Chromatographic Determination of the Effect of Storage on Lycopene

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Abstract

A reverse-phase high-performance liquid chromatography (HPLC) method for the determination of lycopene in an alkaline lipid phase is described, and pigment stability in stored tomato sausage is reported. To avoid and replace the use of nitrite, lycopene from tomato products is added to minced meat and a tomato sausage with natural color is produced. Tomato sausage with and without nitrite were smoked in a smoking compartment and stored (4°C and 8°C) for 25 and 17 days, respectively. Among other factors, the quality of the tomato sausage depends on stability of lycopene during process and storage. Lycopene, being lipophilic, is extracted together with the polar and neutral fat in food. Efforts to purify lycopene from the fatty content will result in loss of pigment. The triacylglycerides obstruct the detection of lycopene by spectrophotometry or by HPLC with diode-array detection. To solve this problem, the triacylglycerides are hydrolyzed to free fatty acids just before analyzing lycopene on a column tolerating alkaline samples. At the end of the storage, loss of pigment in the sausage without nitrite was 26% stored at 4°C and 19% at 8°C. Corresponding results for the sausage with nitrite added as well as tomato paste show the loss of pigment is 20% and 45%. For each type of fatty food extracted, it is important to minimize the use of alkaline solutions because the HPLC equipment may be susceptible to alkaline conditions.

Introduction

Cured meat and meat products often discolor after a short storage. Ascorbic acid or sodium-ascorbate as antioxidant and nitrite are additives used to avoid these undesirable effects. Nitrite is converted into nitroso myoglobine, giving the characteristic pink color to cured meat. The use of nitrite in meat curing has been questioned since the 1970s, and there is a considerable worldwide interest in the development of new natural colorants for use in the food industry, which is apparently due to strong consumers demand for natural products. The consumer's preference for naturally derived colorants is associated with their image of the food being healthy and of good quality. In addition, some synthetic colorants are considered to be responsible for allergic and intolerance reactions.

To avoid and replace the use of nitrite, lycopene from tomato products and crystalline lycopene has been added to minced meat. Increased consumption of tomato products has in recent years been associated with decreased risks of cancer. The benefits of tomatoes and tomato products are often related to the carotenoid lycopene (1). Replacing nitrite with lycopene from tomato product resulted in a meat farce with different taste and color and with a longer shelf life (2). A new functional food could be developed when producing sausage from this meat farce. The quality of this tomato sausage depends on, among other factors, the stability of lycopene. Monitoring lycopene content during process and storage is therefore of great importance.

Lycopene (Figure 1) is a C_{40} tetra-terpenoid with an extended conjugated double-bond system sensitive to light and oxygen but relatively stable in alkaline or acidic solutions. A variety of methods to analyze lycopene in fruits and vegetables (3) and in biological plasma and tissue as well (4,5) have been reported. In fatty food, recovery of the lycopene content is a problem. Lycopene, being lipophilic, is extracted together with the polar and neutral fat in the food product. Efforts to purify lycopene from other lipids will often result in loss of pigment. The triacylglycerides make the detection of lycopene by spectrophotometry or high-performance liquid chromatography (HPLC) with diode-array detection difficult. Botsoglou et al. (6) examined oxidative stability of Japanese quail meat after being fed a diet with added dried tomato pulp. The fact that α-tocopherol, not lycopene, content in the quail meat was determined during storage could be because of a lack of an appropriate method for the determination of lycopene in fatty food and feed.

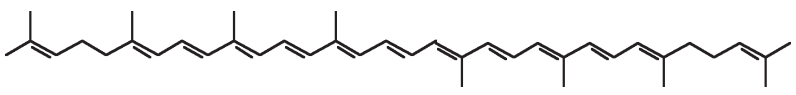

Figure 1. *All-E*-lycopene.

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The objective of this study was to develop an HPLC method for analyzing lycopene in tomato sausage after extraction of the lipid phase without loss of pigment when determining the effect of storage on lycopene. No emphasis of detection of geometrical isomers of lycopene will be done.

Experimental

Chemicals, reagents, and standard

Tomato paste (DIVA, Trondheim, Norway) without any synthetic antioxidant was obtained from the local market. Tomato paste (216.8 mg lycopene/kg tomato paste) was added to minced meat, resulting in a meat farce with 63.3 mg lycopene/kg. It was manufactured at the Department of Food Science (Trondheim, Norway). Sausages were produced from the different meat farces. As a reference, a standard sausage without tomato paste was produced. The sausages were smoked in a smoking compartment (Mulitimat 200, Trondheim, Norway) at 65°C and stored in the dark in plastic bags at 4°C and 8°C for 25 and 17 days, respectively. Sausage stored at 8°C had an extra withdrawal the 10th day and the storage ended after 17 days because of bad microbiological quality.

Crystalline lycopene was a gift from Organic Chemistry Laboratories, Norwegian University of Science and Technology (Trondheim, Norway). All solvents and reagents used were of analytical or HPLC grade. Potassium hydroxide (KOH) was purchased from Merck (Darmstadt, Germany) to make a 10% solution in methanol. The KOH solution was filtered (5951/2, Schleicher & Schuell, Dassel, Germany) before use.

Color measurement and equipment

Tristimuli color [International Commission on Illumination (CIE) (1976) L*, a*, b*] (7) lightness (L*), redness (a*), and yellowness (b*) were measured using a Minolta Chroma Meter CR 300 (Minolta, Osaka, Japan). The hue (H^0_{ab}) was calculated from the formula $H^0_{ab} = \tan^{-1}(a^*/b^*)$ and were $H^0_{ab} = 0°$ for the red hue and $H^0_{ab} = 90°$ for the yellow hue.

Extraction method, equipment, and sample treatment

Equipment for extraction of fat and carotenoids included a mixer (Ultraturax T25, Janke and Kinkel, IKA Laborteknik, Staufen, Germany) and heating block (Reacti-therm, Pierce, Oud-Beijerland, the Netherlands) equipped with a nitrogen evaporating unit (Reacti-vap, Pierce).

Lycopene from tomato paste and sausage was extracted by a modified method using chloroform, methanol, and water (8). Sausage without skin (3 g) was diluted in chloroform (5.0 mL), methanol (10.0 mL), and ultrapure water (3.0 mL) and homogenized for 1 min with an ultraturax (9500 rpm). More chloroform (5.0 mL) was added to the mixture, and it was homogenized (20 s, 9500 rpm) before adding water (5.0 mL) and homogenizing again (20 s, 9500 rpm). Finally the mixture was centrifuged (10 min, 10,000 rpm, 4°C) before an aliquot (2.0 mL) of the lower chloroform layer was transferred to a test tube and evaporated to dryness under a stream of nitrogen at 35°C. The dried residue of tomato paste and sausage were redissolved in dichloromethane (0.5 mL) by a vortex mixer and added the same amount of a 10% solution of KOH in methanol and diluted with acetone to the applicable concentration.

HPLC system and operating conditions

The HPLC system used was an Agilent 1050 liquid chromatograph (Agilent Technologies, Palo Alto, CA) connected to a Hewlett Packard (Palo Alto, CA) photodiode array UV–vis detector.

Samples of sausage and tomato paste were analyzed on a Zorbax Eclipse XDB-C8 (Agilent) column with acetonitrile as the mobile phase (sample amount, 20 µL; flow, 1.0 mL/min) and detected at 471 nm. For comparison with recovering lycopene in tomato paste, samples were analyzed by HPLC both on the Zorbax column and a Suplex pKb-100 C_{18} column (Supelco, Bellefonte, PA) with a mixture of methanol–acetonitril–isopropanol (54:44:2) as mobile phase (sample amount, 20 µL; flow, 1.0 mL/min) and detected at 471 nm.

The Rotor seal (VESPEL part number 0101-0623, Agilent) in the HPLC equipment may be susceptible to alkaline attack, deteriorating rapidly with solutions of pH higher than 10. In this case it is recommended to use a TEFZEL (part number 0101-0620, Agilent).

After a working period (~ 10 samples), it was necessary to rinse the injection valve and column with water at a flow rate at 1 mL/min for 20 min to eliminate alkaline attack, followed by conditioning with acetonitrile for 10 min.

Calculation

In order to quantitate the samples, an external standard method was employed. Standards of known concentrations were prepared from crystalline lycopene and were used as an external standard. The concentration of the standard solution was measured using a spectrophotometer (Pharma Spec. UV-1700, Shimadzu, Kyoto, Japan), and the employed extinction coefficient $E_{1cm,\ 1\%}$ at 471 nm in light petroleum was 3450 (9). Immediately after preparation, 20-µL aliqouts of the standard solution were injected into the HPLC system. The peak area of lycopene was determined, and the response factor for lycopene was calculated from the resulting means and spectrophotometrically measured lycopene concentration.

Statistical analysis

The values obtained for lycopene color and lycopene content are an average of at least triplicate determinations. Comparison of means of the measurements, using a significant level of $P < 0.05$, was performed by one-way analysis of variance.

Amount of lycopene found

To measure recovery of lycopene in the lipid phase dissolved in triacylglycerides and other lipids, extracted tomato paste was dissolved in: (*i*) dichlormetan (0.5 mL) and acetone (4.5 mL); (*ii*) dichlormetan (0.5 mL), maize oil (1 mL), and acetone (3.5 mL); and (*iii*) dichlormetan (0.5 mL), maize oil (1 mL), KOH (10% in acetone, 1 mL), and acetone (2.5 mL) and analyzed by HPLC on the Zorbax Eclipse XDB-C8 column.

The two HPLC methods, the Suplex pKb-100 C_{18}, and the

Zorbax Eclipse XDB-C8 column were compared with respect to the detection of lycopene content in tomato paste.

Results and Discussion

Color stability during storage

The color of the tomato sausages with and without nitrite stored at 4°C and 8°C were quite stable during 17 and 25 days, respectively. When nitrite was added to meat, the color changed from the purple-red color of myoglobin to the brown of metmyoglobin. With time and reduced conditions, the color was converted to the dark red of nitric oxide myoglobin (10). Compared with an ordinary sausage with nitrite, the color of the tomato sausages had quite the same hue and the same stability. Figure 2 shows the color of three types of sausage stored at 4°C for 25 days. Influence of storage at 8°C on color revealed the same tendency. The ordinary nitrite sausage was turning more reddish when nitric oxide myoglobin was produced during storage. The tomato sausage without nitrite was more reddish from the start and most stable. These findings are in accordance with an earlier study on the stability of meat farces from which the sausage were made, showing that a meat farce with crystalline lycopene added and no nitrite had the most reddish hue, and that the color was stable during storage (1). The influence of natural colorants on color attributes of frankfurters and consumer preference was examined by Bloukas et al. (11). Results have shown that the level of nitrite can be reduced from 150 to 100 mg/kg when using betanin as a color additive in addition to nitrite.

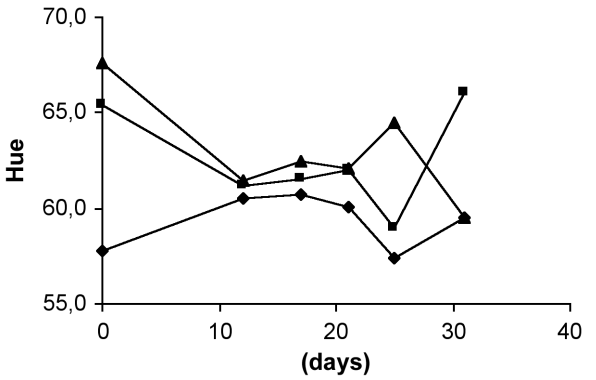

Figure 2. Color (hue) measured by chroma meter for tomato sausage (♦), tomato sausage with nitrite (■), and ordinary sausage with nitrite (▲) stored at 4°C for 31 days.

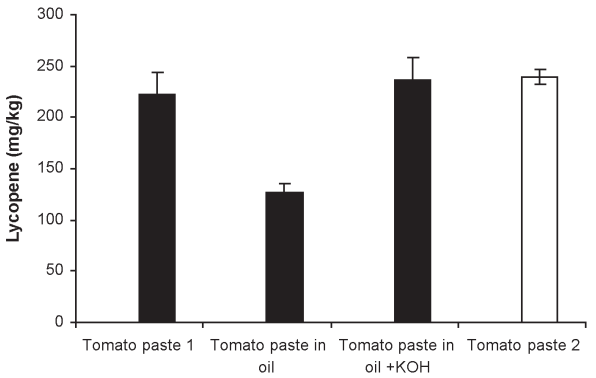

Figure 3. Retrieval of lycopene analyzed using a Zorbax Eclipse XDB-C8 column (dark bars, $n = 3$) in tomato paste (221.6 ± 22.5 mg/kg), tomato paste in oil (126.3 ± 8.6 mg/kg), and tomato paste in oil with KOH added (236.3 ± 22.1 mg/kg). The light bar shows retrieval of lycopene in tomato paste ($n = 3$) (239.6 ± 6.8 mg/kg) analyzed using a Suplex pKb-100 C18 column.

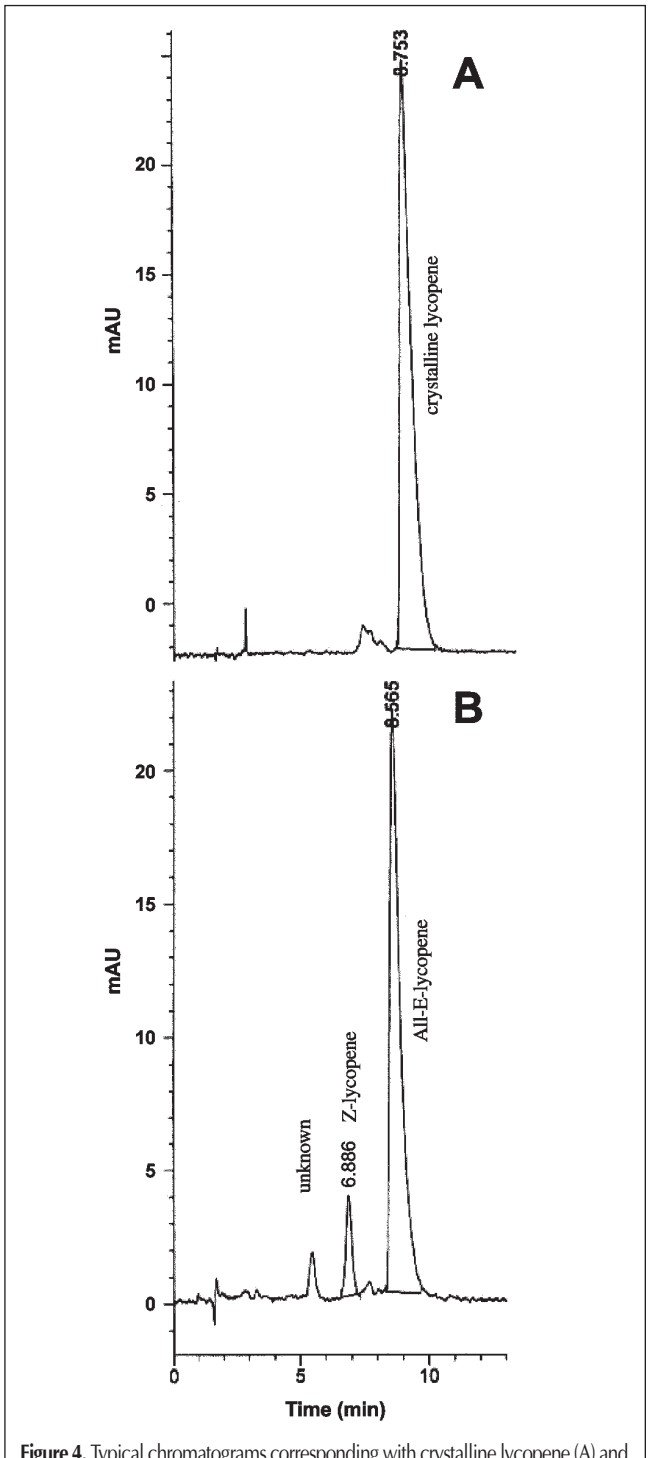

Figure 4. Typical chromatograms corresponding with crystalline lycopene (A) and extract of lycopene in a lipid phase from tomato sausage (B) both added alkaline.

Amount of lycopene

Retrieval of lycopene from tomato paste in different solutions and comparison of two HPLC methods for the detection of lycopene content in tomato paste are shown in Figure 3. Lycopene content in tomato paste analyzed by HPLC on the Suplex pKb-100 C_{18} column and the Zorbax Eclipse XDB-C8 column was 223.6 ± 24.1 and 221.6 ± 22.5 mg/kg, respectively. Comparison of the two HPLC methods for the detection of lycopene shows that there are no significant differences in the amount of lycopene found.

Retrieval of lycopene from tomato paste dissolved in maize oil and analyzed on the Zorbax Eclipse XDB-C8 column was 43.2% of the lycopene found in pure tomato paste without oil, suggesting that the triacylglycerides in oil or fat interfere with the detection of lycopene. As opposed to this finding, 98.6% of the lycopene content of tomato paste in oil (to which KOH was added) was retrieved. When using KOH, triacylglycerides are hydrolyzed to free fatty acids, which do not interfere with the detection of lycopene. It is important to minimize the use of alkaline solutions because of the HPLC equipment's sensitivity to alkaline conditions. To manage this, the admixture of alkali to the sample has to be optimized for each type of fatty food from which the lycopene shall be extracted. Typical chromatograms corresponding with an alkaline extract of lycopene from a lipid phase and crystalline lycopene analyzed on a Zorbax Eclipse XDB-C8 column is shown in Figure 4.

The sausages were produced from meat farce containing 63.3 mg lycopene from tomato paste/kg, with and without nitrite and smoked in a compartment at 65°C. The smoked sausages with and without nitrite had a lycopene concentration of 17.7 ± 3.9 mg and 23.0 ± 2.8 mg lycopene/kg, respectively. Processing conditions like heat and smoke will bring about a loss in lycopene content (12). In addition, the nitrite probably decomposes the lycopene, as approximately 23% less lycopene was found in sausages with both tomato paste and nitrite. Former results from analyses of lycopene in meat farces with added nitrite together with tomato paste and sun-dried tomatoes revealed 91.7% to 102% retrieval, respectively, of the pigment before further processing (1).

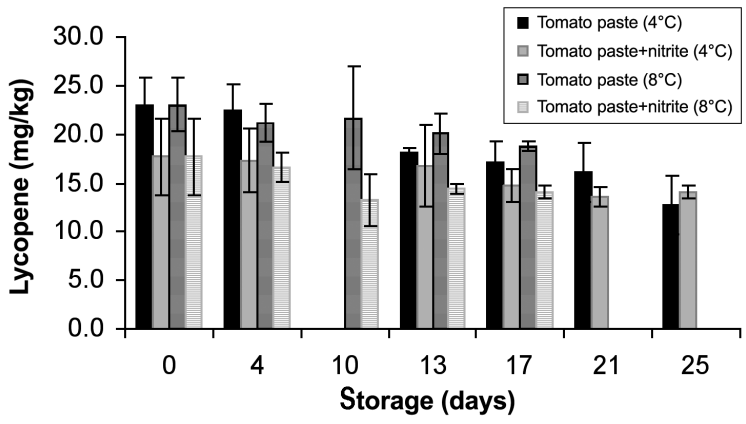

Figure 5. Lycopene content in tomato paste sausage with and without nitrite (n = 3) stored at 4°C and 8°C for 25 and 17 days, respectively. Sausage stored at 8°C had an extra withdrawal on the 10th day and the storage ended after 17 days because of bad microbiological quality.

Effect of storage on lycopene

Storage temperature (4°C and 8°C) has little influence on the lycopene content. Figure 5 shows a loss of pigment in the sausage without nitrite stored at 4°C and 8°C for 17 days of 26% and 19%, respectively. The greatest loss of lycopene was expected to be found in the sausage stored at the highest temperature.

By adding nitrite to the meat farce and processing the sausage, the lycopene is decomposed. Figure 5 reveals a loss of pigment in the tomato sausage with nitrite stored for 25 days at 8°C of 20% and a loss of 45% in the sausage without nitrite, ending up with a lycopene content of 14.1 ± 0.7 mg/kg and 12.7 ± 63.0 mg/kg, respectively. At the end of the storage, loss of pigment in the sausage without nitrite was 26% stored at 4°C and 19% stored at 8°C. The same stability is seen in the color measurement. The lycopene content in tomatoes analyzed by HPLC has formerly been correlated with color measurements in which the Tristimuli color [CIE (1976) L*, a*, b*] (7) value index a*/b* produced the best regressions (13).

Conclusion

The reported HPLC method using a Zorbax Eclipse XDB-C8 column after hydrolysis of the samples facilitates the detection of lycopene in fatty food and feed. For each type of fatty food extracted, it is important to minimize the use of alkaline solutions because the HPLC equipment may be susceptible to alkaline conditions. Work on validation of precision, accuracy, and linearity of the HPLC-method has to be done in the future.

In this experiment it is shown that lycopene added to fatty food as a colorant can be assayed and the storage stability monitored. At the end of the storage, loss of pigment in the sausage without nitrite was 26% stored at 4°C and 19% stored at 8°C. The corresponding results for the sausage with nitrite added and stored for 25 days included the loss of pigment of 20% and 45%, resulting in a lycopene content of 14.1 ± 0.7 and 12.7 ± 63.0 mg/kg, respectively.

These findings enable the detection of lycopene in fatty food. Results from monitoring lycopene content in the storage process indicate that lycopene can completely or partially compensate for admixture of nitrite in sausages.

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