# Factors Affecting the Softening of Pickled Pasteurised Cucumbers

*M. VOLDŘICH*, I. HORSÁKOVÁ, M. ČEŘOVSKÝ, H. ČÍŽKOVÁ and H. OPATOVÁ*

*Department of Food Preservation and Meat Technology, Institute of Chemical Technology in Prague, 166 28 Prague, Czech Republic, *E-mail: michal.voldrich@vscht.cz*

**Abstract**: During the last three seasons the specific softening of pickled cucumbers was observed. The defective samples were analysed, but no microbial contamination was confirmed and no residual enzyme activity as well. The hypothesis of residual activity of microbial pectinases and cellulases as the most probable softening cause was proposed. The cellulolytic and pectolytic activities of nineteen strains of moulds and yeasts isolated from the samples of soils, cucumbers and cucumber plants rests were compared. The inactivation parameters (D and z values) of pectolytic enzymes of the most active strains were determined. The inhibitory effect of $Ca^{2+}$ addition was evaluated within the model experiments. The residual enzyme activities were confirmed as the main cause of the defect, together with other factors such as the characteristic composition of microbial contamination, the stress or other damage of the cucumbers during the postharvest manipulation (chilling injury, humidity stress, etc.), microbial contamination of cucumbers before processing, conditions of washing, heat treatment parameters, etc. The practical recommendations for the prevention of the defect were formulated.

**Keywords**: cucumbers; effect of $Ca^{2+}$; softening of sterilised vegetables; cellulolytic activity; pectolytic activity; mould

Pasteurised cucumbers in acidic brine are one of the traditional Central European foodstuffs (e.g. Znojemské okurky and other brands). During the last three seasons the specific quality defect was observed. The products complied with specifications just after the production process (sorting, washing, pickling, sealing and pasteurisation) but after several months of storage the maceration of surface layers of cucumbers was observed. Similar phenomenon was described in fifties in the production of fermented cucumbers in brine, the softening was caused by fungal polygaracturonases and cellulases, which were introduced into the brine mainly by way of fungus laden flowers that remain attached to the green cucumbers (Raymond *et al*. 1959). Various inhibitory treatments including the use of plants rich in hydrolysable tannins (grape, persimmon, dog wood, blueberry, etc.) were described (Bell *et al*. 1962). Leafs of grapes are often used as a traditional part of the recipe also for the production of pasteurised pickled cucumbers, but more often the calcium is used to improve the texture. The mechanism of calcium effect is known and often studied, divalent cations form bonds with the dissociated free carboxyl groups of adjacent pectin molecules to form more rigid structure (e.g. Fleming *et al*. 1987). The firmness effect of calcium depends on the degree of pectin methylation and amount of calcium added (Hudson & Buescher 1986; Howard & Buescher 1990; Krall & McFeeters 1998), the hardening effect of calcium surprisingly increases with pH reduction, below pH 5 the relative effectiveness of calcium in reducing rate of softening increases as pH decreased (Hudson & Buescher 1985; McFeeters & Fleming 1989, 1990, 1991).

The aim of this study was to evaluate potential causes of the defect and to propose the methods of prevention.

## MATERIALS AND METHODS

***Determination of the cellulolytic activities***. The mineral medium (White & Downing 1951) was prepared and sterilised with cotton ribbon (at

121°C, 15 min). Medium was inoculated by the mould and cultivated at the temperature 25°C for 14 days. 50% ethanol and distilled water was used for washing of the ribbon. The measurement of the power needed to tear across the ribbon was carried on the apparatus Instron (max. power [N]).

***Determination of the pectolytic activities***. The moulds were cultivated in 100 ml of pectin medium (Moyo *et al*. 2003) (8 days at 25°C). The medium was strained after cultivation. It was prepared 1 ml medium with 1 ml test solution containing dinitrosalicylic acid. This solution was boiled 10 min and then was filled up to 10 ml with distilled water. Absorbance was measured at 530 nm.

***Strength measurements***. The strength measurements were carried out on the Instron 5544 tensile strength tester (Instron Ltd., England). The teasing adapter was used for the measuring of the ribbons strength and the punching one was used for the peel firmness evaluation. The power needed for the teasing of the ribbons or the punch through the cucumber peel was determined.

***Determination of the temperature and the treatment influences on the cucumber texture***. For the observation of the temperature influence, the cucumbers were inserted into the can they were overflowed with sweet-sour pickle and heat treated at 90°C and 95°C. For the observation of the influence of handling with raw material, one part of cucumbers was leave to become mouldy and the second one was used healthy. The cucumbers were inserted into the bottles they were overflowed with sweet-sour pickle and heat treated at the temperature of 90°C. After the heat treatment, the firmness of the cucumbers was evaluated as above.

***Determination of the inactivation parameters of pectolytic enzymes***. The temperature influence on the enzymes stability was determined by the metal ampoules method (Quintavalla *et al*. 2001). The enzyme activity was estimated as increasing of the reducing sugars concentration after the enzyme reactions passed through (Mik 2008).

## RESULTS AND DISCUSSION

The surface maceration of cucumbers pasteurised in acidic brine were observed in important part of the production in the seasons 2006–2007. The occurrence of defective products related to the part of cucumbers from one site in South Moravia and Hungary and to the several periods of harvest. The defect was not evident immediatelly after the pasteurisation but the softening occurred after several months of storage of final products. Comparing with the defects of fermented cucumbers described above in our cases the surface layers of cucumbers were macerated mainly (Figure 1). No living microorganisms were found in the products, no residual enzyme activity was observed. The effect of heat degradation was also considered, but the overheating resulted in different texture quality, and no surface maceration was observed. In the case of infected jars, the texture of individual cucumbers in jar was less homogenous comparing with overheated products only (Figures 2 and 3).

Figure 1. Cucumber in jar before and after manipulation

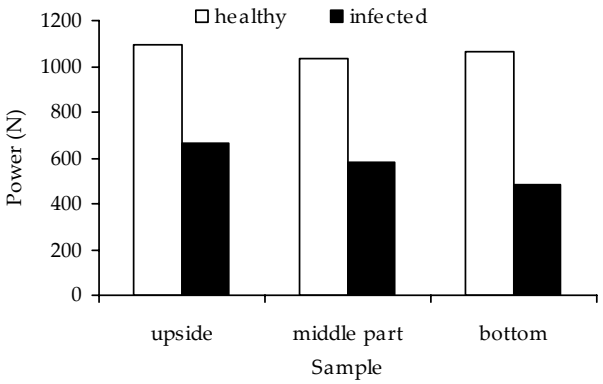


Figure 2. Comparison of the cucumbers firmness

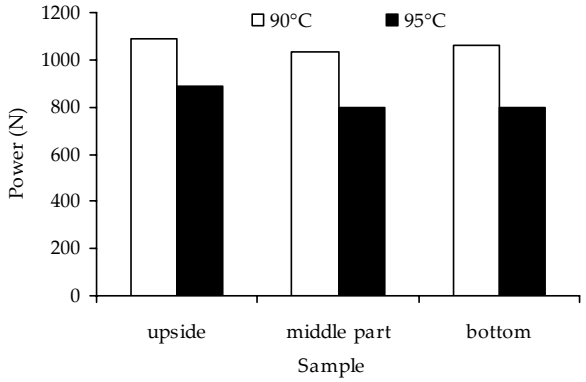


Figure 3. Comparison of the cucumbers firmness between the moulded and healthy cucumbers in one jar between the cucumbers heated on the temperatures of 90 and 9°C in one jar

Considering all available information the most probable reason of the defect is probably residual polygalacturonase and cellulose activity in brine after the pasteurisation. The climatic conditions in the seasons and the cultivation conditions (e.g. fertilisation) could also support the microbial growth on the surface of cucumbers. The conditions of postharvest treatment and storage also affect significantly the surface enzymes activities (Etchells *et al*. 1973). According to the information of producer the cucumbers were harvested within several days and collected in refrigerator and probably stressed by fast chilling below the safe temperatures for cucumbers. The chilling injury could also support the microbial growth with parallel physiological changes in the cellular skeleton due to the stress. The surface contamination of cucumbers increases during the processing, microorganisms and their extracellular enzymes also remain in the damaged surface layers of cucumbers, the washing need not to be efficient enough. During the pasteurisation treatment the microorganisms are destroyed, but residual enzyme activities can stay in the brine and cause the subsequent hydrolysis of already damaged tissues.

To support this hypothesis the 19 mould species were isolated from the cucumbers prepared for the processing in the production plant.

In addition to the characterisation of individual enzyme activities of isolates the thermoinactivation parameters of pectolytic enzymes were determined using the mould isolates possessing the highest pectolytic activities (i.e. No.: 2, 7, 11, 12, and 20). The thermal inactivation parameters for the most resistant isolate No. 7 were: D-65 = 3.8 min, z = 14.4°C. Unfortunately the applied method of cellulase activity determination was not usable to get the corresponding D-t parameters for cellulases. The obtained inactivation data of pectinases do not support the hypothesis of residual enzyme activities, but most of moulds are able to produce extracelullar cellulases, some of them relatively thermoresistant, especially cellulases produced by thermophilic moulds (Kvesitadze *et al*. 1986). Thermoinactivation of such enzymes is difficult, low residual activities can probably remain also after the usual pasteurisation treatment.

In another laboratory experiment fresh cucumbers were pickled in brine and heated using the similar heat treatment as commercial products, then $CaCl_2$ in concentration 20 mmol/l was added into one of the set, the both were inoculated with yeast isolate No. 14 and stored at ambient temperature. The changes in texture were followed during the short storage experiment, the results are given in Figure 5. In agreement with the above cited results the cucumbers with calcium remained firm while the samples without addition exhibited texture qualities close to those of the defective commercial products. The used concentration of calcium did not affect the flavour of the products. The tissue hardening was evident also in the case of $CaCl_2$ treatment of fresh cucumbers infected by mould. In the cited articles (Hudson & Buescher 1985; McFeeters & Fleming 1989, 1990, 1991) the inhibitory effect of calcium on cucumber softening is explained mainly as hardening of tissues by linking of pectines. But the inhibition of enzymes by forming bigger molecules which cannot reach the active sites of the enzymes is also obvious.

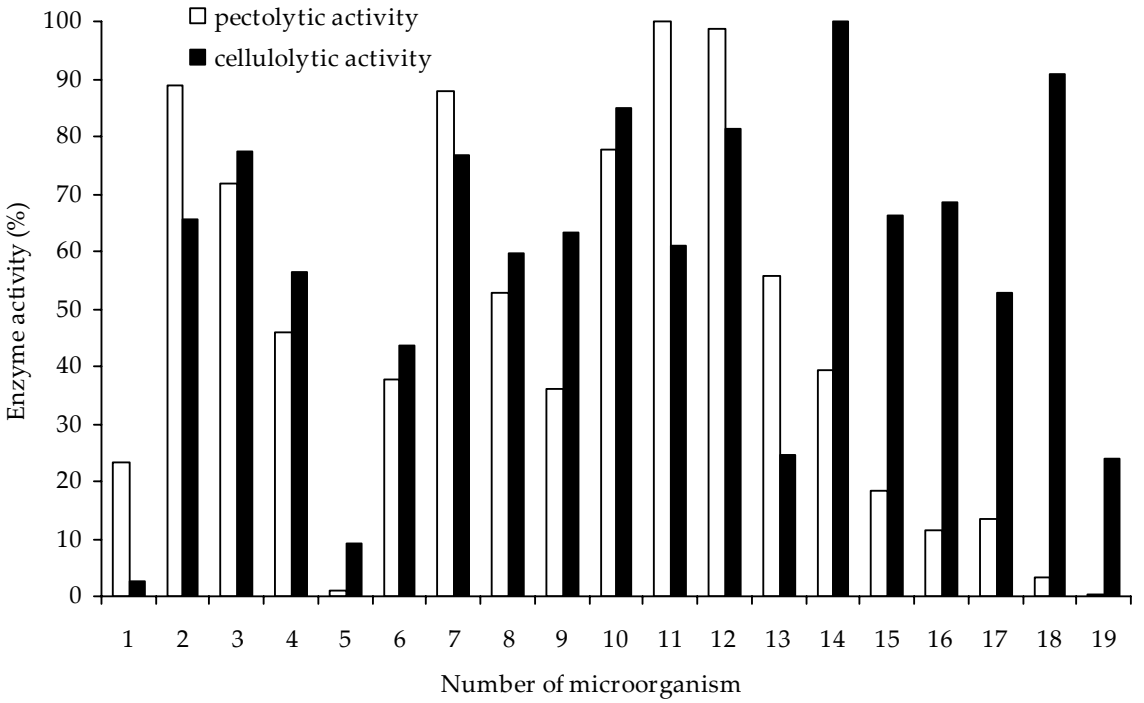


Figure 4. Comparison of cellulolytic and pectolytic activity of the moulds isolated from cucumbers, soil and cucumber plants. 15 of the isolates were identified at the Faculty of Science, Charles University Prague: 2 – *Alternaria* sp., 3 – *Alternaria alternata*, 4 – *Fusarium* sp., 6 – *Mucor mucedo*, 7 – *Alternaria/Ulocladium*, 8 – *Fusarium* sp., 9 – *Alternaria* sp., 10 – *Pleospora*: *Stemphylium*, 11 – *Cladosporium herbarum*, 12 – *Alternaria* sp., 14 – unidentified yeast, 15 – *Epicoccum nigrum*, 16 – *Alternaria* sp., 17 – *Penicillium expansum*, 18 – *Ulocladium* sp., 19 – *Cladosporium herbarum*

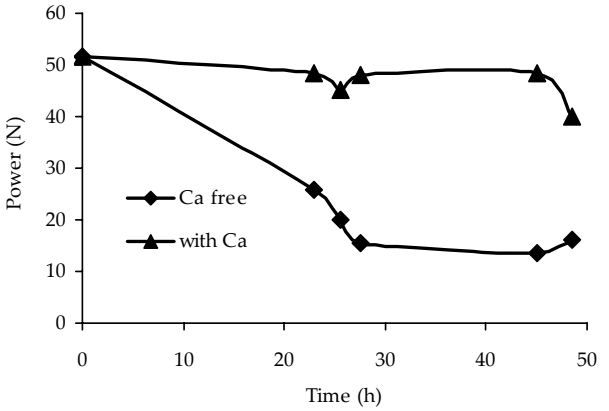


Figure 5. Effect of the $Ca^{2+}$ on the softening of cucumbers surface caused by the yeast isolate (No. 14)

The possible preventive actions avoiding this defect are:

– Selection of superior quality raw material (naturally fertilised, no forced, proper cultivars);
– Elimination of stress (slow chilling and storage at 10°C and high relative humidity at about 95%);
– Addition of $Ca^{2+}$ to washing water and to the brine.

***Acknowledgements***: The authors are grateful for financial support provided by the Ministry of Education, Youth and Sports of the Czech Republic (Project No. MSM 6046137305).

## References

Bell T.A., Etchells J.L., Williams C.F., Porter W.L. (1962): Inhibition of pectinases and cellulases by certain plants. Botanica Gazeta, **123**: 220–225.

Etchells J.L., Bellt A., Costilow R.N., Hood C.E., Anderson T.E. (1973): Influence of temperature and humidity on microbial, enzymatic, and physical changes of stored, pickling cucumbers. Applied Microbiology, **26**: 943–950.

Fleming H., McFeeters R.F., Thompson R.L. (1987): Effects of sodium chloride concentration on firmness retention of cucumbers fermented and stored with calcium chloride. Journal of Food Science, **52**: 653–657.

Howard L.L., Buescher R.W. (1990): Cell wall characteristics and firmness of fresh pack cucumber pickles

affected by pasteurization and calcium chloride. Journal od Food Biochemistry, **14**: 31–43.

Hudson J.M., Buescher R.W. (1985): Pectic substances and firmness of cucumber pickles as influence by $CaCl_2$, NaCl and brine storage. Journal od Food Biochemistry, **9**: 211–229.

Hudson J.M., Buescher R.W. (1986): Relationship between degree of pectin methylation and tissue firmness of cucumber pickles. Journal of Food Science, **51**: 138–140.

Krall S.M., McFeeters R.F. (1998): Pectin hydrolysis: effect of temperature, degree of methylation, pH and calcium on hydrolysis rates. Journal of Agricultural and Food Chemistry, **46**: 1311–1315.

Kvesitadze G., Gogilashvili L., Svanidze R., Buachidze T., Chirgadze L., Nizharadze D. (1986): Exogenous cellulases of thermophilic micromycetes. 11. Thermostability of enzyme preparations. Acta Biotechnologica, **6**: 361–367.

McFeeters R.F., Fleming H.P. (1989): Inhibition of cucumber tissue softening in acid brines by multivalent cations: inadequancy of the pectin "egg box" model to explain textural effects. Journal of Agricultural and Food Chemistry, **37**: 1053–1059.

McFeeters R.F., Fleming H.P. (1990): Effect of calcium on the thermodynamics of cucumber tissue softening. Journal of Food Science, **55**: 446–449.

McFeeters R.F., Fleming H.P. (1991): pH effect on calcium inhibition of softening of cucumber mesocarp tissue. Journal of Food Science, **56**: 730–732.

Mik V. (2008): Faktory ovlivňující maceraci sterilovaných okurek. [Diplomová práce.] VŠCHT, Ústav konzervace potravin a technologie masa, Praha: 32–33

Moyo S., Gashe B.A., Collison E.K., Mpuchane S. (2003): Optimizing growth conditions for the pectinolytic activity of *Kluyveromyces wickerhamii* by using response surface methodology. International Journal of Food Microbiology, **85**: 87–100.

Quintavalla S., Larini S., Mutti P., Barbuti S. (2001): Evaluation of the thermal resistance of different *Salmonella* serotypes in pork meat containing curing additives. International Journal of Food Microbiology, **67**: 107–114.

Raymond F.L., Etchells, J.L., Bell T.A., Masley P.M. (1959): Filamentus fungi from blossom ovaries and fruit of pickling cucumbers. Mycologia, **5**: 492–495.

White W.L., Downing M.H. (1951): *Coccospora agricola* Goddard – its specific status, relationships, and cellulolytic activity. Mycologia, **43**: 645–657.