Effects of Process and Storage Temperature on Browning Index, Furosine, and HMF of Aseptic Cold Break Tomato Paste during Storage Time

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Abstract: Effects of process and storage temperature on concentration of Maillard compounds in aseptic cold break tomato paste were evaluated during 12 months of storage time. Cold break tomato paste was processed at 104 °C or 112 °C, aseptically filled into 5 liter bags, and stored at 21 °C and 33 °C. Level of Browning Index, Furosine, and HMF was monitored in the aseptic cold break tomato paste during the storage time. Browning Index, Furosine, and HMF significantly increased in the aseptic cold break tomato paste during storage at 33 °C, and there was linear correlation between Browning Index and Furosine (or HMF). At the lower storage temperature of 21 °C, no significant increases were observed. Effects of the process temperature on Browning Index, Furosine, and HMF of aseptic cold break tomato paste were less significant than the storage temperature.

Key words: Aseptic cold break tomato paste, process temperature, storage temperature, Browning Index, Furosine, HMF.

1. Introduction

In the US, 75% of tomatoes are consumed in a processed form and California produces most of the processing tomatoes [1]. Processing tomatoes are often manufactured into tomato paste, packed in bulk containers, and stored for use for up to 18 months [1]. Thermal treatment of tomato products as well as storage conditions and transportation can affect the chemical composition and final quality of the product [2]. Undesirable changes occur in the color and flavor of tomato products upon processing, concentration, and storage [3]. Processing and storage of commercial tomato-based products may result in quality degradation, including loss of color and nutrients [4]. Significant oxidative damage has been observed in tomato products during their commercial shelf life [5]. Thermal browning reactions during heat processing and storage lead to changes in the color and flavor of foods [6]. Many food products are darkening during thermal processing and storage, and this is due to non-enzymatic reactions including the Maillard reaction [7]. Maillard browning significantly affects visual quality and consumer acceptance of processed foods [8].

Degree of browning reactions can be measured by the Browning Index at 420 nm and are often used to assess the extent of the Maillard reaction [8]. Formation of Furosine, €-N-(2-furoylmethyl-L-lysine), and HMF, 5-hydroxymethyl-2-furfural, have been reported in heat processed tomato products [9, 10]. Furosine and HMF are intermediate compounds of the Maillard reaction, and they have been used to evaluate non-enzymatic browning of tomato products [11-14].

It is important to understand effects of process and storage temperature on the Maillard browning compounds in aseptic tomato paste. This helps us to identify key control factors for maintenance of the quality of aseptic tomato paste. Commercial aseptic systems require significant volumes of tomato paste to evaluate different process temperatures, therefore a pilot scale aseptic system was used. A pilot scale aseptic system was not available in California where

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tomato paste was produced. Therefore, a frozen tomato paste was manufactured in California, transported to New Jersey, and aseptically packed at a pilot plant.

In this study, two different aseptic processing temperatures (112 °C and 104 °C) were selected. Control temperature, 112 °C, was within normal aseptic processing temperature of the pilot plant’s aseptic system. Test temperature, 104 °C, was selected from lower processing temperatures than the Control. There was a limitation in lowering aseptic processing temperature due to the minimum lethality requirement of aseptic processing. For evaluation of storage temperature, 21 °C and 33 °C were selected to represent indoor storage and outdoor storage temperatures. Level of Browning Index, Furosine, and HMF was monitored in aseptic cold break tomato pastes for quality evaluation during 12 months of storage time.

2. Materials and Methods

2.1 Manufacturing of Aseptic Tomato Paste

Frozen cold break tomato paste (total solids 42%) was manufactured at Campbell’s tomato plant in California. Drums of the frozen tomato paste from the same lot were transported to a pilot plant in Camden, New Jersey. The frozen cold break tomato paste was standardized to 20% total solids to help pumping and blending of an aseptic processing system at the pilot plant. The standardized cold break tomato paste was sterilized using a tube-in-tube sterilizer (Tetra Spiraflo, Tetra Pak Inc., Denton, TX), then filled into 5 liter aseptic pouches using a Rapak Aseptic Filler (Rapak, Romeoville, IL). The entire process diagram and aseptic processing conditions are shown in Fig. 1 and Table 1.

2.2 Microbiological Examination of Aseptic Tomato Paste

Eleven grams of tomato paste per each sample was aseptically transferred into a sterile stomacher bag (Fisher Scientific, Pittsburgh, PA). A 99 mL of sterile Butterfields Phosphate buffer (Fisher Scientific, Pittsburgh, PA) was added for a dilution. The diluted tomato paste sample was homogenized using a stomacher (Smasher, bioMerieux, France). A 0.1 to 0.5 mL of the diluted and homogenized tomato paste was transferred into duplicate sterile Petridish plates (Falcon, Corning Inc., Corning, NY). A 15-20 mL of sterile and tempered Tomato Juice Agar Special agar (TJAS, BD Diagnostic Systems, Sparks, MD) was poured into each of the inoculated Petri dish plates. The TJAS plates were gently swirled and solidified. Solidified TJAS plates were inverted and aerobically incubated at 32 ± 2 °C for up to 5 days. After the incubation, the colonies were counted on each plate. For a smear test, a thin layer of tomato paste sample was applied with a flame sterilized loop to a glass microscopic slide. After drying and quickly passing through an open flame three times, the slide was cooled before staining. The slide was stained with crystal violet stain (BD Diagnostic Systems, Sparks, MD) for 60 seconds and examined under a microscope.

2.3 Storage of Aseptic Tomato Paste and Preparation of Analysis Samples

Five liter aseptic pouches (Control and Test tomato paste at 20% total solids) were stored at 21 °C and 33 °C for 12 months. One aseptic pouch was collected per each Control and Test after each storage time (0, 3, 4, 6, 7, 9, 12 months at 21 °C and 2, 3, 4, 5, 6, 7, 9, 12 months at 33 °C). All tomato paste samples were diluted to 5 °Brix with distilled water for analysis of Browning Index, Furosine, and HMF. Duplicate analysis was conducted with each sample.

2.4 Browning Index Measurement

The diluted tomato paste (5 °Brix) was centrifuged at 12,800 × g for 10 min in an Eppendorf Centrifuge 5412 (Eppendorf North America, Hauppauge, NY) to remove pulp and particles. Supernatant was collected
and clarified using a 0.45 μm syringe filter (Fisher Scientific, Pittsburgh, PA). Browning index of the clarified tomato serum was measured at 420 nm using Beckman DU530 UV/VIS spectrometer (Beckman Coulter, Inc., Indianapolis, IN) at room temperature [15].

2.5 Furosine Analysis

In general, the diluted tomato paste sample (5 °Brix) was hydrolyzed with strong acid and heat, extracted and stabilized in buffer, and then neutralized and analyzed with Gradient HPLC with UV detection at 280 nm [16-19] (see details below).

An Agilent 1100 Series HPLC (Agilent Technologies, Santa Clara, CA) equipped with auto-sampler, binary pump, column compartment, in-line degasser, multi-wavelength UV detector and data acquisition system, Chemstation, was used. The analytical column used was a Polaris C18-A, 4.6 × 250 mm, 5 μm and the guard column was Eclipse

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### Table 1  Pilot plant’s aseptic processing condition.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow Rate (liter per min)</td>
<td>18.5</td>
<td>18.5</td>
</tr>
<tr>
<td>Hold tube length (m)</td>
<td>15.24</td>
<td>15.24</td>
</tr>
<tr>
<td>Hold tube outside diameter (Inside Diameter)</td>
<td>3.81 cm (3.48 cm)</td>
<td>3.81 cm (3.48 cm)</td>
</tr>
<tr>
<td>Feed temperature</td>
<td>72 °C</td>
<td>71 °C</td>
</tr>
<tr>
<td>Product outlet temperature after hold tube</td>
<td>112 °C</td>
<td>104 °C</td>
</tr>
<tr>
<td>Product outlet temperature after cooler</td>
<td>24 °C</td>
<td>23 °C</td>
</tr>
</tbody>
</table>

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**Fig. 1** Pilot plant aseptic processing system.
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XDB-C18, 4.6 × 12.5 mm, 5 µm (Agilent Technologies, Santa Clara, CA). HPLC grade water and organic solvents were purchased from Fisher Scientific (Pittsburgh, PA). All standard solutions and extracted samples were filtered through 0.45 µm Nylon syringe filter or vacuum filtered using 0.45 µm EMD Millipore Nylon hydrophilic membrane filters (Fisher Scientific, Pittsburgh, PA). Furosine standard ([ε-N-(2-furoylmethyl-L-lysine) HCl salt] was purchased from PolyPeptide Group (Strasbourg, France). Concentrated Hydrochloric Acid (12 N), Potassium Phosphate Dibasic, Anhydrous (K₂HPO₄, ACS Grade), Sodium Azide (99%, extra pure), Sodium Hydroxide Solution (2.5 N), and Sodium hydroxide pellets were purchased from Fisher Scientific (Pittsburgh, PA). One to six grams of diluted tomato paste sample (5 °Brix) was mixed with 6 N HCl, then nitrogen gas was flushed before it was placed in an oven at 110 °C for 23 hours. Sodium hydroxide, 10 M, was added to neutralize the extract to pH 6.8. The extract was diluted with potassium phosphate buffer dibasic, 0.05 M. The diluted extract of 2 mL was filtered into HPLC vials through 13 mm, 0.45 µm nylon syringe filter. Mobile phases were potassium phosphate buffer dibasic, 0.05 M, pH 2.9, and 80% acetonitrile. HPLC injection volume was 20 µL and flow rate was 0.3 mL/min. Detector was set at 280 nm. The mobile phase was 2% acetonitrile and 0.2% acetic acid in HPLC water. The HPLC column temperature was 40 °C and run time was 25 min. The peaks and areas were calculated with Chemstation. Duplicate was analyzed for each sample.

2.6 HMF Analysis

Insoluble solids of the diluted tomato paste (5 °Brix) were removed by centrifugation, the supernatant was filtered, and HMF was separated and quantitated by HPLC using reverse phase chromatography on a C18 column [6, 10-12, 20]. The same Agilent 1100 Series HPLC system with a C18 analytical and a guard columns (Agilent Technologies, Santa Clara, CA) was used for HMF analysis. Standard solutions were prepared by dissolving HMF standard (5-Hydroxymethyl furfural, Sigma-Aldrich, St. Louis, MO) in HPLC water. Five grams of diluted tomato paste (5 °Brix) was mixed with 10 mL of HPLC water and refrigerated for 8 hours. Sample was centrifuged for 5 minutes at 2,500 × g using IEC Explosion Proof Centrifuge (Thermo IEC, Needham Heights, MA). The supernatant of 2 mL was collected and filtered through 0.45 µm, 25 mm syringe filter before injection. HPLC injection volume was 20 µL and the flow rate was 1.0 mL/min. Detector was set at 280 nm. The mobile phase was 2% acetonitrile and 0.2% acetic acid in HPLC water. The HPLC column temperature was 40 °C and run time was 25 min. The peaks and areas were calculated with Chemstation. Duplicate was analyzed for each sample.

2.7 Statistical Analysis

An analysis of the variance (ANOVA) was performed to evaluate the effects of processing and storage temperature on Browning Index, Furosine, and HMF of aseptic cold break tomato paste (Minitab v. 17, 2015, Minitab Inc., State College, PA). The level of significance was set at \( p < 0.05 \). Data are the means of duplicate analysis determination and all data were reported as means ± SD.

3. Results and Discussion

3.1 Microbiological Examination of Aseptic Tomato Paste

No aerobic recovery of microorganisms was observed in all tomato pastes (Table 2), therefore the aseptic processing was successfully done at 112 °C (Control) and 104 °C (Test).

3.2 Browning Index, Furosine, and HMF during Storage

Browning Index significantly increased in the Control and Test when stored at 33 °C for 12 months of storage time (Fig. 2). Furosine and HMF also increased significantly during storage at 33 °C (Figs. 3 and 4). However, at the lower storage temperature, 21 °C,
Table 2  Microbiological examination results of aseptic cold break tomato paste.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4.13</td>
<td>4.12</td>
</tr>
<tr>
<td>Product smear</td>
<td>No microorganisms</td>
<td>No microorganisms</td>
</tr>
<tr>
<td>TJAS @ 32 ± 2 °C</td>
<td>No growth</td>
<td>No growth</td>
</tr>
</tbody>
</table>

Fig. 2  Browning Index of aseptic cold break tomato paste stored at 21 °C and 33 °C. Control was processed at 112 °C and Test was processed at 104 °C. All tomato paste samples were diluted to 5 °Brix before analysis.

Fig. 3  Furosine content (µg/g) of aseptic cold break tomato paste stored at 21 °C and 33 °C. Control was processed at 112 °C and Test was processed at 104 °C. All tomato paste samples were diluted to 5 °Brix before analysis.
there was no significant change in Browning Index, Furosine, or HMF (Figs. 2-4). Researchers reported that high storage temperature caused significant increase of nonenzymatic browning compounds in tomato products [13, 21, 22]. It was observed that tomato paste is very sensitive to heat damage with high reaction rates due to the high concentration of the various reaction substrates [13].

Furosine, which is produced by acid hydrolysis of the Amadori compounds, has been previously identified as a heat damage index for tomato products [9, 10]. Furosine concentration is correlated with the intensity of heat treatments during processing and with storage conditions [13]. HMF is an intermediate compound of the Maillard reaction between sugar and aminoacids [11] and it is normally used to evaluate heat damage in food products [10]. In this study, Furosine and HMF showed a linear increase when the aseptic tomato paste was stored at 33 °C (Figs. 3 and 4).

![Graph showing HMF content (µg/g) of aseptic cold break tomato paste stored at 21 °C and 33 °C. Control was processed at 112 °C and Test was processed at 104 °C. All tomato paste samples were diluted to 5 °Brix before analysis.](image1)

**Fig. 4** HMF content (µg/g) of aseptic cold break tomato paste stored at 21 °C and 33 °C. Control was processed at 112 °C and Test was processed at 104 °C. All tomato paste samples were diluted to 5 °Brix before analysis.

![Graph showing correlation between Browning Index and Furosine (µg/g) or HMF (µg/g) of Control during storage at 33 °C. All tomato paste samples were diluted to 5 °Brix before analysis.](image2)

**Fig. 5** Correlation between Browning Index and Furosine (µg/g) or HMF (µg/g) of Control during storage at 33 °C. All tomato paste samples were diluted to 5 °Brix before analysis.
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Fig. 6  Correlation between Browning Index and Furosine (µg/g) or HMF (µg/g) of Test during storage at 33 ºC. All tomato paste samples were diluted to 5 °Brix before analysis.

Browning Index showed a good correlation with Furosine and HMF in Control and Test during storage at 33 ºC (Figs. 5 and 6). Linear correlation between Browning Index and HMF has also been reported previously in cold break tomato paste [23]. Browning Index can be easily measured and it had a linear correlation with the concentration of Furosine and HMF. Therefore, Browning Index might be used as a heat damage index to monitor quality of aseptic tomato paste after process and during storage. According to researchers, color alone was not a good indicator to measure the effect of temperature or storage time in tomato ingredients [24]. Redness of tomato puree did not change by nonenzymatic browning, and previous investigators stated that this was due to the masking effect of lycopene [4].

4. Conclusion

The storage temperature of aseptic tomato paste significantly affected the formation of Maillard compounds, which are important for quality of aseptic tomato paste. However, effects of process temperature were not as significant as storage temperature. It might be due to the length of time. Storage time of the aseptic tomato pastes was much longer (up to 18 months) than aseptic processing time (up to few minutes). Aseptic tomato paste is usually stored at outdoor temperature in California. Average outdoor temperature in Northern California can reach up to 35 ºC during summer [25]. Therefore, it is recommended to store aseptic cold break tomato paste at lower temperatures to keep quality of aseptic cold break tomato paste with less browning compounds development.

Acknowledgement

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References


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