the moisture content was measured using moisture analyzer at 105ºC.

Preparation of the cucumbers to the souring process

To ensure optimal course of the souring process the fresh cucumber fruit were subjected to the preliminary selection that was based on the exclusion of damaged cucumbers, those affected by diseases or characterized by an abnormal staining.

Then, several kg of cucumbers were washed and dried before they were placed into the chamber of the apparatus for the ozone treatment, where they were exposed to gaseous ozone at the concentration of 10 ppm, for half an hour in the closed loop where the flow rate of the ozone-air mixture was $4m^3/h$. The ozonation process was carried out at constant temperature of 20°C. Such process conditions have been chosen based on our previous experience concerning treatment of plant material with ozone [12,13,14,15].

Next the cucumber fruits were placed into the glass jars with twist-off type lids, which before the souring process were subjected to sterilization. Aside of the cucumber fruit, the jars were filled with brine consisting of water at the temperature of 90 °C including sodium chloride and spices. The cucumbers were subjected to souring procedures in two brines with a different composition. One of the brines was enriched with FD-DVS YC-X16 - Yo-Flex (Chr. Hansen, Denmark), this products includes thermophilic yoghurt culture consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* . Before the start of the turbulent fermentation the jars were kept at constant temperature of 20 °C and then they were transferred into the room where the temperature was 5°C. Moreover, control samples were prepared that were subjected to the souring procedures but were not exposed to the gaseous ozone before the souring. The composition of brines used during the souring procedures was as follows: aqueous solution of NaCl 6**%**, fresh dill stems with canopies 2.5**%**, Horseradish root 0.2**%**, Garlic 0.2**%**, Allspice (Galeo), Bay leaves (Galeo) 0.1% (Brine A), in addition brine B included 0.3% of an additive in form of the probiotic.

Measurement of mechanical properties

The cucumber fruit were subjected to the puncture strength test with a pen puncture probe with a diameter φ=5 mm using Zwick/ Roell Z010 machine. The measurements were conducted separately for each size fraction, in 36 repetitions for each of the analyzed cucumber varieties on the fresh fruit and the fruit after 10, 30, 60 and 90 days of souring. Measurements of the mechanical properties were conducted at the following set parameters:

- $Fv = 2N$ (preliminary power),
- $V_1 = 20$mm/min (speed of traverse of the beam load cell during measurement).

Identification of microorganisms

Determination of CFU and cultivation media

Basic dilutions (10^{-2} and 10^{-3}) of brine were prepared. Basic dilution prepared from the stock solution as follows: 1 mL of brine sample was added into test-tube which contained 99 mL of physiological saline solution (0.85%). After that 1 ml of brine was added into 9 mL of the physiological saline and a dilution 10^{-3} was prepared. Plate spread method was used for the determination of colony forming unit (cfu) counts of the respective groups of the microorganisms in 100 µL of brine. The microorganisms were inoculated into the agar medium surface. Each experiment was done in three replications.

The counting of lactic acid bacteria (LAB) was done using de Man, Rogosa, and Sharpe agar (MRS) (Merck, Darmstadt, Germany). MRS agar plates were incubated anaerobically using a CO_2 incubator at 30°C for 48 h. Other bacteria were enumerated using Trypicase soy agar (TSA) (Merck, Darmstadt, Germany). TSA plates were incubated aerobically at 30°C for 48 h.

MALDI-TOF MS sample preparation, measurement and data analysis:

Sample preparation was performed according to the instructions from Bruker Daltonics. The strains were grown on the Trypicase soy agar (TSA) (Merck, Darmstadt, Germany) and incubated for 24 - 30 h at 37 °C. An isolated colony for identification was harvested in 300 µL of sterile water, and 900 µL of ethanol (Roth, Karlsruhe, Germany) was added, and all components were well mixed. The samples in an ethanol and water mixture were centrifuged for 2 min at 20.000 ×g, and next the supernatant was removed and the pellets were dried. For sample extraction, 50 µL of formic acid (70% in water) (Roth, Karlsruhe, Germany) was added to the pellet and mixed thoroughly, followed by an addition of 50 µL of acetonitrile (Roth, Karlsruhe, Germany). All components were mixed and after centrifugation at 14.000 ×g for 2 min, 1 µL of the supernatant was deposited on the target plate (Bruker Daltonics, Bremen, Germany) and allowed to dry at 20 °C. Subsequently, the sample was overlaid with a 1 µL of MALDI matrix (a saturated solution of α-cyano-4-hydroxy-cinnamic acid in 50% acetonitrile - 2.5% trifluoro-acetic acid) (Bruker Daltonics, Bremen, Germany) and dried again. Samples were then analyzed in the MALDI-TOF MS spectrometer microflex LT (Bruker Daltonics, Bremen, Germany). The measurements and data analysis were performed automatically by the MALDI Biotyper 3.0 (Bruker Daltonics, Bremen, Germany). The classification of spectra was obtained by comparison to the Biotyper database version 3.1. The identification by MALDI TOF MS Biotyper, is conducted in a following manner: a list of most similar reference spectra is given as a result and a score value as a measure of confidence. Only score values > 1.7 were counted as identification, the values between 1.7 and 2.0 for genus and > 2.0 for species identification. Each strain was measured two times from three independent agar plates. At least two independent replicates out of three had to be concordant to be taken as valid for species or genus identification. Score values for unique species identification had to differ at least for 0.1.

Statistical analysis.

Statistical analysis of the results was conducted using the Statistica 10 program. To verify the normal distribution and the homogeneity of the variance the data was analyzed using the Shapiro-Wilk test. Confidence level was $\alpha = 0.05$. Then the ANOVA test was used to determine the significance of the differences between the parameter mean values. After the analysis was performed, when the differences between the mean values of the parameters were confirmed, Tukey's post-hoc test was performed to specify and verify them.

EXPERIMENTAL RESULTS AND DISCUSSION

Water content:

The highest water content was recorded for the Izyd variety exposed to ozone, the lowest for the cucumber variety Partner without ozonation. These values were 94.87% and 96.1%, respectively. No statistically significant differences in the water content were found in the ozonated fruit compared to the control sample and between the analyzed fruit varieties.

Investigations of the mechanical properties:

The process of storing and transporting of fresh cucumbers affects the water content within the fruit and as a consequence its firmness and quality of final products. As a result of various technological processes, the biological materials, such as fruit and vegetables are damaged. The determination of mechanical properties allows to accurately assess the stability of the texture of the raw material, which is one of the essential elements that determine the acceptability of horticultural products [5,18].

Considering the variability of the elasticity of the raw material tested, a peel and mesocarp puncture strength test was performed with a modification of the measurement sites, taking into account the size parameters (fruit length). There were no significant differences between the fresh ozonated and control cucumbers. The mean values of the puncture strength of the fresh cucumbers peel and mesocarp were in range between 35.36 N (control for Partner variety) to 38.98 N (Polan variety after ozonation only).

The mean values of puncture strength of the peel and mesocarp for ozonated and control cucumbers after the souring process, that was conducted using brines with two different chemical compositions, were determined after 10, 30, 60 and 90 days of souring procedure. As an example the results obtained for the parameter determined during the souring procedure for the Polan variety are presented on figure 1.

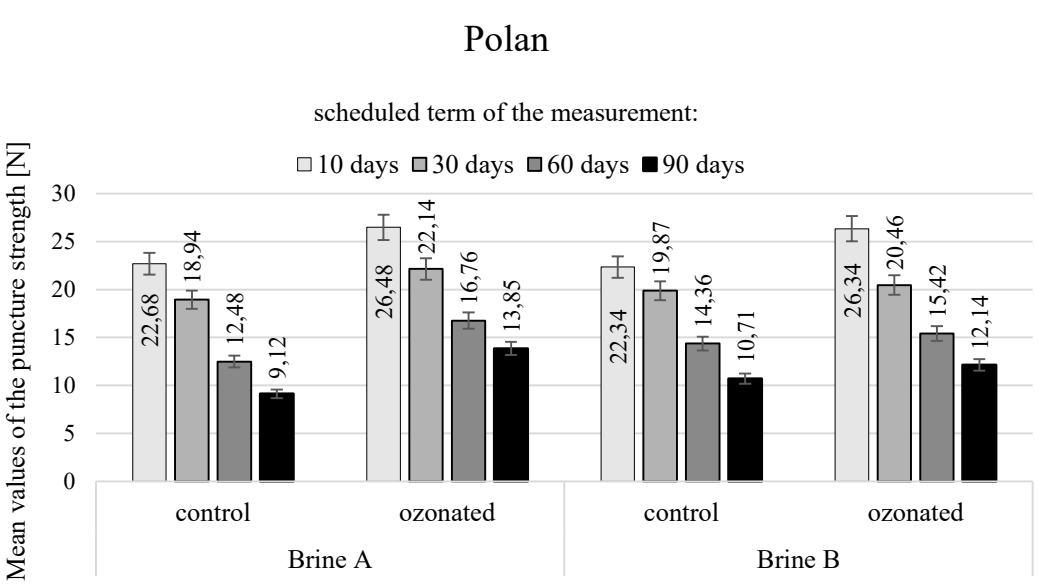

Fig. 1. Mean values of puncture strength of the peel and mesocarp for cucumbers of Polan variety after the souring procedure depending on souring time, the ozone treatment and the chemical composition of brines

It was observed that the mean values of the puncture strength of the peel and mesocarp for the analyzed fruit variety dropped during the souring procedures independent from the brines composition, or the utilization of ozonation process. Also it was observed that the measured parameter was always higher for the ozonated cucumbers in comparison to the cucumbers that were subjected to souring processes only. Furthermore, for the cucumbers of Polan variety, the addition of the probiotic resulted in an increase of the puncture strength only for the cucumbers that were not subjected to ozonation procedure. A combination of ozonation process before the souring, with the addition of the probiotic to the brine used for souring procedure was ineffective, i.e. the puncture strength determined for the cucumbers after the pre-ozonation and the souring in the brine with the additive of the probiotic was lower in comparison to the parameter value for the cucumbers after pre-ozonation only.

For the Izyd and Partner varieties the mean values of the peel and mesocarp puncture strength, were comparable to the results observed for Polan variety. Similarly as for the Polan variety, as a result of ozonation, or the addition of the probiotic to the brine, the values of the determined parameter were higher in comparison to control.

However, the parameter values were no statistically significantly different for the cucumbers after the ozonation procedure in comparison to the combined procedure, which are better results in comparison to the results obtained for Polan variety. When analyzing the parameter value for individual varieties, no statistically significant differences were observed between the obtained values. The mean values of puncture strength of the peel and mesocarp for cucumbers of the investigated cucumber varieties, after the souring procedure, depending on souring time, the ozone treatment and the chemical composition of brines were summarized in table 1.

Table 1. Mean values, standard deviation and the statistical significance of puncture strength values for cucumbers after the 90 days of souring depending on the ozone treatment and the chemical composition of brines. Vertically (the differences between the cucumber varieties) - uppercase letters, Horizontally (the differences between varieties and brines) - lowercase letters

Variety	Brine A				Brine B			
	control		ozonated		control		Ozonated	
	mean	SD	mean	SD	mean	SD	mean	SD
Polan	9.12 A a	0.5	13.85 A d	0.6	10.71 A b	0.5	12.41 A c	0.6
Izyd	9.35 A a	0.5	13.91 A c	0.5	10.74 A b	0.5	13.66 AB c	0.5
Partner	9.47 A a	0.4	13.33 A c	0.5	12.41 AB b	0.4	13.71 AB c	0.5

A statistically significant variations of the puncture strength values were already observed at the stage of turbulent cucumber fermentation [19,20]. The relation of the mean value of the measured parameter to the souring time can be described using a logarithmic function f(x)=log ax. The determination coefficients R^2 were high and their values were in the range from 0.968 to 0.992 for brine A and from 0.979 to 0.995 for brine B. Fleming et

al. [4] and Xiaoyi et al. [17], both also observed the deterioration of the cucumber fruit mechanical parameters during souring procedures.

Identification and enumeration of the microorganisms:

LAB were represented by: *L. plantarum, L. paracasei, L. rhamnosus, L. zeae* and *Le. pseudomesenteroides*. Bacterial species identified using the TSA were *B. simplex, A. lwoffii, B. megaterium, E. cloacae, M. luteus, P. amylolyticus, S. silvestris, S. epidermidis*. *Leuconostoc mesenteroides* and related LAB, including *Weissella* and other *Leuconostoc* species, are important in the initiation of the fermentation of many vegetables, i.e., cabbages, beets, turnips, cauliflower, green beans, sliced green tomatoes, olives, and sugar beet silages. Homofermentative species, such as *Lb. plantarum*, produce exclusively lactic acid from the remaining sugars. In most vegetable fermentations, *Lb. plantarum* will eventually outcompete other LAB because of its superior acid tolerance [21]. It was observed that the addition of probiotic did not result in the promotion of the growth of the species included in the probiotic.

The values of the colony forming units counts determined for the bacteria that were detected in the cucumber souring brine samples are presented on figures 2 and 3. In the case of LAB the determined values ranged from 2.91 to 4.21 log cfu/mL and in the case of the other bacteria (OB) that were grown on TSA they ranged from 2.35 to 3.52 log cfu/mL.

In the case of the brine without the addiction of the probiotic (Brine A) the counts of LAB and OB for cucumbers after the pre-ozonation was lower (Figs. 2 and 3), in comparison to the counts of microorganisms detected in brine were control cucumbers were soured.

These observations correspond with the results of the conducted puncture strength tests, showing higher values of the determined mechanical parameters for the ozonized cucumbers in comparison to the control cucumbers, regardless of the cucumber variety. Ozonation depletes naturally occurring populations of microorganisms, limiting their development, which is confirmed by the research previously conducted by Balawejder et al. [13,14,15]. As a consequence, changes in the values of puncture strength of the fruits are observed.

In turn the presence of the probiotic in brine B resulted in an increase of the OB population counts. The highest counts of OB was observed in the case of the brine with addition of the probiotic without the pre-ozonation. This indicates a disproportionate stimulation of the growth of the determined populations under the impact of the probiotic additon. It follows that both the ozonation process and the addition of a probiotic has independently impact on the imbalance between lactic acid bacteria populations and populations of other bacteria, reducing the percentage of lactic bacteria in the total population of microorganisms. However, the addition of probiotic had a much lesser impact than pre-ozonation process.

CONCLUSIONS

During the research it was observed that the water content in fresh cucumber fruit was not statistically significantly different for the investigated cucumber varieties. The utilization of ozonation process of fresh cucumber fruit had no statistically significant impact on the values of the peel and mesocarp puncture strength. However, the mean values of the measured parameter dropped during the souring in case of all experiments. Moreover pre-ozonation process had a positive impact on the quality of soured cucumber. The ozonated cucumbers after the souring procedure were characterized by statistically significant higher value of the peel and mesocarp puncture strength in comparison to the cucumbers that were processed without the pre-ozonation. Also the addition of the probiotic caused an increase of the puncture strength values for the analyzed cucumber varieties in comparison to the cucumbers that were soured in traditional manner. It was observed that the combination of the ozonation process with the addition of probiotic to the brine used for souring procedure resulted in similar results as the ozonation itself. The puncture strength for the cucumber varieties subjected to both treatments were dependent on the cucumber variety. For the Polan variety addition of the probiotic to the brine caused the reduction of the measured parameter after 90 days of souring, whereas for the Izyd and Partner varieties the values of the measured parameter were not different. The most dominant from the isolated lactic acid bacteria were: *Lactobacillus plantarum* and *L. paracasei*. The highest number of the total count of bacteria was found in control samples. From the total count of bacteria most predominant were: *Bacillus megatherium* and *Paenibacillus amyloliticus*.

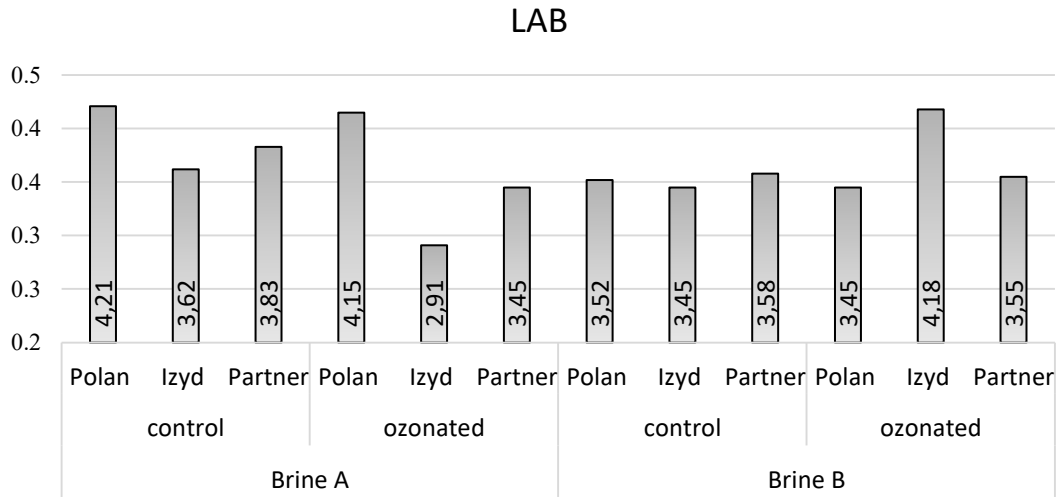

Fig. 2. Number of lactic acid bacteria in brine used for the cucumber souring presented as log cfu/ml

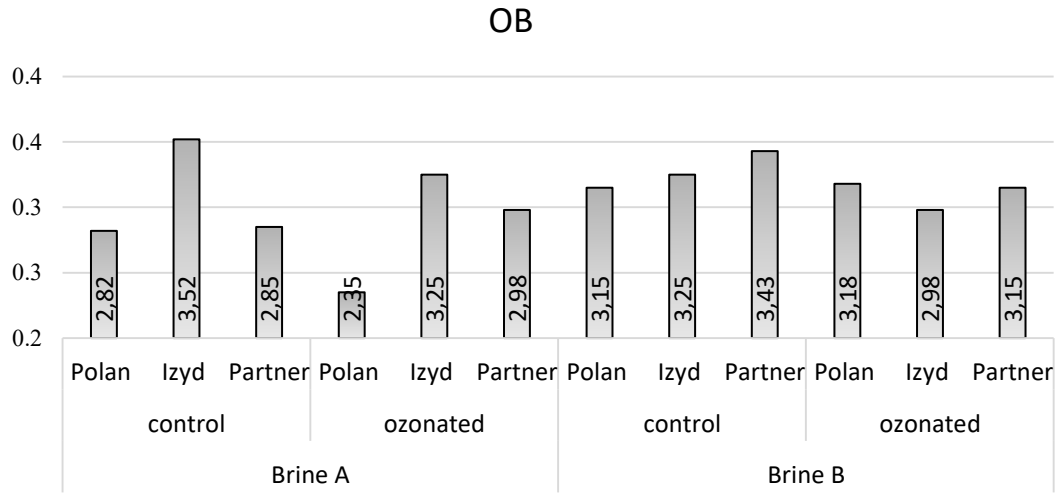

Fig. 3. Total count of other bacteria in brine used for the cucumber souring presented as log cfu/ml

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