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Effect of Thermal Treatment on the Quality of Cloudy Apple Juice

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Apple juice from eight different varieties of apples was heated at high-temperature (60−90 °C) and short-time (20−100 s) (HTST) combinations. To determine the effect of heating conditions on enzymatic browning and cloud stability in apple juices, the activity of polyphenol oxidase and pectinesterase was analyzed and correlated with the thermal treatment conditions and the quality of the juice. Additional investigations included the measurement of pH value, soluble solid content, titratable acidity, color, and turbidity after 3 and 6 months. The results showed that HTST treatment at 80 °C already inactivated polyphenol oxidase, whereas pectinesterase activity was reduced to half and could even at 90 °C not be inactivated completely. In fact, highest residual pectinesterase activity was found at 60 °C. Heating at 70 °C caused stable pectinesterase activity and even a slight increase for 50 and 100 s heating times. Turbidity and lightness increased after HTST treatment. In particular, differences in cloud stability between the varieties were measured. HTST parameters did not correlate with the residual cloud stability after 6 months. The sensory evaluation revealed that only a few combinations were distinguishable. The best stability of cloud and color in relation to heat impact was achieved by HTST treatment between 70 °C/100 s and 80 °C/20 s.

KEYWORDS: Cloudy apple juice; pectinesterase; polyphenol oxidase; cloud stability; color difference

INTRODUCTION

Cloudy apple juice is a product of outstanding sensory and nutritional quality. Natural cloudy apple juice has been available on the market since the mid 1950s when flash pasteurization was first introduced (*1*) and has increasing market value due to sensory and nutritional qualities. Cloud and color stability is an essential quality criterion for natural cloudy apple juice, which is said to be strongly influenced by pectinesterase and polyphenol oxidase.

Optimized processing technology has not completely avoided cloud loss and enzymatic browning during production and storage of apple juice. In practice, a long delay occurs between pressing and thermal processing.

A big step toward solving problems with opalescense and color was steam heating (*2*) and high-temperature−short-time (HTST) treatment. From a practical point of view, it is generally accepted that the cloud loss problem can be easily overcome with an effective HTST stabilization treatment. It is believed that HTST is sufficient to inactivate pectinesterase (PE) and polyphenol oxidase (PPO). However, it appears to be mandatory to inactivate the PE and PPO from the initial phases of the product transformation because of the very fast enzymatic action soon after the mechanical breakage of the fruit during the juice extraction procedure (*3*).

Browning is caused by PPO activity, which catalyzes the oxidation of phenolic compounds in various fruits. The control of enzymatic browning has great importance just at the beginning of these processes. Enzymatic browning starts with the initial enzymatic oxidation of phenols to quinones by the enzyme in the presence of oxygen. Many procedures were proclaimed, including the use of ascorbic acid and nitrogen (*4*), blanching of pulp (*5*), and controlled pectolytic enzyme treatment (6). Another approach for the prevention of enzymatic browning of fruit juices has been the use of antibrowning agents (*7*), pH variation (*8*), and heat treatment (*9*).

It has been reported that the cloud destabilization process and the cloud loss are caused by pectinesterase (*3*). PE de-esterifies the methyl group of pectin (methyl ester of polygalacturonic acid) and converts it into low-methoxy pectin or pectic acid. PE is added during commercial apple juice processing to facilitate juice extraction and filtration and to produce clear apple juice (*10*).

There are many studies about PE, especially in citrus juices and fruits that suggest more types of PE of different heat stabilities (*11*−*14*). Although PE from apples was detected a

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long time ago by Mehlitz and Maas (*15*), only a relatively small amount of research has been done so far about apple pectinesterase. Castaldo et al. (*16*) demonstrated two forms of the enzyme that differed in both their charge and molecular weight. Denes et al. (*17*) identified only one type of PE with an optimum pH above 7.5 and a classical first-order denaturation process.

The model of the stability of cloudy apple juice is supposed to consist more or less on the same assumption: a solid phase (cloud) in a liquid phase (serum). The cloud particles are mainly proteins (positively charged), which are covered by pectin (negatively charged due to galacturonic acid) and therefore do not aggregate (*18*, *19*). Due to the fact that there is only very little pectin in the cloud (0.5%), other components with negative charge (i.e., phosphatid acids of cloud lipids) have to be taken into consideration for stabilizing the cloud in the serum. A hydrophilic surface seems to be most important to ensure cloud stability. Polysaccharides provide a highly hydrophilic surface to the hydrophobic core consisting of proteins and lipids. As a result, the aggregation of hydrophobic components is inhibited (*16*). Therefore, the importance of PE is to be questioned.

The objective of this study was to investigate the effect of HTST on enzymatic browning and cloud stability considering eight different varieties of apples of high importance in the market and in fruit juice production (Florina, Gala, Golden Delicious, Idared, Jonagold, Pilot, Pinova, and Topaz). Many factors influence cloud stability and color. Therefore, the impact of PE and PPO on changes in cloud stability and color was analyzed with regard to HTST conditions. Analysis included measurement of pH value, soluble solid content, titratable acidity, PE activity, PPO activity, and further measurement of color and turbidity after 6 months.

So far, much work has been done on citrus fruits. Only a few publications concentrate on PE or PPO and even fewer on apple, apple juice, and cloudy apple juice. On the basis of these research data, producers can get an idea of optimized conditions of production to ensure best quality and to optimize reduction of enzyme activity.

MATERIALS AND METHODS

Apples. Florina (Fl), Gala (Ga), Golden Delicious (GD), Idared (Id), Jonagold (Jg), Pilot (Pl), Pinova (Pn), and Topaz (To) apples were harvested from the experimental orchard of the Federal College and Institute for Viticulture and Pomology (Klosterneuburg, Austria) in 2004.

Apple Juice Production. About 300 L of juice was obtained from 650 kg of apples for each variety. After washing, milling (Raetz-Mill, Voran), pressing (belt press, Stoissier), addition of 150 mg/L ascorbic acid, and HTST treatment (60, 70, 80, and 90 °C for 20, 50, and 100 s) with a tubular heat exchanger (400 L/h, Fischer), the juice was cooled and immediately filled into prepasteurized bottles and then stored at 8 °C. The duration of heating was measured from the point when the juice reached the desired temperature until cooling. Ascorbic acid was used to reduce browning of the juice and to be in accordance with the standard conditions of industrial and commercial production. Citric acid was not added to preserve the apple's original profile.

pH Value. The pH value of apple juice was determined with a pH-meter (WTW) in combination with a pH-electrode SenTix 41-3 (WTW, Germany)

Total Soluble Solid. Brix value (°Brix) was measured by a hand refractometer (Atago) and corrected according to temperature and acidity.

Titratable Acidity. Titratable acidity was measured at the titration endpoint of pH 8.1 (0.1 N NaOH) and expressed as grams per liter of tartaric acid (factor = 0.75).

Color Measurement. L^*, a^*, and b^* values of the cloudy apple juice samples were measured with a Minolta Chroma Meter CR-200. The instrument was calibrated using the standard white reflector plate no. 11033069. The changes in color components ($L^*a^*b^*$) were analyzed statistically.

In addition, the difference in color (ΔE) was calculated (eq 1). The

$$\Delta E = \sqrt{(\Delta L^2 + \Delta a^2 + \Delta b^2)} \quad (1)$$

average, casual viewer can notice a difference between two colors only when they differ by >2−3.5 ΔE. A trained eye is capable of differentiating two colors that differ by <2 ΔE. The ability to distinguish two colors is also dependent on the color itself. The calculation of the difference in color between two stimuli is based on the Euclidean distance between the two points in the three-dimensional space.

PPO Activity. PPO activity was determined following the method developed by Zemel et al. (*21*) and Özoglu and Bayindirli (*7*). An aliquot of 2.5 mL of McIlvane buffer solution (pH 6.5) was mixed with 0.4 mL of deionized water in a magnetically stirred cuvette. Measurement was started and absorbance values were recorded at 420 nm (Photometer Cintra 10e, Software Spectral 1.7). After 30 s (constant baseline), 0.1 mL of apple juice and, after a further 30 2, 1 mL of catechol solution (0.2 M) were added. The enzyme activity was calculated on the basis of the slope of the linear portion of the curve plotted against time. One unit of enzyme activity per milliliter of juice was calculated using eq 2, where Abs(0) is the initial absorbance after

$$\text{PPO (units/mL)} = \frac{\text{Abs}(1) - \text{Abs}(0)}{t(1) - t(0)}/\text{mL of juice} \quad (2)$$

the addition of catechol and Abs(1) is the absorbance at the end of linearity.

Measurement of Turbidity. Measurement of turbidity (TE/F) was carried out with a Dr. Lange LTP5 laboratory turbidity photometer. Apple juice was diluted 1:100 and measured in a 5 cm cuvette with color compensation.

PE Activity. PE activity was determined by acid−base titration based on the method of Tajchakavit and Ramaswamy (*22*) and Riahi and Ramaswamy (*10*). The pH of titration was constantly maintained at 7.5 at 25 °C considering the results of Castaldo et al. (*16*), Denes et al. (*17*), and the 1989 more reproducible assay routine at 25 °C. One unit of PE activity is defined as 1 μmol of carboxyl group produced per minute. Exactly 15 mL of apple juice sample was made up to 50 mL with 1% pectin solution containing 0.1 M NaCl. The pectin solution (Pectin Classic AF 101, Herbstreith & Fox) was prepared previously and adjusted to pH 7.5 with 0.1 N NaOH. The consumption of NaOH during a 10 min reaction time was recorded. A blank sample containing pectin solution and water was prepared. Activity of PE was not influenced because the pH value was maintained at 7.5. The PME activity was expressed as microequivalents of ester hydrolyzed per milliliter of juice sample at pH 7.5 and 25 °C. The units per milliliter were multiplied by 1000 for easy interpretation and were calculated according to the following equation:

$$\text{PE (units/mL)} = \frac{(\text{mL of used NaOH})(\text{N of NaOH})(10^3)}{(\text{mL of juice})(\text{time in min})} \quad (3)$$

Sensory Evaluation. Triangle differences tests were performed with each variety and heating temperature to determine if panelists were able to detect differences between juices heated for 20 or 100 s. The sensory panel (aged 20−50 years) was experienced with apple juice. The tests were conducted with black-coated white wine glasses because there was an obvious color difference between the samples. At $p = 0.05$ 9 of 15 (10 of 18, 12 of 21, respectively) judgments were needed for a significant difference between two juices (*23*).

Statistical Analysis. Analyses of pH value, soluble solid content, titratable acidity, color, and turbidity were run twice. Analyses of PE activity and PPO activity were run in triplicate and averaged. Statistical

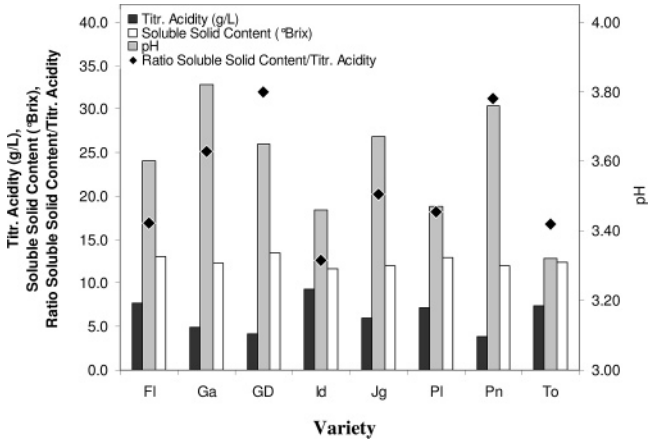

Figure 1. Spectrum of pH value, titrable acidity, and soluble solid content of eight varieties. Conditions of apple juice production: washing, milling, pressing, addition of 150 mg/L ascorbic acid, no citric acid.

analysis was carried out using SPSS 12.0 (Statistical Package for the Social Sciences). Significance of differences was defined at $p < 0.05$.

RESULTS AND DISCUSSION

Cloudy Apple Juice. During the production of the juices the pH value, titratable acidity, and soluble solid content were evaluated. **Figure 1** illustrates the resulting quality of the juices. Among the apple varieties the pH value differed from 3.32 (Topaz) to 3.82 (Gala), sugar from 11.70 °Brix (Idared) to 13.50 °Brix (Golden Delicious), and titratable acidity from 3.8 g/L (Pinova) to 7.7 g/L (Florina).

Due to the low variability of the juices' acidity (6.3 ± 1.9 g/L), pH (3.6 ± 0.2), and soluble solid content (12.5 ± 0.5 °Brix), the activity of PPO and PE, as described by Tajchakavit and Ramaswamy (*22*, 1997), was not influenced.

All juices represented directly the apple's characteristic because no citric acid was added. The ratio of soluble solid content (grams per 100 g; °Brix) compared to acid (grams per liter) should range from 10 to 16, respectively, depending on the consumer's preference (*24*). The sweetest juice (ratio of °Brix/titratable acidity) was Golden Delicious (32.0), followed by Pinova (31.2) and Gala (25.1). A desirable ratio was achieved by Idared (12.6), Topaz (16.7), and Florina (16.9) and more or less by Pilot (18.1).

Topaz and Idared are widely used for apple juice production, whereas Florina is a relatively new breeding. Pilot is seldom used as a dessert fruit because of its higher acid content. The well-balanced ratio of sugar and acid found in the juice produced from Pilot could have been caused by a longer storage period that may have led to a fast reduction of acid.

Color Measurement. Generally, during browning the L^* values decrease while the a^* and b^* values increase. Metaphorically speaking, the juice's color becomes darker with more red and yellow components. Although it was reported that the changes in browning are best and sufficiently represented by the a^* value (*27*), the L^* and b^* values were considered within the scope of this study as well.

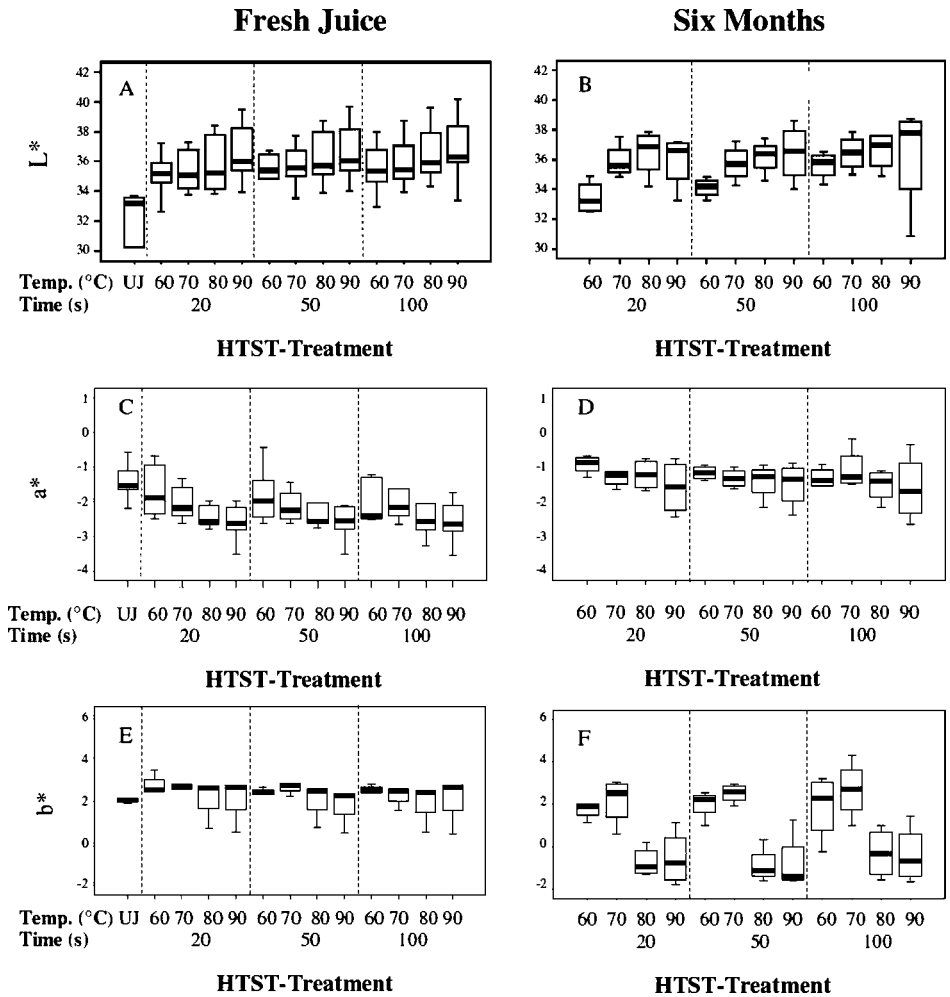

Figure 2. Changes in values for L^* (**A**, **B**), a^* (**C**, **D**), and b^* (**E**, **F**) during 6 months of storage (UJ, untreated juice). Black bars represent medians.

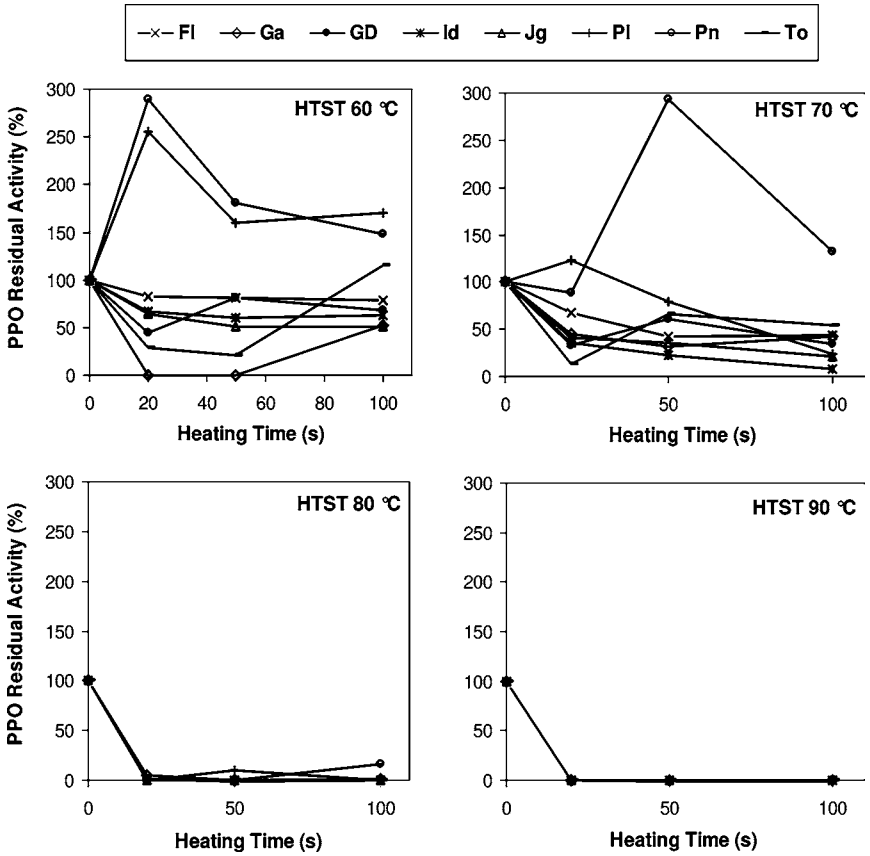

Figure 3. Changes in residual activity of PPO for each variety depending on HTST conditions.

The L^* values (**Figure 2A**) of all freshly produced juices of all varieties increased and the a^* values (**Figure 2C**) decreased slightly with higher HTST temperature but not necessarily with longer duration of the HTST treatment.

After 6 months of storage at 8 °C, the b^* values changed to a greater extent than the a^* and L^* values. Juices heated at 60 and 70 °C contained more yellow components (higher b^* value) than the juices heated at 80 and 90 °C after 6 months (**Figure 2F**). Also, the a^* values increased during the 6 months of storage (more red components), but they differed not as much as immediately after production. Juices heated at 80 and 90 °C were still slightly "greener" (lower a^* value) (**Figure 2D**). Regarding lightness, L^*, especially the samples treated for 20 and 50 s at 60 °C HTST became darker (lower L^* value) (**Figure 2B**).

Analysis of the ΔE values revealed that the color difference within the HTST-treated juices was not easily distinguishable after production ($\Delta E < 2$). The highest ΔE value was shown for HTST treatment at 60 versus 90 °C for 20 s ($\Delta E = 2.00$) and HTST treatment for 60 °C/20s versus 90 °C/100 s ($\Delta E = 2.18$). Untreated fresh juice showed a ΔE of at least 2.69 compared to the HTST juice.

After 6 months, the following samples showed higher deviation: 20 and 50 s/60 °C versus 100 s/90 °C ($\Delta E = 3.86$ and 4.33, respectively) and 50 s/60 °C versus 50 s/90 °C ($\Delta E = 3.68$). Comparing the color differences of samples after HTST treatment and 6 months of storage, only those treated for 20 s at 90 °C had a ΔE value >4 ($\Delta E = 4.18$). Values lower than 2.2 were found only for samples treated for 100 s at 70 °C ($\Delta E = 1.60$) and for 100 s at 60 °C ($\Delta E = 2.14$). All other samples resulted in ΔE values between 2.15 and 3.40. Tendentiously, the color changes of samples treated at 60 and 70 °C were lower than those treated at 80 and 90 °C.

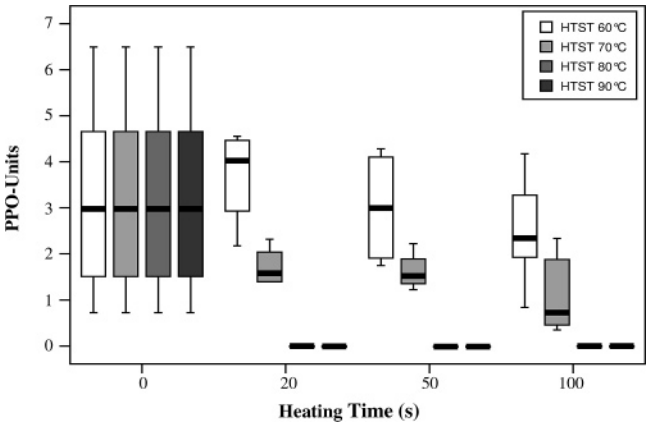

Figure 4. Statistical analysis of changes in PPO activity of all varieties depending on HTST conditions. Black bars represent medians.

Generally, juice heated at 80 and 90 °C was brighter (higher L^* values) and had more green components (lower a^* values) than juice heated at 60 and 70 °C. After 6 months, the higher heated samples showed more red and blue components. Juices of 60 °C HTST (20 and 50 s) became darker after 6 months. Especially, the L^* value showed a steady increase, which correlated to the heat impact during HTST. Determination of the L^* value seems to be particularly suitable for indicating the heat impact and correlates with TE/F as well. ΔE values are more capable as an indicator for color stability.

PPO Activity. Figure 3 shows the kinetic of the heat inactivation of the enzyme PPO, and **Figure 4** gives a overview of the statistical evaluation. The highest initial activity of PPO in the juice was found in Idared and Florina, which was ≈7 times higher compared to Topaz. HTST treatment at 60 and 70 °C resulted in an incomplete inactivation of PPO. In some juices,

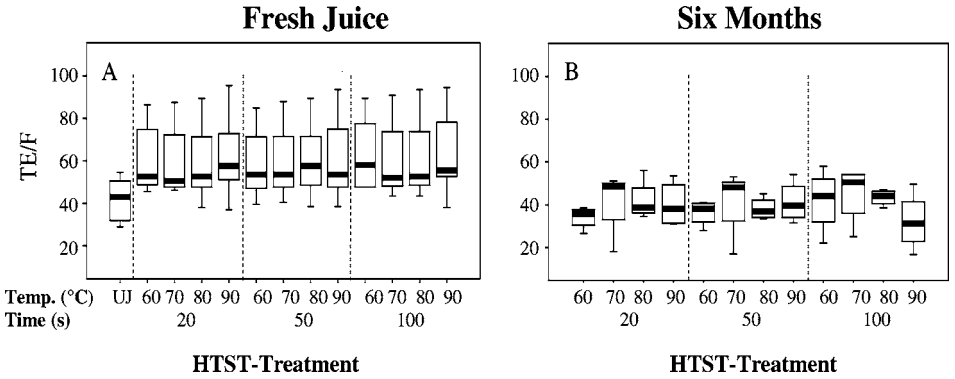

Figure 5. Statistical analysis of changes in cloud stability of all varieties during 6 months of storage (dilution of samples: 1:100).

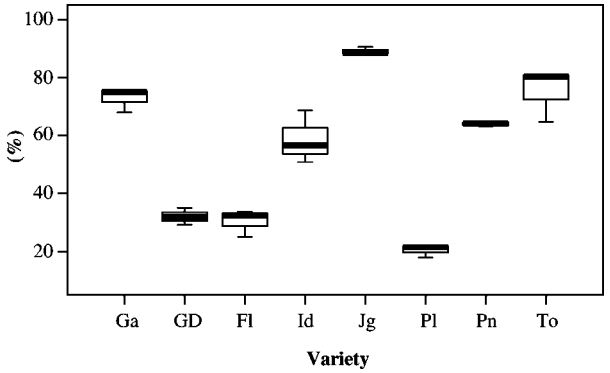

Figure 6. Remaining cloud of all varieties (all HTST combinations) after 6 months of storage (percent).

activity of PPO increased, for example, HTST at 60 °C, Pilot, Pinova, and Topaz juices heated for 100 s; HTST at 70 °C, Pilot juice heated for 20 s and Pinova juice heated for 50 or 100 s. The HTST treatment at 80 and 90 °C successfully inhibited PPO activity.

Compared to the approach of PPO inactivation by low pH treatment (20 min at pH 2 to denature PPO completely) by Zemel et al. (*21*) or by antibrowning agents (ascorbic acid, isoascorbic acid, benzoic acid, cinnamic acid, sorbic acid, β-cyclodextrin, and cysteine; 0.3, 1, and 1.8 mM) by Özoglu and Bayindirli (*7*), the HTST treatment at 80 and 90 °C (complete inactivation of PPO) had an apparently greater effect.

The statistical analysis proved that there was a significant reduction of enzyme activity already at 70 °C after 20, 50, and 100 s of heating time, but no difference was observed between the duration of heating (**Figure 4**). At 60 °C there was no significant decrease of PPO activity. Between 80 and 90 °C HTST temperature there was no difference and always zero activity. The juice with 70 °C HTST treatment showed a significant decrease of enzyme activity compared to the juice treated at 60 °C.

HTST heating for 20 s at 70 °C decreased PPO activity. Above 80 °C enzyme activity is reduced completely. *D* and *Z* values (thermal resistance parameters) could not be calculated because the number of samples and points of measurements were too low. Nevertheless, it was quite evident that longer heating time did not reduce enzyme activity as efficiently as higher HTST temperature (80 and 90 °C).

Measurement of Turbidity. The turbidity increased as soon as the juice was thermally processed (**Figure 5A**). It was shown that the raw juice had less turbidity than all other HTST juices where no significant difference could be found. After 6 months of cold storage, cloud decreased from about 60 TE/F to approximately 40 TE/F (**Figure 5B**). This difference was obvious but not significant. HTST treatment at 70 °C resulted in slightly higher residual values. There was no correlation between cloud stability after 6 months and the parameters during HTST treatment.

Highest cloud stability was apparently achieved by HTST treatment of 70 °C for 100 s. Worst cloud stability was shown for the juices treated at 60 °C for 20 and 50 s and at 90 °C for 100 s. Zimmer et al. (*26*) pointed out that cloud stability of >50% is generally accepted as good. After 6 months, cloud stability of Jonagold, Topaz, and Gala juices was >70% and ≈50% in Idared and Pinova juices, respectively (**Figure 6**).

Table 1. Overview of All Initial and Residual PE Activities Depending on HTST Temperature and Heating Time

	PE units (untreated juice)	HTST temp (°C)	PE residual activity (%) at heating time of 20 s	50 s	100 s
Fl	0.071 ± 0.001	60	81.2	98.3	80.5
		70	50.6	54.9	62.2
		80	30.8	34.2	36.3
		90	52.7	46.8	46.3
Ga	0.042 ± 0.003	60	NA[a]	NA	87.3
		70	94.3	70.3	92.7
		80	66.5	60.4	81.1
		90	50.5	67.0	63.7
GD	0.050 ± 0.000	60	86.1	92.0	90.7
		70	71.4	91.8	99.0
		80	70.4	54.9	40.8
		90	34.0	45.3	41.0
Id	0.490 ± 0.001	60	80.4	70.3	55.2
		70	67.8	57.8	69.2
		80	46.2	53.2	51.7
		90	48.3	30.5	36.3
Jg	0.440 ± 0.002	60	96.3	113.5	96.1
		70	89.3	95.9	113.5
		80	79.0	105.3	123.1
		90	73.5	67.6	81.3
Pl	0.018 ± 0.000	60	333.3	100.0	105.6
		70	238.9	94.4	155.6
		80	155.6	66.7	44.4
		90	61.1	77.8	61.1
Pn	0.033 ± 20.00	60	87.9	124.2	54.5
		70	54.5	142.4	60.6
		80	30.3	81.8	48.5
		90	48.5	81.8	36.4
To	0.032 ± 0.002	60	84.4	81.3	59.4
		70	64.1	40.6	34.4
		80	40.6	34.4	37.5
		90	43.8	28.1	34.4

[a] Not analyzed.

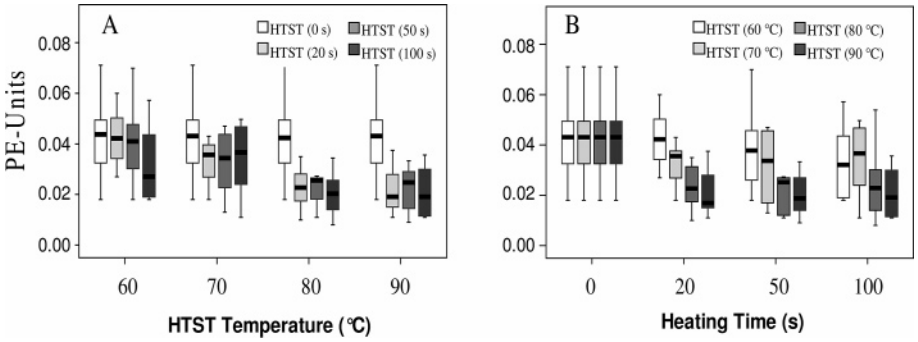

Figure 7. Statistical analysis of changes in PE activity of all varieties depending on temperature (**A**) and heating time (**B**) during HTST treatment. Black bars represent medians.

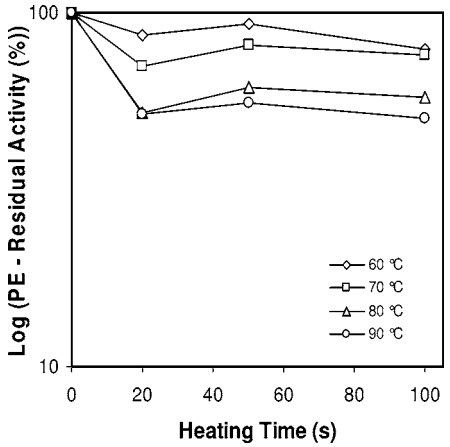

Figure 8. Isotherm inactivation curves of PE.

PE Activity. Table 1 gives detailed information on the initial and residual PE activities depending on HTST temperature and heating time. Maximum activity of untreated juice was obtained by Idared (0.490 PE unit). The initial residual PE activity of Pilot was lower than those of the other varieties, but the residual PE activity of Pilot was much higher than the average of all other juices.

Intermediate HTST conditions tended to result in a constant or slightly increasing PE activity for some varieties. The statistical analysis of PE activity proved that the heat impact at 60 °C was not sufficient to cause any decline (except a slight decrease at 100 s). At 70 °C and 50 or 100 s, PE activity was stable or increased slightly. A decrease of PE activity was observed at 80 and 90 °C at all HTST periods (**Figure 7A**), which is consistent with Castaldo et al. (*16*). At 70 °C PE activity was tendentiously higher for 50 and 100 s than for 20 s (**Figure 7B**)

On the basis of the available data it was not possible to calculate *D* and *Z* values accurately. The denaturation process was not a first-order one as Denes et al. (*17*) reports on studies with purified PE. Due to the fact that there are at least two isoenzymes of PE in oranges, Chen et al. (*13*) proposed a first-order kinetic model of a two-component system. Collet et al. (*25*) used a three-parameter−multicomponent−first-order model to fit the nonlinear regression, which also would be consistent with **Figure 8**.

Denes et al. (*17*) demonstrated that PE of apples, as in other fruits, is ionically bound to the cell walls and therefore would require a high ionic strength buffer for extraction of PE from these cell walls. Besides the effect of different types of heat-resistant isoenzymes, the increase in PE activity especially at intermediate HTST conditions (70 °C, 50−100 s) could therefore be the result of two counteractive reactions as well: extraction of PE from cell walls and delivery into the serum versus inactivation of free PE with increasing temperature. These observations indicate that there was no impact at 60 °C, a counteracting reaction at 70 °C (50 and 100 s) that led to increased soluble PE activity and a decrease at 80 and 90 °C. In this context Anthon and Barrett (*28*) put forward a proposal that heating of PE to >45 °C causes an irreversible change in the structure of the enzyme and therefore activates PE. This should also be considered to influence the extent of PE activity after HTST treatment. Especially the high activity in the Pilot juice could be caused by this activation, although all varieties

Table 2. Triangle Taste Test of Cloudy Apple Juice (HTST 20 s versus 100 s)

		HTST 20 s vs 100 s		
		responses		
	HTST temp (°C)	correct	total	signif diff ($p < 0.05$)
Fl	60	7	18	no
	70	7	18	no
	80	4	18	no
	90	6	18	no
Ga	60	NA[a]	NA	no
	70	6	18	no
	80	7	18	no
	90	5	18	no
GD	60	11	18	yes
	70	8	18	no
	80	11	18	yes
	90	9	18	no
Id	60	4	15	no
	70	4	15	no
	80	10	15	yes
	90	3	15	no
Jg	60	12	21	yes
	70	11	21	no
	80	13	21	yes
	90	10	21	no
Pl	60	9	15	yes
	70	11	15	yes
	80	7	15	no
	90	9	15	yes
Pn	60	5	15	no
	70	4	15	no
	80	7	15	no
	90	3	15	no
To	60	9	15	yes
	70	8	15	no
	80	7	15	no
	90	4	15	no

[a] Not analyzed.

were heated at the same levels and should therefore show similar changes in activity.

When the statistical evaluation is taken into consideration, PE activity was significantly reduced at 80 and 90 °C. HTST conditions of 70 °C and 20 s or 60 °C and 100 s tended to result in a decrease of PE activity. Cloud stability increased in particular at 70 °C with higher activity of PE. These observations indicate that there might be no significant correlation between cloud stability and activity of PE in HTST-treated cloudy apple juice as there are also other components responsible for cloud stability (phosphatid acids of cloud lipids) (*20*).

Sensory Evaluation. Table 2 summarizes the results of the triangle test conducted. For Florina, Gala, and Pinova juices, there was no significant difference ($p < 0.05$) between HTST treatment for 20 or 100 s. Differences were found for the varieties Golden Delicious, Idared, and Jonagold treated at 80 °C and for the varieties Golden Delicious, Pilot, and Jonagold treated at 60 °C. At 70 and 90 °C, only for Pilot was a significant difference observed.

The results showed that the duration of HTST treatment did not lead to great sensory differences. Most of the significant differences were found at relatively low-temperature treatment (60 °C) and at 80 °C (accompanied with changes in activity of PE/PPO and color). An important result was that all of the panelists that correctly identified the odd sample noted that the apple juice heated for 20 s was more similar to the untreated fresh juice than the apple juice heated for 100 s.

In conclusion, titratable acidity, sugar content, and pH value had no significant impact on color and cloud stability. The apple varieties showed different properties. Nevertheless, it was not possible to make a distinction in terms of PE and PPO activity, although Jonagold, Topaz, and Gala showed highest cloud stability.

Unpurified PE activity is not comparable to prior results with purified PE. The impact of HTST treatment did not seem to follow a classic kinetics due to different circumstances in the natural system "cloudy apple juice" and/or the presence of more types of PE. PPO showed inactivation kinetics that was easily comprehensible. On the other hand, PE activity was supposed not to effect cloud loss in a significant way. Furthermore, cloud was more stable at higher PE activity.

When all results are taken into account, a HTST treatment between 70 °C/100 s and 80 °C/20 s seems to ensure the best stability of cloud and color in relation to heat impact.

More studies need to be carried out to define the best conditions of HTST treatment to maximize product quality and minimize cloud loss and enzymatic browning. Our own investigations, which have not been published so far, reveal a strong relationship between color and expected product quality. Due to the objective of the presented study, a smaller range of optimized HTST temperatures with following pasteurization should create more detailed sensory differences between the combinations and thus give an idea of the importance of various HTST treatment and pasteurization conditions. On the other hand, a broader range of temperature in shorter time periods would be needed to calculate more kinetic parameters.

ABBREVIATIONS USED

Fl, Florina; Ga, Gala; GD, Golden Delicious; HTST, high-temperature−short-time; Id, Idared; Jg, Jonagold; PE, pectinesterase; Pl, Pilot; Pn, Pinova; PPO, polyphenol oxidase; To, Topaz; UJ, untreated juice.

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