ELSEVIER

Food Chemistry 52 (1995) 35–41

0308-8146/95/$9.50

Effects of ultra-high hydrostatic pressure treatments on the quality of tomato juice

Sebastiano Porretta,[a] Alessandra Birzi,[a] Claudio Ghizzoni[c] & Enzo Vicini[b]

[a]*Department of Tomato Products,* [b]*Department of Microbiology, Stazione Sperimentale per l'Industria delle Conserve Alimentari, Viale F. Tanara, 31/A, 43100 Parma, Italy*
[c]*Fructamine SpA, Milan, Italy*

(Received 12 January 1994; accepted 9 February 1994)

The effects of ultra-high hydrostatic pressure (UHP) treatments on some physicochemical, microbiological and sensory properties of single-strength natural tomato juice were evaluated using response surface methods. Application of graphical optimization techniques showed that UHP processing yields products which, beside being microbiologically stable, have improved viscosity and colour properties in comparison with their conventional heat-processed counterparts. Enzyme inactivation was found to be less than that caused by the conventional hot-break treatment. During UHP processing, very high *n*-hexanal and *cis*-3-hexenal concentrations were formed from free fatty acid oxidation.

INTRODUCTION

As traditional high-temperature processing methods for food and drinks can cause thermal damage and reduce fresh flavour, low-temperature alternatives are being sought.

The demand for minimally processed tomato products of rich flavour and high consistency has risen markedly in these last years.

Consistency of tomato products refers to their viscosity and the ability of their solid portion to remain in suspension throughout the shelf-life of the product; a high-consistency juice has almost no syneresis, i.e. no separation into pulp and serum. Consistency is normally improved using technological processes which minimize pectin-breakdown by enzymes (polygalacturonase and pectinmethylesterase) in pectin- and cellulose-rich tomato cultivars. Several reports on such processes are available (Porretta & Leoni, 1990; Porretta *et al.*, 1992*a*; McCalloch *et al.*, 1950; Smith & Nortje, 1958).

Syneresis, the most conspicuous defect in conventionally processed tomato products, can normally be reduced by using hot-break (or even 'super hot-break' when very high temperature–short time treatments are applied) techniques plus, sometimes, a partial removal of excess serum by centrifugal decantation. Many papers (Watanabe *et al.*, 1991; Horie *et al.*, 1992; Oxen & Knorr, 1993) report that microorganisms are inactivated by high pressure, and progress in ceramic processing has led to the development of equipment for high-pressure treatment of food on a commercial scale.

In recent years, particular attention has been given to the adaptation of pressure treatment to food preservation, with particular reference to inactivation of microorganisms. The response of food enzymes to hydrostatic pressure is now beginning to receive equitable study; contrary to what is usually found with the application of high pressures to microorganisms, enzymes may display activation or enhancement of activity, in addition to possible inactivation (Kunugi *et al.*, 1982; Fukuda & Kunugi, 1985; Ogawa *et al.*, 1990; Murao *et al.*, 1992).

The possibility of producing preserved high-consistency 'fresh-like' tomato juice by means of ultra-high hydrostatic pressure (UHP) treatments was evaluated.

The aim of this study was to determine, by means of response surface analysis, optimum processing conditions (pressure value, processing time, pH of juice) and to evaluate the effects of the treatments on some physicochemical, microbial and sensory components of single-strength tomato juice.

The response surface method (RSM) can be expressed, for a two-component system, in terms of the following least squares estimating equation, which considers the linear and quadratic effects of each compound and the interaction between the two compounds as follows:

$$y = \beta_{k0} + \sum_{i=1}^{3} \beta_{k1} X_i + \sum_{i=1}^{3} \beta_{kii} X_i^2 + \sum_{i=1}^{3} \sum_{j=i=1}^{3} \beta_{kij} X_i X_j \quad (1)$$

where X_i X_j are the coded variables linearly related to the uncoded variables (P, t, pH); and β_k are the partial regression coefficients (constants).

MATERIALS AND METHODS

Response surface methodology (RSM) and experimental design

Sterilization and pectolytic-enzyme inactivation, measured

Table 1. Process variables

Input process variables	Symbol		Levels		
	Coded	Uncoded	−1	0	1
Pressure (MPa)	x_1	P	500	700	900
Treatment time (min)	x_2	t	3	6	9
pH	x_3	pH	4·0	4·5	5·0

indirectly by total pectin content determination, processes were assumed to be a system of three input factors x_i (pH of juice, processing pressure, (P), processing time, (t)), while the output responses were total pectin content, colour, viscosity, glutamic acid and some sensory attributes normally associated with heat treatment (natural, cooked, off-flavour).

The coded and uncoded variables, and their respective levels are presented in Table 1. An experimental plan was chosen from the family of three levels design, as suggested by Box and Behnken (1960). The design, presented in Table 1, is specific for the study of quantitative variables by RSM.

Sample preparation

Fresh tomato juice (90 kg), prepared on an experimental line (350 kg h^{-1}) by crushing and sieving (0·8 mm holes) washed tomatoes, was divided into three 30-kg batches, the pH of which was adjusted to 4·0, 4·5 and 5·0, respectively, with 1 M NaOH (these values represent well the range of pH variation of fresh tomato juice).

The pH-adjusted juice of the three lots was filled into polyethylene pouches (10 cm × 13 cm, capacity 200 ml) which were heat-sealed after removal of the air and subjected to 500, 700 and 900 MPa pressures, respectively, for treatment times of 3, 6 and 9 min in all three cases.

Single-strength tomato juice (100 kg) was prepared on an experimental line (350 kg h^{-1}) from washed tomatoes by crushing, hot-break (92°C) enzymic inactivation, sieving (0·8 mm holes), hot-filling (80°C) into 1·0-kg cans and heat processing at 98°C for 15 min. This sample was used as a control for the enzymic-inactivation comparison between the two techniques (UHP and traditional).

High-pressure equipment

An ABB (Asea Brown Boveri, Vastera, Sweden) hydrostatic high-pressure test machine was used. The equipment consisted of a pressure container (110 mm i.d. × 260 mm). Pressure was applied by operating a piston with an oil hydraulic motor of a high-pressure generator and then feeding a fluid (distilled water) into the high-pressure treatment equipment. After discharging the air from the high-pressure equipment through a drain, a valve was closed and the presssure was increased to the desired level.

Chemical and physical analysis

On each sample the following determinations were performed (Porretta *et al.*, 1992*b*). Colour, by means of a model XL 800 colorimeter from Gardner Laboratory Division (Bethesda, MD, USA) with the C-CIE illuminant and the BCR (Community Bureau of Reference, Brussels) reference tile (L=25·8; a_L=33·9; b_L=14·8; a_L/b_L=2·29) (Kent & Porretta, 1990). Viscosity, with a GOSUC viscometer (Gould, 1983) by measuring the efflux of tomato juice between the two graduated marks. Pectins (pectic acids, pectates, protopectins, expressed as grams of monohydrate galacturonic acid, g kg^{-1} TS), according to the official Italian methods of analysis (1989). Glutamic acid (g kg^{-1}), by enzymic analysis (Boehringer, Mannheim, Germany).

Microbiological analysis

Total viable count (cfu ml^{-1}) was determined using a Plate Count Agar (Oxoid, Basingstoke, UK) after incubation at 30°C for 48 h. Enterobacteria by Violet Red Bile Glucose Agar (Oxoid, Basingstoke, UK) after incubation at 30°C for 24 h. Lactic acid bacteria by Rogosa Agar (Oxoid, Basingstoke, UK) after incubation at 30°C for 72 h. Yeasts and moulds, by Malt Extract Agar (Oxoid, Basingstoke, UK) acidified at pH 4·0 after incubation at 30°C for 72 h.

Sensory analysis

Sensory tests were carried out by a seven-member panel selected and trained in assessing for tomato products, natural (characteristic) taste, cooked (slightly sulphuric taste) taste and off-taste on a 1–9 (nil to extreme) category scale. Each attribute is related to hedonic assessment as follows: naturalness (1 = taste very similar to the fresh one; 9 = taste very different from the fresh one), cooked (1 = absent; 9 = burnt), off-taste (1 = absent; 9 = strong).

The attributes used by the panel derived from the main objective to obtain a general evaluation of the product (Porretta, 1992).

The assessors were selected from a group of people consisting of experts on the products and consumers. In each session, two single samples were given in random order; the samples were evaluated at 25°C under red light illumination to avoid colour interferences on assessment.

Volatile compounds

The main volatile compounds were detected using a purge and trap injectors (PTI) system (Chrompack, Middleburg, CA, USA) mounted on to a HP5890 gaschromatograph .

Sampling was performed by a continuous flow of helium applied for 20 min at 22°C. The organics stripped out from the sample were cryofocused using liquid nitrogen as cooling agent before injection into a 30 m

Table 2. Analysis of variance for the overall effect of the three process variables on the four responses

Input process variables	Sum of squares			
	Total pectin content	Viscosity	Glutamic acid	Colour (a_L/b_L)
pH	6·30***	1·40***	0·51 ***	0·41
Pressure of treatment, P	16·8***	1·05**	0·72**	13·22***
Time of treatment, t	2·42*	0·18	0·05	8·18*

*Significant at 10% probability level.
**Significant at 5% probability level.
***Significant at 1% probability level.

Table 3. Regression coefficients of the second-order polynomials for the four response variables

Regression coefficient[a] β_k	Total pectin content	Viscosity	Glutamic acid	Colour (a_L/b_L)
β_{k0}	44***	5·00***	1·58**	2·42***
β_{k1}	−3·14**	1·78**	−0·51**	0·08**
β_{k2}	13·69***	2·90***	0·06**	−0·07**
β_{k3}	5·70	0·18	0·01	−0·02
β_{k11}	6·86	2·25***	0·04	−0·05
β_{k22}	−1·22	−0·41	0·01	−0·06*
β_{k33}	−1·67	−0·75**	−0·07*	−0·08*
β_{k12}	−9·68	−1·25**	−0·45**	−0·205**
β_{k13}	8·06*	1·82**	−0·05*	0·12**
β_{k23}	2·21	−0·35*	−0·03	−0·01

[a]Coefficients of eqn (1).
*Significant at 10% probability level.
**Signficant at 5% probability level.
***Signficant at 1% probability level.

DB-5 column (J & W Scientific, CA, USA; 0·25 μm i.d.).

HP5970 mass spectrometer was used as detector.

The experimental parameters were set up as follows. PTI system: purge valve flow 10 ml min^{-1}; condenser temperature −110°C; pre-cooling time 2 min; cold trap temperature −140°C. GC: temperature programming from 40°C (held for 5 min) at 15°C min^{-1} to 200°C (held for 5 min). Detector: transfer line temperature 240°C; ion source temperature 140°C; mass spectra recording at 70 eV.

Spectral identification was performed with the aid of the NBS library.

Statistical analysis

Data were processed by the response surface method of least squares using Statgraphics (Statistical Graphics System Co., Portland, OR,USA, 1992) and VPLOT subprogram of SIMCA (University of Umea, Sweden, 1984).

RESULTS AND DISCUSSION

Estimation of the overall effect of the three factors (P, t, pH) on response variables revealed that, unlike pressure value P, time of treatment t, was not a parameter of primary importance as it significantly affected all four responses and juice pH (Table 2).

Three-dimensional surfaces were developed on the basis of predictive models.

The regression coefficients (βk) for the four statistically significant models are presented in Table 3.

Microbiological aspects

The control juice proved to be naturally contaminated by the microorganisms normally occurring in tomato juice, at the same levels as those found in this product. The effects of UHP treatments on the microorganisms in the fresh tomato juice used as control are presented in Table 4. The mildest treatment (500 MPa for 3 min) already yielded a microbiologically stable product regardless of pH value.

Total pectin content

Total pectin content was obtained by summing the

Table 4. Results of microbiological analyses carried out on fresh tomato juice (control) and after UHP treatment

Treatment			cfu/ml				
MPa	Minutes	pH	Total viable count	Entero-bacteria	Lactic acid bacteria	Yeasts	Moulds
500	3	4·0	$7·3 \times 10^{2a}$	0	0	0	0
500	3	4·5	$8·3 \times 10^{2a}$	0	0	0	0
500	3	5·0	$3·7 \times 10^{2a}$	0	0	0	0
900	9	4·5	$5·0 \times 10^{2a}$	0	0	0	0
Control:			$4·0 \times 10^{4}$	$5·6 \times 10^{2}$	$1·8 \times 10^{4}$	$7·0 \times 10^{4}$	$8·0 \times 10^{3}$

[a]*Bacillus* sp.

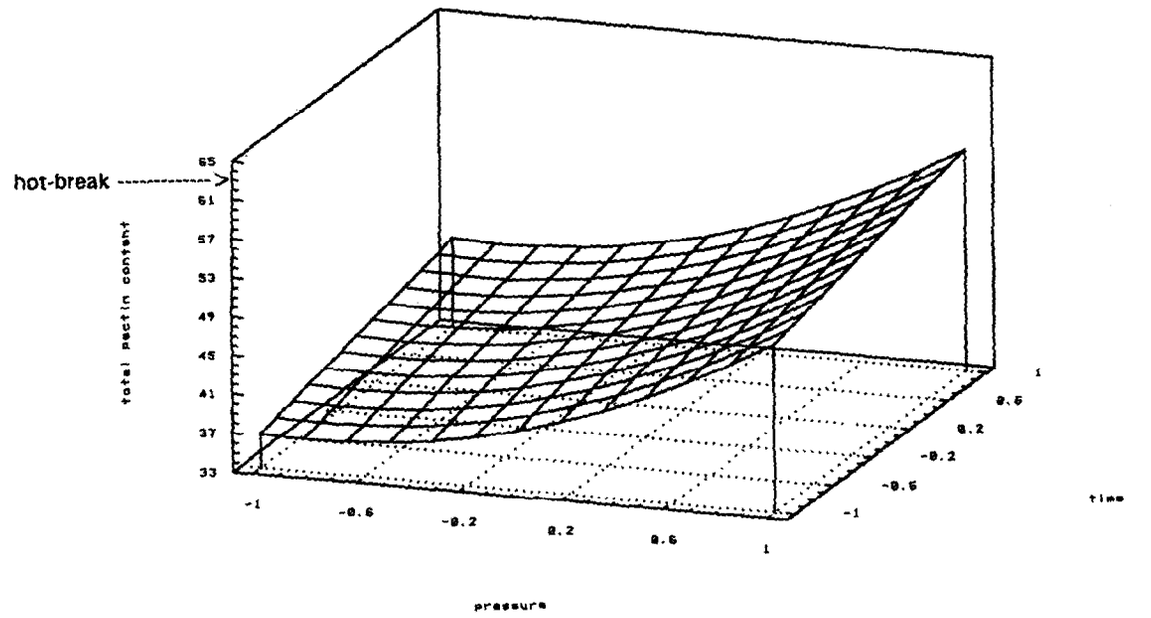

Fig. 1. Effect of ultra-high hydrostatic pressure (UHP) and time of treatments on the total pectin content of single-strength tomato juice and its comparison with conventional hot-break processing.

three fractions analytically determined, viz. pectic acids, pectates and protopectin.

In general, in UHP treatments the enzymes that survived the process reduced pectic acids contents and increased pectates and protopectin in comparison with the control.

The significant lack of fit observed in the case of pH (Table 2) revealed that this parameter was not an important factor for the variation of total pectin content under the experimental conditions used.

Total pectin content increased with increasing pressure and was not greatly affected by treatment time, even if maximum pectin content corresponded to the highest processing pressure and time. The same occurs with the conventional hot-break treatments for enzyme inactivation, which are normally carried out at decidedly higher (up to 110°C) temperatures than those theoretically required for pectolytic-enzyme inactivation. As shown in Fig. 1, UHPs inactivate enzymes to a smaller extent than does hot-break processing: the maximum value for total pectin content (61·7 mg per 100 g fresh weight), corresponding to a treatment at 900 MPa for 9 min, is inferior to the one obtained by the hot-break method (63·7 mg per 100 g fresh weight).

Viscosity

As already seen for pectic substances, viscosity also is strongly dependent on the pressure applied, but independent of treatment time.

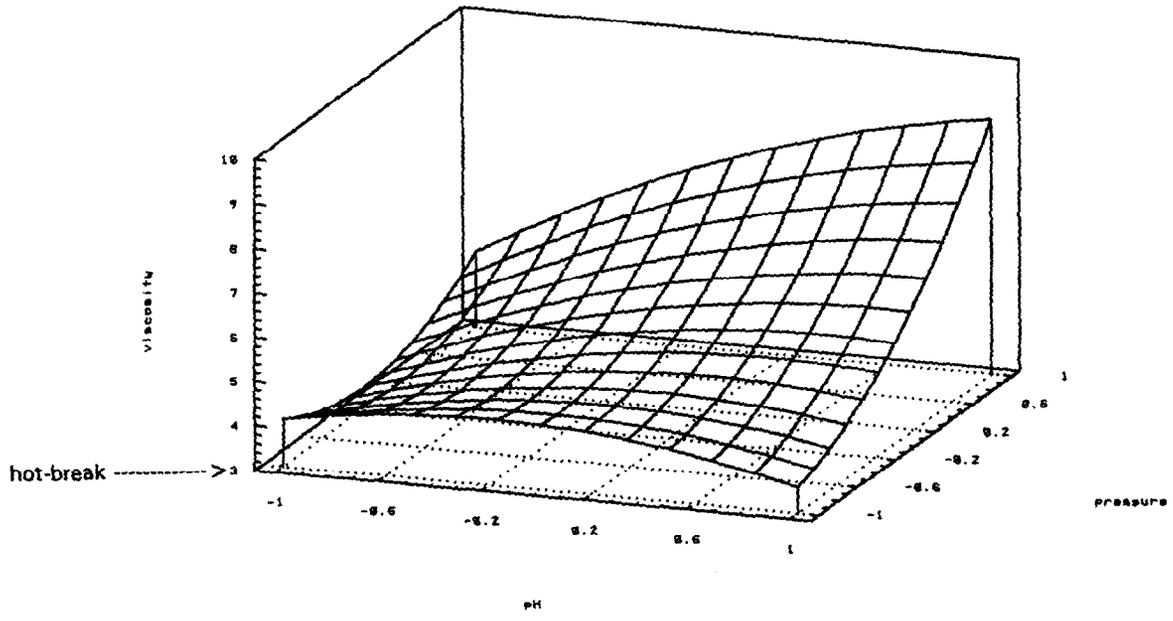

Fig. 2. Effect of UHP treatment and pH value on the viscosity of tomato juice and its comparison with conventional hot-break processing.

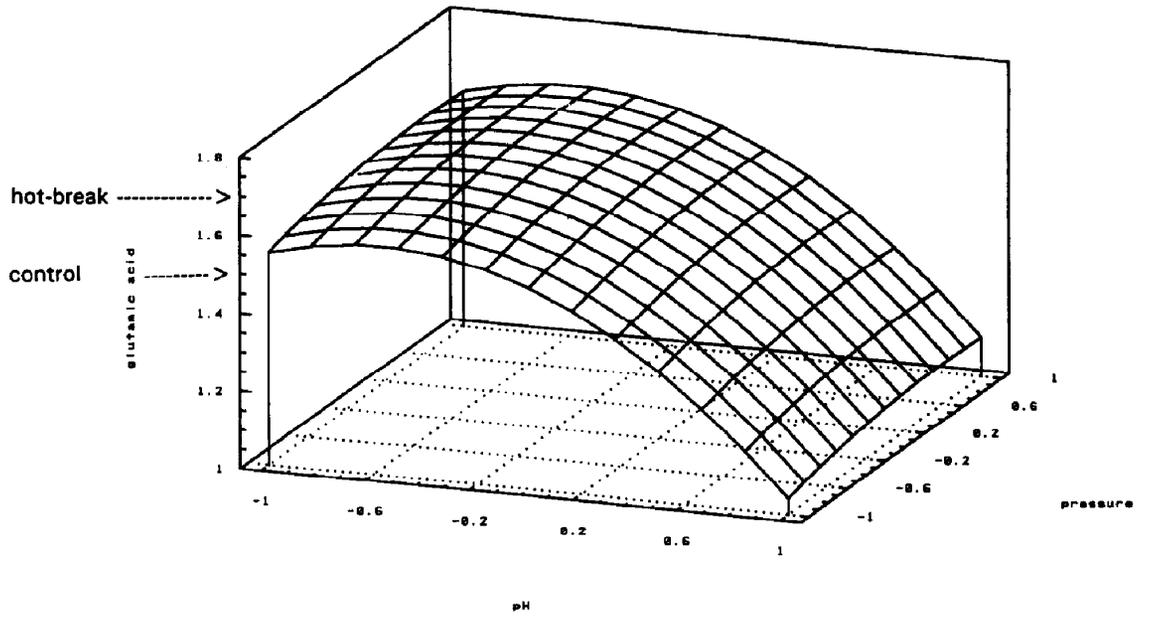

Fig. 3. Effect of UHP treatment and pH value on glutamic acid content of tomato juice and its comparison with conventional hot-break processing and with the control (untreated) sample.

High pressure effect on tomato juice, and more generally on vegetable juices, gives rise to a jelly-like translucent structure due to protein-tissue coagulation and compacting.

As Fig. 2 shows, viscosity increases with increasing pH value only for pressure above 700 MPa. This fact could lead to the consideration that, as reported by other authors (Kunugi *et al.*, 1982; Fukuda & Kunugi, 1985; Murao *et al.*, 1992), pH ranges exist where enzymes are either activated or enhanced in their activity, with changes in viscosity as a consequence. As confirmation of this assumption, the same was observed for pectic substances, even if with less statistical significance ($P = 0{\cdot}13$).

Free glutamic acid

Free glutamic acid comprises up to 49% of the total weight of amino acids in fresh tomato juice. Conventional concentration by heat gives rise to an approximate 10-fold increase in free amino acids as a result of protein denaturation and partial hydrolysis. In heat-treated single-strength tomato juice this increase is very small, albeit still significant.

As shown in Fig. 3, the above-mentioned effect was not observed with UHP treatments. The highest values for free glutamic acid (*c.* 1·6 g kg^{-1}) were obtained at pH <4·5, regardless of the pressure used. Free glutamic acid contents determined in the control and in the hot-

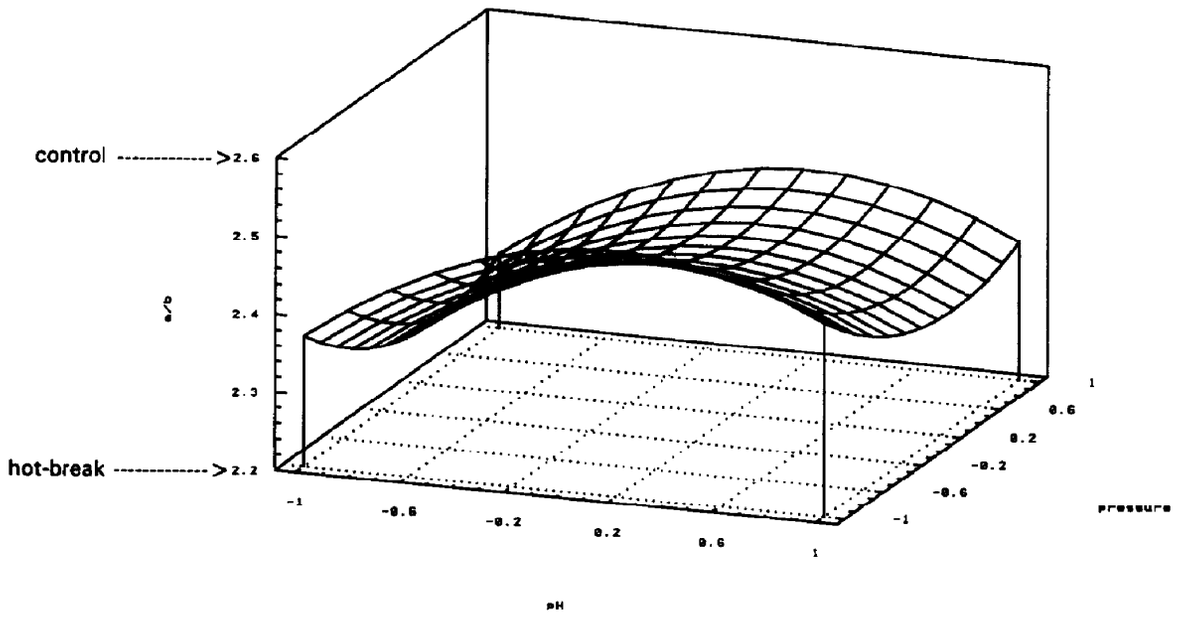

Fig. 4. Effect of UHP treatment and pH value on colorimetric a_L/b_L ratio of tomato juice and its comparison with conventional hot-break processing and with the control (untreated) sample.

Table 5. Identification of chromatographic peaks

Peak no.	Compound
1	Methanol
2	Ethanol
3	Ethyl acetate
4	Isopropylacetate
5	Pentanal
6	Hexanal
7	Column bleeding
8	*trans*-2-Hexenal
9	*cis*-3-Hexenol
10	Hexanol
11	3-Methylbutanenitrile
12	2-Heptenal
13	5-Hepten-2-one-6-methyl
14	2-Pentylfuran
7	Column bleeding
15	Limonene
16	2-Isobutylthiazole
17	*trans*-2-Octenal
7	Column bleeding

break juices were 1·48 and 1·70 g kg^{-1}, respectively. Free glutamic acid values for samples subjected to UHP and hot-break treatments were not found to be statistically different ($P \geq 0{\cdot}05$).

High free glutamic acid levels could have had negative effects during storage because of the high reactivity of this acid in a Maillard-type condensation reaction with sugars (Eichner & Ciner-Doruk 1981; Porretta, 1991).

Colour

As Fig. 4 shows, the partial increase in colour of tomato juice (expressed in terms of a_L/b_L ratio, as is now common in trade contracts and in technical papers) obtained by UHP treatments in comparison with conventional hot-break, can be attributed to compacting and homogenizing effects of the former as already ascertained for viscosity. The highest a_L/b_L ratios (2·52 and 2·50) were observed at pH 4·5, irrespective of the pressure applied. This effect should be regarded as exclusively physical in nature and not due to differences in lycopene content of the UHP and hot-break-treated samples, as shown by results of quantitative analysis.

Sensory analysis and volatile compounds

Samples treated at any value of input process variables (P, t, pH) proved to be absolutely inedible owing to a strong rancid taste, which made it impossible to perform sensory evaluations.To find out the reason of this irreversible change, volatile compounds were analysed immediately after UHP treatment. Figure 5 shows the comparison of two chromatograms for fresh untreated tomato juice and for the same juice after treatment at 500 MPa for 3 min, respectively. There exists a marked difference in the concentration of one of the most important components responsible for the typical fresh flavour of tomato, *n*-hexanal. All UHP-treated samples showed a remarkable increase in *n*-hexanal content, regardless of treatment time and pressure.

Table 5 reports the identification of chromatographic peaks. *n*-Hexanal values ranged from 0·3 (± 0·2 s.d.) mg kg^{-1} in untreated samples to 6·4 (± 0·7 s.d.) mg kg^{-1} in treated ones.

As appears from Fig. 5, other chromatographic differences (not quantified) were in *trans*-2-hexenal, which increased, and in hexanol, which decreased, in UHP-treated samples.

As already mentioned, the typical fresh flavour of tomato is due to *n*-hexanal, when present at concentra-

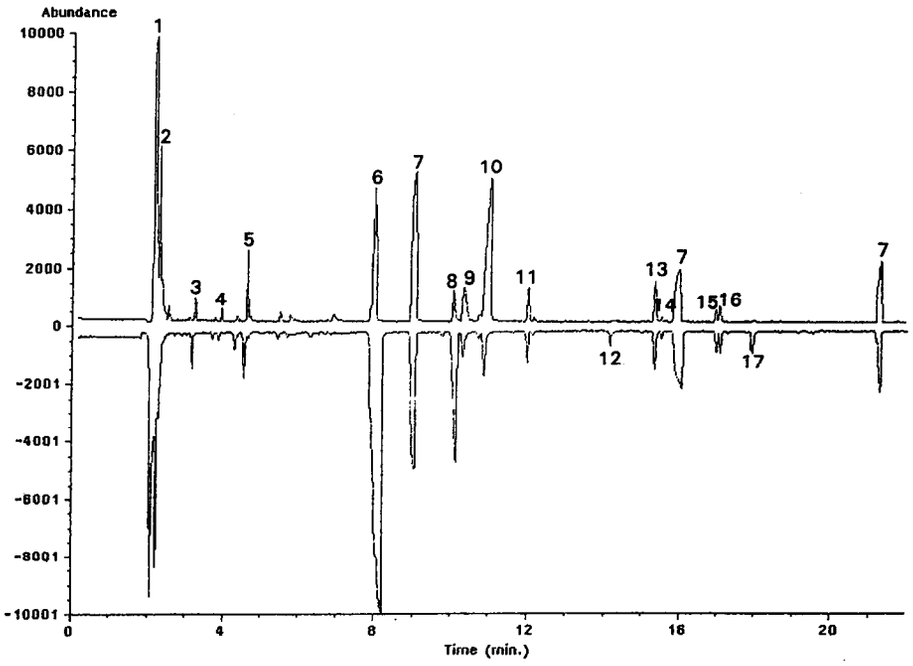

Fig. 5. Comparison of two chromatograms for fresh untreated single-strength tomato juice (top) and for the same juice after treatment at 500 MPa for 3 min (bottom)

tion lower than 1–1·2 mg kg^{-1}; higher concentrations impart a rancid flavour. This undesirable effect is mainly due to the oxidation of free fatty acids and in particular of linoleic (40%) and linolenic (26%). Lipid content of fresh ripe tomatoes with seeds removed has been shown to be about 1 g kg^{-1} (Wills *et al.*, 1984).

A similar effect occurs also in tomato concentrates obtained by reverse osmosis or in high-pressure-homogenized tomato products. Even in these cases, high pressure (about 10–20 MPa in the first case and about 30–50 MPa in the second) are the cause of free fatty acid oxidation.

The undesirable effects, both of reverse osmosis and of high-pressure homogenization, however, can be counteracted by mixing a high-pressure-treated product with a conventionally heat-treated one, in such a ratio as to obtain colour and consistency characteristics typical of mildy processed products. In the case of UHP-treated products, however, free fatty acid oxidation is so extensive as to make mixing ineffective.

CONCLUSIONS

UHP treatment of tomato juice under the experimental conditions used did not allow a quality improvement of the product. Equipment manufacturers are investing a lot of money in this technique and its potentialities, even if experimentation develops slowly because of the high costs of UHP pilot plants.

After a general evaluation of the applicability of UHP treatments to various food products, interest is now directed to its use in association with other mild technologies. In the particular case of tomato juice, membrane technology could be used for concentration and UHP technology for stabilization of products intended for reconstitution for final consumption (reconstitution of concentrates is not permitted in some countries, including Italy).

ACKNOWLEDGEMENT

This work was financially supported by National Research Council of Italy, Special Project RAISA, Sub-project 4, Paper No. 1434.

REFERENCES

Box, G. E. P. & Behnken, D. W. (1960). Some new three level designs for the study of quantitative models. *Technometrics*, **2**, 455–75.

Eichner, K. & Ciner-Doruk, M. (1981). Early indication of the Maillard reaction by analysis of reaction intermediates and volatile decomposition products. In *Progress in Food and Nutrition Science*, ed. C. Eriksson. Pergamon Press, Oxford, pp. 115–35.

Fukuda, M. & Kunugi, S. (1994). Pressure dependence of thermolysin catalysis. *Eur. Biochem.*, **142**, 565–70.

Gould, W. A. (1983). Composition of tomatoes. In *Tomato Production, Processing and Quality Evaluation*. AVI Publishing Co., Westport, CT, pp. 344–58.

Horie, Y., Kimura, K., Ida, M., Yoshida, Y. & Ohki, K. (1992). Identification and pressure-sterilization of a *Candida* strain resistant to high pressure. *Nippon Nogeikagaku Kaishi*, **66**(4), 713–18.

Kent, M. & Porretta, S. (1990). Standardisation of the measurement of tomato paste colour using a new colour reference tile. *Industria Conserve*, **63**, 9–16.

Kunugi, S., Fukuda, M. & Ise, N. (1982). Pressure dependence of trypsin-catalyzed hydrolyses of specific substrates. *Biochim. Biophys. Acta.*, **704**, 107–13.

McCalloch, R. J., Nielsen, B. W. & Beavens, E. A. (1950). Factors influencing the quality of tomato paste. Pectic changes during processing. *Food Technol.*, **5**, 691–3.

Murao, S., Nomura, Y., Yoshikawa, M., Shin, T., Oyama, H. & Arai, M. (1992). Enhancement of activities of cellulases under high hydrostatic pressure. *Biosci. Biotech. Biochem.*, **56**(8), 1366–7.

Official Italian Methods (1989). Ministero dell'Agricoltura e delle Foreste Metodi Ufficiali di analisi per le conserve vegetali — Parte Generale. Gazzetta Ufficiale della Repubblica Italiana, No. 168 del 20/07/'89 Roma. Istituto Poligrafico dello Stato, Rome.

Ogawa, H., Fukuhisa, K., Kubo, Y. & Fukumoto, H. (1990). Pressure inactivation of yeasts, molds, and pectinesterase in Satsuma mandarin juice: Effects of juice concentration, pH, and organic acids, and comparison with heat sanitation. *Agric. Biol. Chem.* (Tokyo), **54**(5), 1219–25.

Oxen, P. & Knorr, D. (1993). Baroprotective effects of high solute concentration against inactivation of *Rhodotorula rubra*. *Lebensm. Wiss. Technol.*, in press.

Porretta, S. & Leoni, C. (1990). Preparation of high-quality tomato products using enzyme inactivation by microwave heating. In *Engineering and Food*, Vol. II, ed. W.E.L. Spiess & H. Schubert. Elsevier Applied Science, London, pp. 251–8.

Porretta, S. (1991). Nonenzymatic browning of tomato products. *Food Chem.*, **40**, 323–35.

Porretta, S. (1992). *L'Analisi Sensoriale*. Tecniche Nuove, Milano.

Porretta, S., Carpi, G., Dall'Aglio, G. & Ghizzoni, C. (1992*a*). Use of ultrafiltration for preparing improved tomato pulp. *Int. J. Food Sci. Technol.*, **27**, 427–33.

Porretta, S., Sandei, L., Crucitti, P. M., Poli, G. M. & Attolini, M. G. (1992b). Comparison of the main analytical methods used in quality control of tomato paste. *Int.J.Food Sci. Technol.*, **27**, 145–52.

Smith, C. J. B. & Nortje, B. K. (1958). Observations on the consistency of tomato paste. *Food Technol.*, **12**, 356–8.

Watanabe, M., Makino, T., Kumeno, K. & Arai, S. (1991). High pressure sterilization of ice nucleation-active bacterial cells. *Agric. Biol. Chem.* (Tokyo), **55**(1), 291–2.

Wills, R. B. H., Lim, J. S. K. & Greenfield, H. (1984). Composition of tomato. *Food Technol. Aust.*, **36**, 78–80.