Microbiological studies on some pickles

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Pickles are considered one of the popular foods in Egypt. Most consumers prefer pickled pepper and turnip. They are not only tasty but also cheap and available for all standards. This study was carried out for producing a high quality product with a long storage period.Many treatments were chosen aiming to encourage lactic acid fermentation and inhibit contaminants which may cause spoilage to pickles. This was achieved by using preservatives which are accepted from the public health point of view and didn't affect the activity of lactic acid bacteria and inhibit spoilage organisms.Saline solution (7% NaCI) was used only or with any of theunpreservatives, which were used either individually ide and or in combination. Potassium sorbate was used as a fgic yeastcide, also, CaCl2 was used to make Cx-cellulase enzyme inactive. A pure culture of L. plantarum was used as a starter. This strain is well known for it's ability of salt resistant in addition t wasit'sability to produce best taste and flavor compounds. Acetic acid used not only as a preservative factor but also gives a gAn attempt was carried out for the production of salt- free pickles which will be safe product for persons suffering from hypertension.Also, this study included the use of ultra filtration whey (permeate) as a carbon source for lactic acid bacteria. Also, the investigation included studying the anti-microbial activity of starter strain (L. plantarum) against 5 test strains included both Gram-positive and Gram-negative microorganisms.Many treatments were carried out once in brine solution 7ati% NaCI and another in permeate 7% NaCI with using preservative agents either individually or in combinations.emhTo choose the best treatment, Microbiological, chemical and organoleptic tests were carried out in the brines during pickling period (90 days) at intervals yzero, 3, 7, 15, 30, 60 and 90 das.113 Microbiological analyses comprised: counts of total microbial flora, lactic acid bacteria, yeasts and molds counts, pectin decomposing micro-organisms and coliforms count. Chemical assays included: determination of pH values, NaCI concentration, total titratable acidity (T.T.A.), non volatile acids (N.V.A.), total volatile acids (T.V.A.), lactic acid and reducingsugars. Anti-microbial activity studies included : determination the diameter of inhibition zones for all filtered (cell-free) brine solutions on the test microorganisms namely, B. cereus, Staph. aureus, Staph. epidermides, Salmonella typhi and E. colt . Organoleptic tests comprised: taste, texture, color, appearanceand odor. Results can be summarized as follows - A- Water solution (7 % NaCI) treatments and salt free treatment in both types of pickles: 1-Microbiological estimations of the brine of pickling :1-Control treatment showed higher microbial counts than allpreservatives treatments, except the starter which exhibited a higher total microbial count than control up to 15 th day. Total microbial counts were increased with increasing the fermentation period up to 15 th day and decreased thereafter. Acetic acid treatment showed sharp reduction in total microbial count, when the treatment was free from NaCl.2-Lactic acid bacteria were increased with the increasing offermentation period up to 15-30 days and gradually decreased thereafter. Control treatment showed the lowest counts of lactic acid bacteria, while combination of preservatives showed higher lactics than all individual preservatives at earlier periods but showed lowest counts at late periods. Starter treatment exhibitedthe highest counts of tactics, while acetic acid treatment showed lower counts of lactic acid bacteria.3-Yeasts and molds count were the lowest at zero time as comparedwith other periods in pickled pepper, while they were not detected in all turnip treatments at the same period. Control treatment recorded the highest counts, while starter or acetic acid treatments exhibited the lowest yeasts and molds counts in pickled pepper, whereas in turnip acetic acid treatment recorded the lowest counts of yeasts and molds.4-Pectinolytic microorganisms

counts in control treatment werethe highest , while treatments included either CaC12 or K- sorbate showed reduction in the densities of pectinolytic micro-organisms. Combination of the aforementioned preservatives sharply decreased the pectinolytic counts than that of individual ones. Starter treatment showed lower counts than that without inoculation. Acetic acid treatment exhibited moderate densitiesof pectinolytic microorganisms.5-Control treatment recorded higher counts of coliforms thanother applied treatments. Coliforms were detected up to 7th day in control treatment, but was detected till the third day in all other treatments. This means that coliforms disappeared after the 3 rdday in all treatments except control. 2-Chemical assays of the brine solutions and salt free treatment of pickling :1-pH values were gradually decreased to reach their minimumlevels at 30 days samples in all treatments except acetic acid treatments. pH values increased with increasing the pickling period till the end of experiment, this means that it recorded the lowest values at the early stages. Compared with control, the pH values were lower in treatments contained either individual orcombination of preservatives.2-NaC1 concentration gradually decreased till the end of theexperiment in all treatments. Individual preservatives treatments exhibited higher concentration of NaCI than that of control. In pepper, combination of preservatives showed relatively higherconcentration of NaCl compared with that of individual ones. whereas, in turnip opposite result was observed.3- Control treatment showed the lowest levels of total titratableacidity at all experimental periods than other treatments. At zero time total titratable acidity (T.T.A.) was not detected in all treatments except starter or acetic acid treatments, the acidity was not detectable even at the third day in CaC12, K-sorbate or CaC12 + K-sorbate treatments as well as in control. Acidity was increased with increasing the fermentation period up to 30-60 days and decreased thereafter in all treatments except acetic acid treatments showed pronounced increase of acidity. Combination of preservatives treatments exhibited a lower acidity than the individual ones. Starter treatment showed higher T.T.A. than control, CaCl2, K-sorbate and the combination of CaC12 + K-sorbate treatments. Acetic acid treatments (including treatment without NaCI) exhibited the highest level of total titratableacidity.4-Lactic acid was not detected in all treatments at zero timeexcept starter treatment. Lactic acid was increased with the increasing of fermentation period up to 60 days and decreased thereafter. Lactic acid directly proportionated with the counts of lactic acid bacteria. Starter treatments showed higher level of lactic acid than the brines treated with either CaC12, K-sorbate or with combination of them. Acetic acid treatments (including treatment without NaCI) exhibited the lowest lactic acidpercentage.5-Total volatile acids were increased with the increasing offermentation period up to 30-60 days and decreased thereafter in almost all applied treatments. CaC12 treatment in pepper reached the peak earlier. Except the control, the lowest concentration of total volatile acids were observed in pickled pepper treated with CaCl2 at all experimental period, while in turnip were in CaC12+ K-sorbate treatment. Starter treatment showed increase in concentration of total volatile acids. Combination of preservatives showed moderate increase in total volatile acids, while the highest percentage recorded in acetic acid treatments (including treatment without NaCI), decreased gradually thereafter.6-Non volatile acids were increased with the increasing offermentation period up to 30 days, then decreased thereafter. It was not detected at zero time samples except starter treatment. Lowest concentration was recorded in control treatment. Either individual preservatives or combination of them showed moderate increase of N.V.A. while both of starter and acetic acid treatments (including treatment without NaC1) produced the highest concentration of non volatile acids in both types ofpickles.7-Reducing sugars were increased with the increasing offermentation period up to 15th day and decreased thereafter. The highest values of reducing sugars in turnip pickled resulted from the application of acetic acid treatments (including treatmentwithout NaCl). 3-Anti-microbial activity of pickling solutions (water 7% NaC1) with different treatments : A- Pickled pepper treatments:,1-Except acetic acid treatments including treatment withoutNaC1, the inhibition zone of various test organisms were gradually increased with the increasing of fermentation period up to 30 days and decreased thereafter.2-Control and CaC12 treatments didn’t exhibit any inhibitionzone at the early periods against test organisms, even at the later periods on Staph. epidermides and E. colt.3-Combination of preservatives showed higher inhibition zone than the using of individual ones.4-Starter and acetic acid treatments including treatment withoutNaC1 recorded the highest inhibition zones of various

test organisms and at all experiment periods.5-Gram-negative strains exhibited higher sensitivity than Gram-positive.117 B- Pickled turnip treatments:1-In treatments which included either CaC12 or K-sorbate and bothof them exhibited an increase in the inhibition zone with the increasing of fermentation period and reach their maximum at 90 days, mostly similar in all test organisms.2-Inhibition zone of starter treatment increased up to 30 daysfor B. cereus, Staph. aureus and Staph. epidermides, while, inhibition zone was highest at 60 days for Salmonella typhi andE. coli.3-Acetic acid treatments including treatment without NaC1showed higher inhibition effect during the early stages than thelater ones with all test organisms. 4-Organoleptic evaluations of pickles in brine solution and treatment without NaCI:1-Starter and acetic acid treatments (including treatment without NaC1) exhibited the best quality (highest organolepticvalues) than other treatments. This was true at all experimental periods.2-Control treatment showed the lowest quality of pickles, including bad texture and appearance.3-Individual preservatives gave lower quality than using of preservatives in combinations. B- Permeate 7% NaCI treatments in both types of pickles. B-1-Microbiological studies of the salted permeate used in pickling:1- Total microbial counts were increased with increasing thefermentation period up to 15th day and decreased thereafter in both pepper and turnip treatments, but turnip showed lower densities than pepper. Control treatment recorded the highest counts compared with other treatments. Treatments containingeither CaC12 or K-sorbate exhibited a moderate decrease in total microbial count, but higher decrease was observed when combination of them was used. Starter treatment showed higher densities than treatment included CaC12 + K-sorbate.2- Lactic acid bacteria were increased with the increasing offermentation period up to 7-15 days and decreased thereafter till the end of experiment in most investigated treatments of both of pepper and turnip. Control treatment showed lower densities of lactics than other treatments. Brines treated with either CaC12 or K-sorbate showed moderate increase of lactics but the combination of them gave higher populations. Starter treatment showed the highest counts of lactic acid bacteria, while acetic acid treatment recorded lower counts than starter at all experimental periods. Lactics disappeared in K-sorbate and aceticacid treatments at the storage period.3- The densities of lactics were higher in all applied treatments of permeate than of brine solutions.4- Yeasts and molds were very low at zero time in pickled pepper, while they were not detected in the turnip treatments at the same period. Control treatment showed the highest counts of yeasts and molds. Addition of preservatives especially in combination reduced the yeasts and fungi counts, since the lowest counts were obtained with CaC12 + K-sorbate treatmentsat all experimental periods.5-The highest counts of pectinolytic microorganisms were in control treatment. Brines (permeate) treated with either CaC12 or K-sorbate showed reduction in the population of pectinolytic microorganisms, but combination of them highly decreased pectin decomposing microorganisms. The lowest count, was observed in brines (permeate) treated with CaC12 + K-sorbate +acetic acid .6-All applied permeate treatments contained coliform bacteria at zero time and three days samples, and was present up to the 7 th day only in control treatment. Under all investigated treatmentscoliform group colonies disappeared after the 7 th day till the end of experiment. Control treatment exhibited the highest coliform counts compared with other treatments in both types of pickles. 2- Chemical determinations of the salted permeate used in pickling:1-pH levels decreased gradually with the increasing of fermentation period up to 15-30 days and increased thereafter. Acetic acid treatments showed an increase in the pH values with increasing the pickling period. Control treatment showed the highest pH values, while preservatives treatments showed lowerpH than control treatment .2-Except the treatment of acetic acid, the total titratable acidity gradually increased with the increasing of fermentation period up to 30-60 days and decreased thereafter, while, it was high at the early stage of acetic treatment and decreased thereafter . Control treatment showed the lowest value of total titratable acidity than preservatives treatments. Combination of preservatives exhibited higher values of total titratable acidity than the individual ones. Starter treatment showed the best percentage of total titratableacidity.3-Percentage of lactic acid gradually increased with the increasingof pickling period up to 30-60 days and decreased thereafter . Preservatives treatments showed higher values of lactic acid especially the treatment including CaCl2 + K-sorbate. The highest percentage of lactic acid occurred with starter treatment, while , acetic acid treatment showed lower values of lactic acid thanstarter

treatment.4-Except acetic acid or starter treatments, total volatile acidswere not detected at zero time and increased with the increasing of pickling period up to 30-60 days. Except the control, the lowest concentration of total volatile acids were recorded in brines (permeate 7% NaCI) treated with the combination of CaC12 + K-sorbate. Starter treatment showed an increase in totalvolatile acids , while acetic acid treatments showed the highest percentage of total volatile acids at the early stage of pickling.5-Non volatile acids gradually increased with the increasing offermentation period up to 30-60 days. Compared with the control, individual or combination of preservatives exhibited increase in non volatile acids. Both starter and acetic acid treatments recorded the highest percentage of non volatile acids.6-NaCI gradually decreased with the increasing of picklingperiods till the end of experiment. Combination of preservatives treatments exhibited relatively higher values of NaCI than the brines (permeate 7% NaC1) treated with individual preservatives.7-Reducing sugars increased with the increasing of picklingperiod up to 7-15 days and decreased thereafter till the end of experiment. Control treatment showed the lowest values of reducing sugars . Brines treated with different preservatives either individual or in combination increased reducing sugars content in brine solutions. Acetic acid treatments showed the highest values of reducing sugars . Comparing with the application of either CaCl2 or K-sorbate, starter treatment exhibited an increase in the concentration of reducing sugars. 3- Anti-microbial activity of the salted permeate used in pickling: A- Pickled pepper treatments:1-Gram-negative and Gram-positive bacteria were affected withusing various preservative substances.2-Inhibition zone (mm) of test organisms increased with theincreasing of pickling period up to 30-60 days and then decreased.3-Control treatment didn’t exhibit any inhibition zone at the early periods with all test organisms, thereafter, it recorded thelowest inhibition zone, while, starter and acetic acid treatmentsshowed the highest inhibition zones.4- Inhibition zone of various test organisms increased with theapplication of preservatives as compared with control. B-Pickled turnip treatments.1-Inhibition zones gradually increased with the increasing of pickling period up to 30-60 days and then decreased.2-Sensitivity of test organisms were increased with theapplication of preservatives.3-CaCl2 + K-sorbate treatment gave higher inhibition zone thaneither CaCl2 or K-sorbate alone.4-Starter and acetic acid treatments showed the highestinhibition zone, starter was very effective against Staph. aureus, whereas, acetic acid treatments was very effective against E. coli. 4- Organoleptic evaluations of pickles in salted permeate:1-Control treatment gave the lowest quality of pickles comparedwith various investigated treatments.2-CaCl2 either alone or in combination with K-sorbate gave higher quality pickles than using of K-sorbate alone.3-Starter and acetic acid treatments gave the highest quality ofpickles.4-Permeate treatments were better than brine solution in qualityof the pickles as estimated by the organoleptic tests.