Lebensm.-Wiss. u.-Technol., 27, 463–467 (1994)

Ultra-High Pressure Processing of Onions: Chemical and Sensory Changes

P. Butz, W. D. Koller, B. Tauscher and S. Wolf

P. Butz, B. Tauscher, S. Wolf: Federal Research Centre for Nutrition, Institute of Chemistry and Biology, Engesserstr. 20, D-75131 Karlsruhe (Germany)

B. Tauscher: Federal Research Centre for Nutrition, Institute of Technology, Engesserstr. 20, D-75131 Karlsruhe (Germany)

(Received February 7, 1994; accepted May 2, 1994)

The microbial load of fresh onions was considerably reduced by a short pressure treatment at temperatures of 25 and 40 °C and pressures up to 350 MPa. These conditions are not sufficient to inactivate undesirable enzymes. Pressure above 100 MPa damages cell structures like membranes and provokes a massive release of enzymes leading to numerous enzymatic reactions that influence flavour and product stability. UHP processing (30 min/300 MPa/25 and 40 °C) changes the odour of fresh onions towards that of braised or fried onions. Pressure above 100 MPa induces browning reactions by release of polyphenoloxidase (PPO). The activity of isolated onion PPO is even stronger after pressure treatment up to 700 MPa.

Introduction

Recently, ultra-high isostatic pressure as a pasteurization process has been introduced into food industry (1). Pressures of 300–900 MPa at ambient temperatures may inactivate enzymes and destroy most food-spoiling yeasts, moulds and vegetative micro-organisms while nutrients and the food's natural taste are not affected. For economic reasons it is advisable to not apply pressures higher than 400 MPa with treatment times of only a few minutes at room temperature (2). As onions have recently been suspected of being responsible for microbial contamination of minced meat in hamburgers, applicability of ultra-high pressure to decontaminate fresh onions was to be investigated.

Materials and Methods

Onions, species 'Stuttgarter Riese', were stored at 10 °C.

Ultra-high pressure treatment

Experiments were performed in a high pressure device consisting of a series of thermostated micro-autoclaves (10 mL, ID 16 mm) connected via valves (3). For microbiological assays samples of 1 mL were filled into flexible polyethylene bags which were heat-sealed afterwards. Samples for subsequent gaschromatographic aroma analysis were pressure treated in teflon tubes (ID 6 mm, AD 8 mm) which do not absorb volatile compounds. For sensory analysis 100 mL samples in laminated plastic bags (PET\|Al\|LDPE, 12\|9\|90µm) were pressurized in a 200 mL autoclave (ID 40 mm; 400 MPa max.); under the conditions used no delamination of the plastic bags due to pressure was found. For the investigation of pressure effects on isolated onion polyphenoloxidase the enzyme was diluted in TRIS-buffer (0.01 mol/L, pH 6.5) to an activity of 800 U/mL according to the conditions in the cell free extract. Samples of 0.4 mL were treated in polyethylene reaction tubes. Directly after pressure release the samples were tested for PPO activity. Water was used as pressure transmitting medium. Process parameters are given in the legends to the figures. All experiments were carried out in triplicate.

Determination of the microbial count

Surviving micro-organisms were determined by the Koch pour plate method using media and conditions listed in **Table 1**.

Table 1 Micro-organisms in the outer peel of onions

Organism	Medium	Conditions	log cfu/g
Total number	Standard-1-Agar	aerobic, 30 °C, 24 h	5.8
Thermotolerant	Standard-1-Agar	aerobic, 55 °C, 24 h	1.5
Lactobacillae	MRS-Agar	aerobic, 37 °C, 24 h	negative
Enterococci	CATC-Agar	aerobic, 37 °C, 24 h	negative
Anaerobics	Thioglycollate Agar	anaerobic, 37 °C, 24 h	4.7
Spores of anaerobics	Thioglycollate Agar	anaerobic, 37 °C, 24 h	1.6
Spores of aerobics	Standard-1-Agar	aerobic, 30 °C, 24 h	2.7
Yeasts and moulds	Malt Extract Agar	aerobic, 30 °C, 24 h	5.5

0023-6438/94/050463 + 05 $08.00/0

Sensory analysis

A trained sensory panel of 8 to 10 assessors examined the smell of pressurized and non-pressurized diced onions (300 MPa; 30, 60 min; 25, 40 °C) in three subsequent sessions. The tests were performed immediately after the pressure treatment.

Headspace gas chromatography

A Perkin Elmer Sigma 3B gas chromatograph equipped with HS 6 headspace sampler and flame ionization detector (FID) was used. Analyses were carried out on a 60 m × 0.25 mm ID Supelcowax 10 fused silica capillary column. Gas Chromatography (GC) conditions were: carrier gas: helium at 2 bar column pressure; initial oven temperature: 60 °C for 2 min. The oven temperature was programmed to increase from 60 °C to 200 °C at 4 °C/min; injector temperature: 200 °C; detector temperature: 200 °C. For analysis samples were equilibrated in gas-tight septum crimped head space vials at 60 °C for 30 min. Than an aliquot (about 1.6 mL) of the headspace gas was injected electronically controlled into the column of which a short section was immersed in liquid nitrogen. After another 5 min the cryotrap was removed and the GC program started. Peaks were identified under the same GC conditions by mass spectrometry on a Hewlett-Packard 5985 B GC/MS with 70 eV electron energy and 200 °C ion source temperature.

Onion polyphenoloxidase purification

Extraction and purification procedures were carried out according to Kimberley and Wissemann (4).

Onion polyphenoloxidase activity test

Enzyme activity was measured spectrometrically by the change in extinction at 420 nm with 1,2-benzenediol as substrate. One unit is the amount of enzyme producing a change of 0.001 in extinction at 420 nm in 1 min.

Gel electrophoresis

SDS-PAGE was performed according to the method described by (5) using a 150 g/kg running gel and a tris-glycine electrode buffer. The gels were stained with Coomassie Blue.

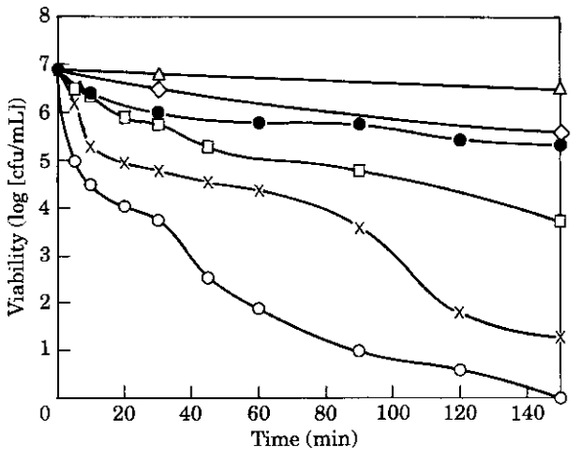

Fig. 1 High-pressure decontamination of diced onions shown as decrease in the logarithm of colony forming units (cfu). Sample composition: mixture of 0.5 g of onion cubes and 0.5 mL of a suspension of bacteria isolated from onion peel in NaCl solution (8.5 g/L). 0.1 MPa, 25 °C (△); 0.1 MPa, 40 °C (◇); 150 MPa, 25 °C (●); 150 MPa, 40 °C (□); 350 MPa, 25 °C (×); 300 MPa, 40 °C (○)

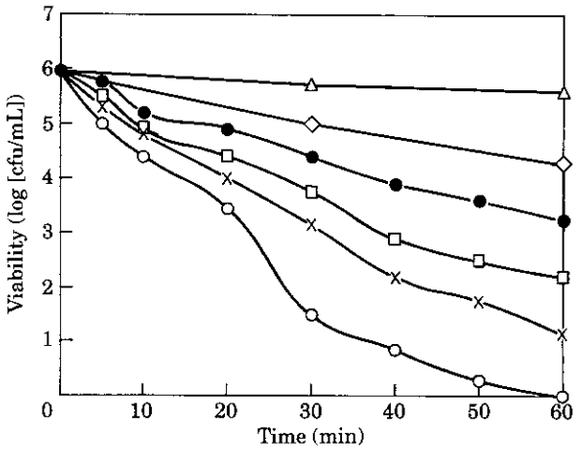

Fig. 2 High-pressure decontamination of diced onions shown as decrease in the logarithm of colony forming units (cfu). Sample composition: mixture of 0.5 g of onion cubes and 0.5 mL of a suspension of yeasts and moulds isolated from onion peel in NaCl solution (8.5 g/L). 0.1 MPa, 25 °C (△); 0.1 MPa, 40 °C (◇); 150 MPa, 25 °C (●); 150 MPa, 40 °C (□); 350 MPa, 25 °C (×); 300 MPa, 40 °C (○)

Results and Discussion

Reduction of microbial counts

Diced onion samples prepared from inner slices of onions and containing up to 10^3 micro-organisms per gram were decontaminated already after treatment of 10 min at 150 MPa and 25 °C. For experiments with higher initial microbial counts the natural occurring germs of the brown onion peel (see **Table 1**) were cultivated in nutrient broth and subsequently pressure treated in the presence of diced onions. **Figure 1** shows the results of the treatment of onion samples in enriched bacterial suspensions at different pressures and temperatures. Viability data shown as decrease of the logarithm of colony forming units (cfu) represent mean values of three experiments, deviations in log cfu were always below one unit. Three hundred megapascals and 40 °C were most effective; under these conditions microbial counts were reduced from 10^7 to less than 1000 within 30 min. The results of similar experiments in diced onions in enriched suspensions of yeasts and fungi are shown in **Fig. 2**. At 300 MPa and 40 °C the microbial load is reduced by five orders of magnitude within 30 min. As the inactivation kinetics of both series of experiments are not linear a more complicated inactivation mechanism can be assumed.

Macroscopic and microscopic changes

Diced onions showed no major changes in appearance immediately following 30 min pressure treatment at 300 MPa and 25 °C (**Fig. 3**). There was not much leakage of cellular fluids. A slight tendency towards a more glassy appearance, typical of steamed onions was observed. The smell had changed accordingly.

After 24 h in storage at 20 °C the diced onions pressurized at 350 MPa had turned brown while those exposed to 300 MPa treatment started browning. The 250 MPa samples showed the same effect with a 24 h delay whereas samples treated

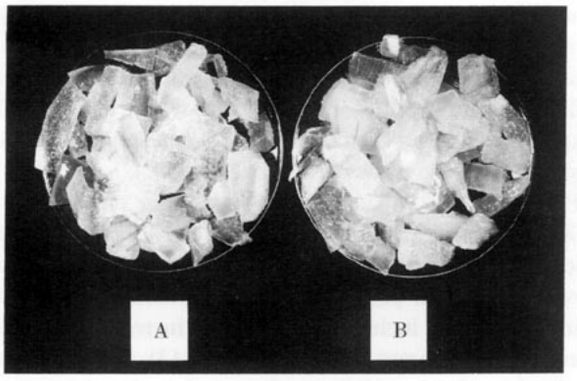

Fig. 3 High-pressure processed diced onions. Freshly cut onion cubes were pressurized in plastic bags (no additives). Photographs were taken immediately after treatment. Plate diameter: 40 mm. (**a**) 30 min, 0.1 MPa, 25 °C. (**b**) 30 min, 300 MPa, 25 °C

by pressures up to 100 MPa remained unchanged. Storage at 4 °C delayed the browning by 1 wk. Systematic investigation of this effect at pressures from 0.1 to 350 MPa has shown that any pressure treatment above 100 MPa induces browning of diced onions and that the rate of the browning reaction increases with increasing pressure. This effect is due to an enzymatic browning reaction involving polyphenoloxidases. Polyphenoloxidase is often described as a soluble enzyme predominantly localized in the cytosol of plant cells; however, the enzyme is as frequently found to be associated with particulate cell fractions (6). In intact cells phenolic compounds, the substrates of polyphenoloxidase, are confined to vacuoles and are spacially separated from the enzyme by the tonoplast. When the cell and the tonoplast are disrupted, phenolic oxidation products are formed (7). This enzymatic browning is usually observed on the cut surfaces of light-coloured fruit and vegetables exposed to air. Due to the influence of pressure onion epidermis cells and cellular components like vacuoles are also found to be affected. Microscopic studies revealed severe damage to the vacuoles of onion epidermis cells treated by 300 MPa at 25 °C (**Fig. 4a**) independently of the duration of treatment. Polyphenoloxidase is thus no longer separated from its natural substrates (phenols which are oxidized to orthoquinones, which in turn polymerize to form brown pigments). Addition of 10 g/L sucrose solution

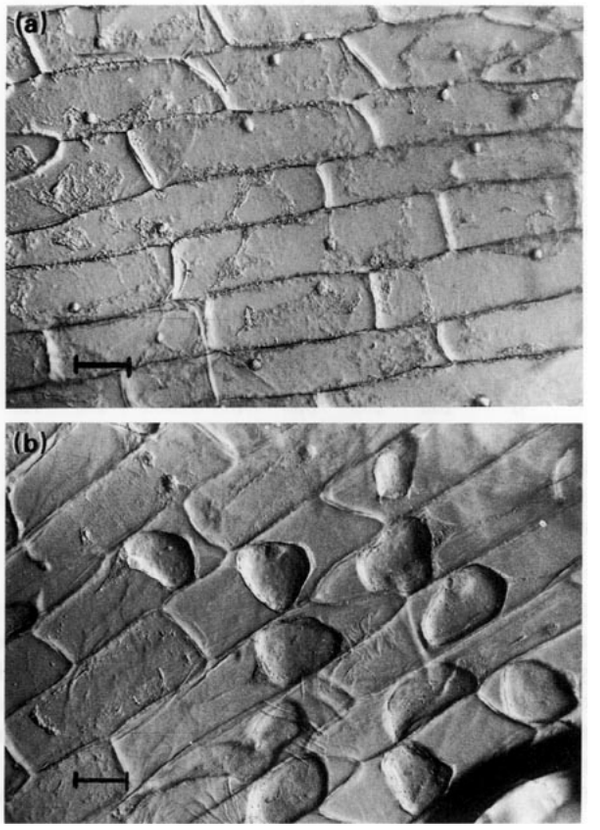

Fig. 4 Epidermal cells of onions transferred into sucrose solution (100 g/L) after high pressure treatment at 25 °C. (**a**) 30 min, 300 MPa; (**b**) 30 min, 100 MPa; bar: 0.1 mm

caused no plasmolysis in contrast to untreated cells. In cells treated at 100 MPa and 25 °C the ability to respond to sucrose by plasmolysis was only affected to a minor degree (**Fig. 4b**). As isostatic pressure acts immediately and uniformly on all cells pressure-induced browning occurs throughout the onion cubes provided enough oxygen is present.

Sensory and chemical changes

In three subsequent sessions the sensory panel without exception judged the pressure-treated onions to smell less intensely and rather like cooked or fried onions.

Therefore a headspace gaschromatographic analysis of

Table 2 Chemical changes in the volatile compounds of onions after 30 min UHP treatment at 25 °C measured as changes in peak areas of headspace gaschromatograms (reproducibility: +/− 15%)

No.	Retention time (min)	Compound	Peak area 0.1 MPa	Peak area 300 MPa	change (%)
1	4.3	Propanal	333	387	+ 16
2	4.7	1-Propanethiol	1548	5.2	− 99.7
3	5.25	Methanol	10.9	92.9	+ 750
4	8.6	Hexanal	1.0	1.4	+ 40
5	11	2-Methyl-pent-2-enal	6.9	20.6	+ 199
6	13.4	Methylpropyl disulfide	30.8	4.2	− 86
7	14.1	3,4-Dimethylthiophene	0.1	0.5	+ 400
8	14.5	Methyl cis-propenyl disulfide	0.25	0.25	+/− 0
9	15.4	Methyl trans-propenyl disulfide	2.8	0.5	− 82
10	18.6	Dipropyl disulfide	648	203	− 69
11	20	Propyl cis-propenyl disulfide	11.4	10.0	− 12.3
12	20.7	Propyl trans-propenyl disulfide	43.3	73.6	+ 70
13	24.1	Methyl propyl trisulfide	2.75	0.25	− 91
14	28.9	Dipropyl trisulfide	12.8	2.1	− 84

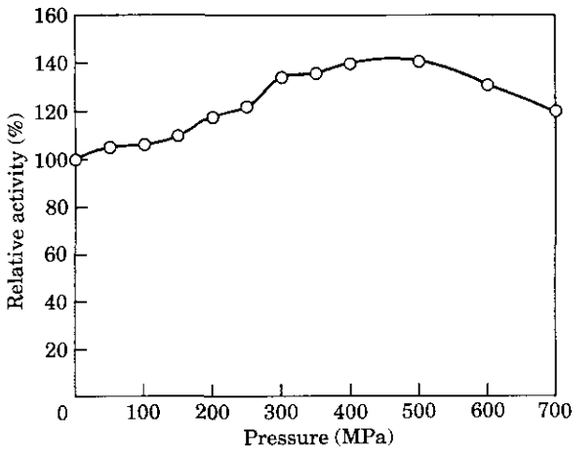

Fig. 5 Relative activity of onion PPO after 10 min incubation at different pressures and 25 °C in 0.01 mol/L TRIS buffer, pH 6.5 (activity at 0.1 MPa taken as 100%).

aroma volatiles of pressure treated and untreated onions was carried out to find out whether these results were paralleled by perceivable chemical changes. **Table 2** shows the changes in concentrations—determined by the comparison of peak areas in headspace gaschromatograms—of some aroma components after pressure treatment of 30 min at 300 MPa and 25 °C. Deviations in peak areas in three repetitive experiments were always below 15 %.

UHP leads to a strong decrease of dipropyldisulfide, a compound considered typical of the pungency and characteristic odour of fresh onions (8).

The pressure-induced change in flavour towards braised and fried onions, respectively, is explained by the increase of the proply transpropenyldisulfide and of 3,4-dimethylthiophene (9).

These changes are accompanied by a strong increase in 2-methyl-pent-2-enal, the aldol condensation product of propanal, one of the main products of alliinase action (9,10). This product forms also without pressure treatment about 2 h after the onions were cut (9,10), probably catalysed by an aldolase which is released after cell injury. The acceleration of this reaction by pressure may be due to stronger decompartimentation of the enzyme as discussed above with polyphenoloxidase. A possible pressure activation of onion aldolase similar to onion PPO remains to be studied.

In samples treated at 40 °C and 300 MPa, in contrast to those at 25 °C, the concentration of methylpropyl disulfide, typical of heated onions (11), increases (data not shown).

As with many pressure-treated fruit and vegetables an increase of hexanal has been found also in pressurized diced onions. Formation of hexanal by lipid oxidation of linoleic acid is enhanced by pressure treatment (12).

Effect on polyphenoloxidase

In order to find out whether the accelerated browning reaction with increasing pressures above 100 MPa is due to pressure activation of polyphenoloxidase (PPO), the influence of pressure on onion PPO was investigated first in cell-free extracts to exclude an apparent activity increase due to pressure-induced release of enzymes from the tissue. However, these experiments were not satisfactory because of undesirable chemical reactions occurring. Therefore PPO had to be isolated. The enzyme was isolated by Hydrophobic Interaction Chromatography (HIC) (4). Measurements of the enzyme activity as a function of pH yielded a maximum at pH 5 to 7. **Figure 5** shows the results of UHP experiments with isolated onion PPO (mean values of four independent assays, deviations below 10 %).

PPO activity is enhanced with increasing pressure up to 500 MPa where a maximum activity of 142 % compared to the untreated enzyme is reached. Higher pressure results in lower activation, possibly due to beginning denaturation. Similar effects, including a five-fold increase in activity, have been reported for pear PPO (13). High pressure activation of onion PPO can thus explain the phenomenon of pressure induced browning. However, one has to bear in mind that enzymes in whole onions are present in different chemical environments, e.g. bound to membranes or as enzyme-substrate-complexes. The resistance of such conjugates to pressure may be much higher as that of isolated enzymes in solution. The mechanism underlying the pressure activation of onion PPO remains to be explored. It may be that pressure induces a change in protein conformation resulting in higher enzyme activity, or separation of a part of the protein molecule and subsequent liberation of a second active site. However, there were no indications of major changes in native and SDS-PAGE of pressure-treated onion PPO.

Conclusion

The microbial load of onions is considerably reduced by short pressure treatment at up to 400 MPa at moderate temperatures. These conditions are not sufficient to inactivate undesirable enzymes. Pressures above 100 MPa damage cell structures like membranes and induce a massive release of enzymes leading to many enzymatic reactions which influence flavour and product stability. The enzyme activity of onion PPO is even higher after pressure treatment.

References

1 CHEFTEL, J.-C. Applications des hautes pressions en technologie alimentaire. *Industries Alimentaires et Agro-Alimentaires (IAA)*, **3**, 141–153 (1991)

2 HAYASHI, R. Use of high pressure to food processing and preservation. In: HAYASHI, R. (Ed.), *Use of High Pressure in Food*. Kyoto: San Ei Publication Co., pp. 1–30 (1989)

3 BUTZ, P., HABISON, G. AND LUDWIG, H. Influence of high pressure on a lipid coated virus. In: BALNY, C., HAYASHI, R., HEREMANS, K. and MASSON, P. (Eds), *High Pressure and Biotechnology*. Colloque INSERM/John Libbey Eurotext Ltd. pp. 61–64 (1992)

4 KIMBERLY, W. AND WISSEMANN, L. C. Y. Purification of grape polyphenoloxidase by hydrophobic chromatography. *Journal of Chromatography*, **192**, 232–233 (1980)

5 BERTRAM, S. AND GASSEN, H. G. (Eds) *Gentechnische Methoden*. Stuttgart: Gustav Fischer Verlag (1991)

6 BUTT, V. S. Direct oxidases and related enzymes. In: STUMPF, P. K. and CONN, E. E. (Eds), *The Biochemistry of Plants, Vol. II*. New York, London, Toronto, Sydney, San Francisco: Academic Press, pp. 95–112 (1980)

7 ANDERSON, J. W. Preparation of subcellular fractions from plants. In: ANDERSON, J. W. and BEARDALL, J. (Eds),

Molecular Activities of Plant Cells. Oxford, London: Blackwell Scientific Publications, pp. 46–47 (1991)

8 TEWARI, G. M. AND BANDYOPADHYAY, C. Pungency and lachrymatory factor as a measure of flavour strength of onions. *Lebensmittel-Wissenschaft und-Technologie*, **10**, 94–96 (1977)

9 BOELENS, M., DE VALOIS, P. J., WOBBEN, H. J. AND AN DER GREN, A. Volatile flavor compounds from onion. *Journal of Agricultural and Food Chemistry*, **19**, 984–991 (1971)

10 MAZZA, G., LE MAGUER, M. AND HADZIYEV, D. Headspace sampling procedures for onion aroma assessment. *Canadian Institute of Food Science and Technology*, **13**, 87–96 (1980)

11 TOKARSKA, B. AND KAROWSKA, K. Untersuchungen über die Zusammensetzung eines Geschmacks- und Geruchsextraktes aus Zwiebeln. *Die Nahrung*, **25**, 565–571 (1981)

12 BUTZ, P., KOLLER, W. D., BRAUN, M. AND TAUSCHER, B. Untersuchungen über chemische und sensorische Veränderungen bei der Hochdruckbehandlung von Grapefruitsaft. In: *Gesellschaft Deutscher Chemiker, 24.* Hauptversammlung, Kurzreferate. Weinheim: VCH, p. 321, ISBN 3-527-29083-4 (1993)

13 ASAKA, M. AND HAYASHI, R. Activation of polyphenoloxidase in pear fruits by high pressure treatment. *Agricultural and Biological Chemistry*, **55**, 2439–2440 (1991)