

Microbiological studies on some pickles

Mahmoud Zenhom Mahmoud Hamed

SUMMARY Last years showed an increase in pickling of various vegetables. The green olives were one of the most acceptable pickled production. Therefore the great companies increased their production of pickled green olives which resulted in the appearance of the problems throughout pickling and storage. Therefore, this investigation used the method of the production in El-Badrachin factory of Kaha company as a pilot experiment and studied some laboratory treatments to encourage lactic fermentation. This was done by usage of chemical preservatives such as potassium sorbate (0.1%) and acetic acid to pH4 and by the inoculation of the brines by starter of lactic bacteria (*L. plantarum*, *Leuc. mesenteroides*, *Str. lactis*). These preservatives, natural (starter) or synthetic were investigated either individually or in combinations (sorbate+acetic, sorbate+starter or sorbate+acetic+ starter). Also, this study included the use of the brined whey instead of the salt brine to enhance the lactic fermentation. The used whey was modified by NaCl to reach 13% NaCl, which is the concentration used for brines of all treatments. These treatments were done by using olives untreated and treated with 1.6% NaOH solution. In order to evaluate the best treatment valid to produce pickled olives under factory conditions (either early or lately package) microbiological, chemical and organoleptic investigations were done during fermentation and storage. Azizi-Axe the most widely produced olives in Egypt was selected to this study. The pilot experiment which was similar to the line production in the factory, which used the olives untreated with NaOH. Control experiments were done corresponding to each variety of the olives (untreated and treated with NaOH). This study included microbiological and chemical estimations in the brines which were taken under aseptic conditions at the following intervals: zero time, 15, 30, 60, 90 days (during fermentation) 180 and 270 days (during storage). Organoleptic evaluations were done in olives at intervals: 60, 90, 180 and 270 days. The microbiological determinations were: Total bacterial counts, lactic acid bacteria counts, yeasts counts, lipolytic bacterial counts, pectinolytic bacterial counts, cellulolytic bacterial counts and coliform group counts. The isolates which were taken throughout yeasts counts and lactic acid bacteria counts were identified. The chemical estimations included The determination of pH values, total titratable acidity, lactic acid, total volatile acids, tannins, vitamin C, NaCl concentration and pectic substances in the brine. On the other hand, soluble acids, total sugars, reducing sugars, total crude-protein, true-protein, ether extract, tannins, vitamin C, NaCl concentration, pectic substances and moisture were determined in the fruits. The taste, colour, texture, appearance and odor were determined through organoleptic evaluation of the pickled olives. Results can be summarized in the following: I. Microbiological studies: 1. The total microbial counts in the brines of olives treated with NaOH were higher than the corresponding brines of olives untreated. The total microbial densities in the brines treated with combination of preservatives were lower than that treated with individual preservatives. On the other hand, the highest counts were in the brined whey treatments, pilot and control experiments. 2. Brines untreated with preservatives including whey treatments and starter treated brines contained the highest counts of lactic acid bacteria. On the other-hand, the brines treated with chemical preservatives contained lower counts especially that treated with combination of preservatives. Therefore, the later exhibited lower quality. The highest counts recorded in whey treatments than all treatments, which resulted in obtaining quick pickling. 3. Yeasts counts were competitive with lactic acid bacterial densities, hence they were the first ones exhibited competition with lactic acid fermentation. Yeasts counts were higher in brines of olives treated with NaOH than that of untreated. The inoculation of the brine with

starter only didn't show any effect on the yeasts densities, but the counts increased due to the presence of the nutrients in the whey used in the preparation of the starter. Pilot experiment counts were lower than whey treatments. Sorbate treated brines exhibited potential effect on yeasts densities. The brines treated with combination of preservatives were more affected than that treated with individual preservatives. Yeasts densities in whey treatments were higher than other preservatives treatments. 4. Starter treated brines among individual preservatives didn't also exhibit any effect on lipolytic bacterial counts but the densities increased in the brines of olives untreated with NaOH. The counts of pilot experiment were higher than that of control. These microorganisms were relatively resistant to the effect of acetic acid. There was a decrease in the counts from the 30 days samples to the end of the experimental period. The great decrease was in sorbate and starter treated brines and the same effect showed in the brines of olives treated with NaOH. The counts of whey treatments were higher than that of control, pilot experiments and all other treatments except starter treatment. 5. Pectinolytic bacterial counts were higher in pilot experiment than control experiment and the two experiments were higher than individual preservatives treatments. Sorbate was more effective against these microorganisms. The counts in the brines of the olives treated with NaOH were higher than that of the corresponding brines of olives untreated. The brines treated with combination of preservatives were more effective than that of individual ones. The counts were lower in the brines of untreated olives with NaOH than that of the treated. Sorbate + acetic + starter treated brine were more effective on the pectinolytic bacterial counts. The whey treatments counts were lower than pilot, control experiments and most individual preservatives treatments. Whey treatments counts were higher than combination of preservatives treatments. 6. Pilot and control experiments of olives untreated showed higher cellulolytic bacterial densities than preservatives treatments. The counts of pilot experiment were higher than control. Sorbate and acetic treated brines exhibited the lowest densities. The counts of combined preservatives treated brines were lower than the counts of individual preservatives ones. The counts of whey treatments were higher than other treatments. 7. Starter treated brine and whey treatments showed high effect on the densities of different microorganisms investigated at the last periods of pickling and during storage period due to the accumulation effect of the inhibitors on the counts. 8. After zero time the coliform group disappeared in preservatives brines but the counts increased in pilot control, starter and whey treatments for 15-30 days then disappeared there after. 9. Presence of preservatives accompanied with the disappearance of some sensitive strains of yeasts and/or raise in the percentage of resistance ones. 10. Species of family Cryptococcaceae were more predominated than that of Saccharomycetaceae in individual preservatives treated brines. 11. Brines treated with individual preservatives showed *P. membranaefaciens* as a resistant strain. 12. The application of combined preservatives caused the disappearance of some species observed in the brines treated with individual ones and control. Brines treated with combined preservatives of olives treated with NaOH showed more species than that of untreated. 13. Whey treatment exhibited species had not been found in other brines such as *Klu marxians*, *klu. fragilis* and *C. pseudotropicalis*. Also, whey treatments rendered higher percentage of pectinolytic species which was higher in whey treatment of olives treated with NaOH than untreated. 14. Sorbate treatment of untreated olives with NaOH exhibited great inhibitory effect on *L. brevis*, *S. lactis* and *Leuc. mesenteroides*. Acetic acid treatment of the same olives was effective against *L. casei*. 15. *Ped. cerevisiae* was the most resistant strain against individual preservatives brines. 16. Sorbate + acetic treatment of olives untreated with NaOH exhibited inhibitory effect on cocci lactics, especially *Leuc. mesenteroides*, while sorbate + starter treatment didn't inhibit *L. plantarum*, *S. lactis*, *Ped. cerevisiae* and *L. casei*. Sorbate + acetic + starter treatment exhibited great inhibitory effect on cocci lactics, but increased *L. plantarum*. The same trend was shown in brine of olives treated with NaOH. 17. *L. lactis* was found in whey treatments, but *L. casei* increased. II. Chemical determinations 1. The pH level decreased during pickling and storage periods. The lowest pH values were exhibited in acetic acid and starter treated brines among brines treated with individual preservatives of olives untreated and treated with NaOH. The pH values decreased in the brines of olives treated with NaOH than that of untreated. The whey treatments exhibited quick decrease in pH values during the first 30 days then increased due to the activity of yeasts. 2. Starter

treated brine exhibited the highest mean value of acid produced among individual preservatives treatments. This was followed by sorbate treatment of the olives untreated and treated with NaOH. The total acidity was higher in the preservatives treated brines. The acidity was higher in the combination of preservatives treated brines than individual ones and was the highest in whey treatments.

3. Percentage of lactic acid in starter treatment was higher than other individual preservatives treated brines of the olives untreated and treated with NaOH. The percentage of lactic acid in the combination of preservatives treated brines was lower than that of individual ones due to the lactic activity. Sorbate + starter treated brine showed the highest percentage of lactic among combination of preservatives, but whey treatments exhibited the highest percentage of lactic.
4. Percentage of volatile acids in sorbate treatment of olives treated with NaOH was higher than individual and combined preservatives treatments including pilot and controls experiments. On the other hand, whey treatments exhibited the highest percentage of volatile acids.
5. Sorbate treated brine of olives treated with NaOH contained the highest percentage of soluble acids than individual preservatives treatments including pilot and control. On the other hand, "sorbate + starter" treated brine of the olives treated with NaOH was higher than sorbate treatment. Whey treatments exhibited superiority than all other treatments.
6. The highest percentage of total sugars remained in the olives untreated with NaOH than that treated. The highest percentage remained in control experiments, followed by olives untreated with NaOH in the whey, then that found in sorbate + acetic acid treated brine than other treatments.
7. The percentage of reducing sugars remained in the olives untreated with NaOH of sorbate + acetic + starter brine was higher than all other treatments including pilot, control and whey treatments.
8. Percentage of total crude-protein remained in olives untreated with NaOH was higher than that in olives treated. The highest percentage remained in olives untreated in sorbate + acetic treated brine. This was followed by that remained in olives untreated in the whey. True-protein showed the same trend.
9. Ether extract in olives untreated with NaOH was higher than that treated. The highest percentage was in untreated olives in sorbate + acetic + starter treated pickles. The percentages of ether extract in the fruits of preservatives treated brines were higher than corresponding controls and pilot experiment, while whey treatments showed lower percentage than corresponding controls.
10. The fruits in the brines of individual and combined preservatives exhibited the highest percentage of pectin than pilot and control fruits. The fruits treated with NaOH showed lower percentage than untreated. The olives untreated in sorbate and sorbate + starter treated brines exhibited the highest percentage of pectin, while fruits of whey treatments showed the lowest percentage of pectin.
11. The percentage of tannins in the olives untreated with NaOH was higher than treated olives. . The highest percentage showed in olives untreated with NaOH of sorbate and sorbate + acetic treated brines.
12. Vitamin C was lost from the fruits during pickling. The fruits in the acidified brines with acetic acid exhibited the highest percentage of the vitamin. The vitamin C in the brine was lost in all treatments during storage.
13. The fruits of preservatives brines showed lower percentage of NaCl than that of pilot and control in most treatments including whey treatments. Fruits in acetic treated brines exhibited the lowest percentage of NaCl. The percentage of NaCl in the brine exhibited reverse relationship with that of the fruits.
14. The percentage of the moisture increased with time till the end of the experimental period. The highest increase was in the fruits of the preservatives brines than pilot and control experiments.

III. Organoleptic tests:

1. Olives untreated with NaOH of acetic treated brine recorded the best evaluation than olives of other individual preservatives.
2. Olives of combined preservatives exhibited lower quality than that of individual preservatives.
3. The best treatment among olives untreated with NaOH in the combined preservatives treated brines was in the fruits in sorbate + acetic + starter, while the best treatment with treated olives was sorbate + starter treatment.
4. Whey treatments exhibited the lowest quality than all treatments after storage period. Olives treated with NaOH in brined whey produced lower quality than that of untreated.
5. Whey treatments may be suitable for quick pickling but not for the storage of pickles.
6. Olives in individual preservatives treated brines were valid for the storage for the herein experimented period (270 days), while olives in combined preservatives treated brines were valid for storage for longer period.