Szent István University

DEVELOPMENT OF HPLC AND GC-MS METHODS FOR THE DETERMINATION OF CAROTENOIDS AND AROMA COMPOUNDS IN TOMATO AND SPICE PAPRIKA PRODUCTS

Thesis for PhD Degree

Zsuzsanna Bori

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INTRODUCTION

The nutritional benefit of tomato- and spice paprika-based products has been attributed to their being rich in bioactive compounds such as carotenoids and antioxidant vitamins (vitamin E and C) and polyphenols. Adequate daily intake of carotenoids and other antioxidants especially from fruits and vegetables has been recommended to sustain optimal health, moreover, data from epidemiological studies consistently showed correlation between the intake of fruits and vegetables and the incidence of several diseases such as cardiovascular, ophthalmological, gastrointestinal or neurodegenerative disorder and some type of cancer.

The attractive red colour of red-bell pepper and tomato fruit is due to a diverse composition of several yellow- and red-coloured carotenoids, which occur esterified with fatty acids in a form of mono- and di-esters in paprika and in free (un-esterified) form in tomato. The deterioration of colour in paprika and tomato products can make these products, even though it is not accompanied by a change in test or flavour, unacceptable for the consumers. From the technological point of view, the content and composition of carotenoids as well as presence of another antioxidant compounds in red paprika and tomato products can affect, to a high extent, the colour intensity and stability of the products.

Quality of both paprika and tomato products is extremely linked to the concentration and composition of aroma compounds formed during ripening and processing. Current research is also focusing on the flavour as an important parameter. Hungarian spice paprika is world-wide famous for its characteristic aroma that makes the products preferred as spice and natural colours in many food industries. Recently, there is a growing tendency to produce and to use smoked spice paprika products in food industries. Such products should be controlled from the point of food safety.

OBJECTIVES

The objectives of the present work were:

1. To develop an HPLC separation of carotenoids from spice paprika using cross-linked C18 column with working out the suitable elution that provides the best separation of the individuals of saponified paprika pigment.

2. To work out the efficient elution system for the separation of tomato carotenoids and their geometrical isomers on the new core C30 column.

3. To identify by LC-MS/MS technique the unidentified carotenoids that appears on the HPLC profile.

4. To apply the developed procedures to the determination of carotenoids in different tomato and spice paprika products produced in Hungary and other countries.

5. To develop a GC-MS method for the efficient separation of aroma compounds in tomato and spice paprika products.

6. To evaluate some Hungarian spice paprika samples and to distinguish the conventional products from the smoked ones on the basis of aroma profile.

MATERIALS AND METHODS

In this work, Hungarian, Argentinian, Chinese, South African conventional and smoked spice paprika samples were subjected to analysis of carotenoids and aroma compounds. In addition, fresh Heirloom American tomatoes and different tomato products were obtained and analysed.

The aroma compounds were extracted by steam distillation protocol and determined by GC-MS method using Thermo Trace 1300 Series Single Quadrupole GC-MS instrument. The separation was performed on SLBTM-5ms fused silica 30m x 0,25mm x 0,25 μm capillary column. During measurement, the temperature was 200°C with split mode. The injected volume was 1 μl using helium as the carrier gas at a flow rate of 1 ml/min. The oven temperature program started at 50°C and increased stepwise to 230°C in 30 min. The MS detector measured the masses between 35 and 350 Da. The temperatures of detector and ion source were 260°C and 240°C, respectively.

The carotenoids were separated on different reversed-phase column including cross-linked C18, 1.8μ, 5 cm UPLC column and core C30, 2.6 μ, 15 cm column. The mobile phases used in the gradient elution were: (1) water in acetone (for spice paprika extracts) and (2) *tert*-butyl-methyl-ether in methanol (for tomato and saponified spice paprika extracts). The flow rate was 0.4 ml/min for UPLC of paprika carotenoids and 0.7 ml/min for tomato and paprika carotenoids on core C30 column.

Summary

The results achieved in my research work are summarised as follows:

1. A GC-MS methods for the determination of aroma compounds in tomato and spice paprika products was developed and validated. Precision, recovery, LOD and LOQ were 0.22, 95.6, 2.9-6.4 ng/ml, and 10.1-14.5 ng/ml, respectively. Regression factor for the straight relation between peak area and concentration of standards was 0.9499-1.0000.
2. In spice paprika samples, 143 aroma compounds could be separated and detected. Three of them were found only in Hungarian paprika, these are: 2-methyl-tetradecan, α-normetadol, and hexylcinnamic acid-aldehyde.
3. In Spanish paprika 108 compounds could be detected, of those compounds 5 were found only in Spanish product. These are: *p*-methyl-anizol, methylcycloheptanol, D-Camfor, *p*-acetyltoluol, and verbenon.
4. In two Argentinian paprika samples 96 compounds were separated and determined. Fenylpropan type, myrecitine, β-iso-safrol, elemicin, iso-elemicin, kumaldehyde; bisabolane type ar-turmeron and curlone; sesquiterpenes D-germakrene and α-kubeba; cyclic monoterpene; sesquiterpene α-fellandrene; monoterpene alcohol α-terpinolene; bicyclic monoterpene β-pinene were found only in Argentinian spice paprika.
5. 2-bornene, elemol, γ-eudesmol, among 121 aroma compounds detected, were found only in paprika from Republic of South Africa.
6. In case of paprika from China, the certificating compounds were found to be 2,4-heptadiene-1-al, 5-ethyl-3-heptene-2-on, isopropyl-cyclohexane, 2-*trans*-4-*trans*-dekadienal.
7. In the Spanish smoked paprika, the smoke was more intensive than the original aroma profile of paprika. The Spanish paprika contains typically a lot of compounds having a naphthalene skeleton.
8. In the case of fresh tomatoes and tomato products, almost all samples analysed were found to contain much hexanal. Besides it, *trans*-2-hexene-1-al is of remarkable concentration. This component is responsible for the formation of mature tomato

aroma. The other terpenes present in an amount less than decomposition products of carotenoids, cyclic molecules, alcohols, aldehydes, ketones, acids, hydrocarbons, esters. Such a complex presence causes the tomatoes' characteristic flavour.

9. Excellent separation of paprika carotenoids could be achieved on cross-linked ultra HPLC C18 column. Precision was 2.36% for lutein and 6.27% for β–carotene. The recovery was 93% and 92% for lutein and β-carotene respectively. The detection limit of lutein was 19.44 ng/ml and limit of quantitation was 64.79 ng/ml. For β-carotene, the detection limit was 8.28 ng/ml, and the quantitation limit was 27.61 ng/ml. The linearity range with lutein was between 0 and 6 mg/ml concentration, and with β-carotene standard between 0-15 mg/ml. The capacity factor has a value between 0.4 and 13 and the number of theoretical plate number was between 920 and 270000.

10. With core C30 column, the precision was 1.91-2.38%, recovery was 94-99%, LOD was 0.008-0.17, LOD was 0.029-0.095 μg/ml and the regression factor for standard materials was between 0.9974 and 0.9999.

11. With use of cross-liked C18 column 26 compounds could be separated, whereas core C30 column provided good separation of 43 compounds from spice paprika extract. Most of these compounds were identified and quantified.

12. LC-MS/MS technique was successfully used to identify the compounds that were not identified on the basis of the spectral characteristics in the extract of tomatoes and tomato products. These carotenoids were found to be di-esters of both lycopene and β-carotene.

13. Yellow- and orange-coloured Heirloom tomatoes were found to have pro-neurosporine and pro-lycopene as the dominant carotenoids followed by zeta-carotene.

14. Commercially available foreign and Hungarian tomato products have been analysed for their carotenoid composition and content. The developed method showed the true composition of carotenoids, which assisted to accurately evaluate the tomato products. The highest concentration (343.5 μg/g) of lycopene was recorder for LS Pomil product among the examined tomato juice. The highest level of β-carotene was 20.3 μg/g and found in Kecskemét product. Tomato puree from Tesco had the highest lycopene (728.9 μg/g) and other carotenoids among the investigated purees. In case of ketchup,

the highest concentration of lycopene was in the Globus 1099.1 μg/g and the highest content of β-carotene was 31.7 μg/g in the Univer products. In tomato paste products, the highest concentration of lycopene (1507.2 μg/g) and β -carotene and 95.1 μg/g was found in Carloni S5 product.

NEW SCIENTIFIC FINDINGS

1. A GC-MS method was developed and validated for the separation and determination of aroma compounds in spice paprika and tomato as well as their products. With the developed method more compound could be detected as compared to previous protocols. In case of spice paprika more than 140 compounds, while in tomatoes more than 100 compounds could be detected and identified. Compound existing in a certain product, but not in others, were found.

2. Thirty-five compounds formed as a consequence of smoking of paprika could be detected and identified. Detection of such compounds can certify the measure of smoking.

3. It is confirmed that tomatoes do not differ substantially in their aroma profile, even although the is a considerable difference in their colour and taste. In addition, processing of tomato caused a significant decrease im the concentration of the characteistic hexanal and *trans*-2-hexene-1-al compounds.

4. Two HPLC methods could be developed and validated for the determination of carotenoids in tomatoes and saponified spice paprika extracts. In one method, a less polar cross-linked UPLC column was used with gradient elution of water in acetone. In the other method, the new core C30, 2.6 μ column was used with optimised gradient elution of tert-butyl-methyl-ether in methanol. With cross-linked UPLC and core C-0 column 26 and 43 carotenoids could be separated and determined in spice paprika samples.

5. By hyphenation of LC-DAD-MS/MS di-epoxides of lycopene and β-carotene in tomatoes and tomato products could be identified. Based on only diode-array detection it was difficult to identify such compounds.

6. By the newly developed method, the *cis* isomers of the major and minor carotenoids could be efficiently separated and determined in tomato products. Both all-*trans* and *cis* isomers have biological and nutritional importance. With the developed HPLC methods new data about the real carotenoid composition in tomatoes and tomato products were provided.

Publications on the topics

With Impact factor

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2. Adányiné Kisbocskói Nóra, Bori Zsuzsanna, Szendrő István: OWLS alapú mikrobiális szenzorok fejlesztése. MKE 1. Nemzeti Konferencia (2011. május 22-25. Sopron) Program és előadás összefoglalók p. 299.

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1. N. Adányi, Zs. Bori, I. Szendrő, K. Erdélyi, X. Wang, H.C. Schröder, W.E.G. Müller: Bacterial sensors for food safety based on biosilica immobilization for label-free OWLS sensors. EUROanalysis 2011 16th European Conference on Analytical Chemistry (Belgrad, Serbia, 11-15 Sept. 2011) SE01. (abstr)