SEPARATION AND IDENTIFICATION OF 
CIS/TRANS β-CAROTENE ISOMERS OF 
VIRGIN OLIVE OILS

Author: María E. Escuderos
(IFAPA Venta del Llano, Jaén, Spain)
Where is IFAPA?

IFAPA

Food & Beverages
Euro Global Summit and Expo on
June 16-18, 2015 Alicante, Spain
IFAPA was born to help the demands of the Andalusian agricultural, fishing, aquaculture and agri-food sectors.

IFAPA programs are the tool to boost the agricultural and fishery sector to bring the knowledge and innovation as new ways of work aiming to create wealth, quality employment, and social welfare in Andalusia.
IFAPA NETWORK (18 centres)

http://www.juntadeandalucia.es/agriculturaypesca/ifapa
IFAPA VENTA DEL LLANO (JAÉN-SPAIN)

- It was founded in 1902.
- Specialized in:
  - Olive oil technology and industrial process.
  - Quality virgin olive oil.
  - Olive cultivation.

Instituto de Investigación y Formación Agraria y Pesquera
CONSEJERÍA DE AGRICULTURA Y PESCA
IFAPA VENTA DEL LLANO (JAÉN): Equipments

100 Ha olive farm

Experimental olive oil mill

Classroom

Laboratories

Olive oil tasting room (COI T 20 / Doc 15 / Rev 7)
SEPARATION AND IDENTIFICATION OF
CIS/TRANS β-CAROTENE ISOMERS OF VIRGIN OLIVE OILS

INTRODUCTION

[Image of olive oil and olives]

98% 2%

- Fatty acid compounds
- Minor components

β – carotene pigment
INTRODUCTION

- Cultivars
- The degree of ripeness of the olive fruit
- Environmental conditions
- Processing techniques
- Storage conditions
The carotenoid profile of virgin olive oil can be used as a parameter of quality and authenticity for this product.
Actually, chromatographic methods disadvantages:
1. β-carotene extract contains others components that can interfere in the subsequent identification and separation.
2. The oil amount used is very low, so some cis isomers are not detected.

A new, rapid and precise analytical method was developed for the quality and quantity determination of trans-β-carotene and cis-isomers to detect olive oils subjected to soft temperature process.
MATERIALS

Abencor system

Olives cv. Picual

Extra-Virgin Olive Oil cv. Picual

Extra-Virgin Olive Oil (blank sample)

β – carotene standard

Fortified Extra-Virgin Olive Oil
METHODS

Cold-saponification

Liquid-phase distribution

Evaporation in rotavapor (30°C)

Re-dissolution in 2.5 ml acetone

HPLC (UV-Visible detector)

- C30 column (3 µm)
- MetOH:TBME:H2O
- λ 450 nm
RESULTS AND DISCUSSION

Linearity and detection limit

Calibration line: \( y = 0.1745x + 0.0617 \)

\[ R^2: 0.9986 \]

<table>
<thead>
<tr>
<th>Concentration range (µg/mL)</th>
<th>LOD (µg/mL)</th>
<th>LOQ (µg/mL)</th>
<th>Repeatability (%)</th>
<th>Recovery (%)</th>
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<tbody>
<tr>
<td>0.050 – 10.000</td>
<td>0.18</td>
<td>0.62</td>
<td>3.18</td>
<td>79.57</td>
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</table>

LOD: 3 \times \) standard deviation of low concen/slope of the calibration line.

LOQ: 10 \times \) standard deviation of low concen/slope of the calibration line
RESULTS AND DISCUSSION

Identification of β-carotene isomers (150°C heating)

<table>
<thead>
<tr>
<th></th>
<th>13,15-di-cis</th>
<th>15-cis</th>
<th>13-cis</th>
<th>9,15-di-cis</th>
<th>All-trans</th>
<th>9-cis</th>
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Bibliography: Strohschein et al., 1999; Qiu et al., 2009; Qiu et al., 2012; Achir et al., 2010.
RESULTS AND DISCUSSION

Changes (ppm) in the carotenoid profile in virgin olive oils (150°C)

Bibliography: Chen et al., 1994; Achir et al., 2010; Qui et al., 2012.

*Mean of three replicates
RESULTS AND DISCUSSION

Changes (%) in the carotenoid profile in virgin olive oils (150ºC)

EVOO

- All-trans: 94.40%
- All-cis: 5.60%

OO 150ºC 20 min

- All-trans: 46.60%
- All-cis: 53.40%

Detailed breakdown of carotenoid profile:

- 9-cis: 1.12%
- 9,15-di-cis: 0.14%
- 13-cis: 3.43%
- 15-cis: 0.73%
- 13,15-di-cis: 0.18%
- Total: 94.40% (EVOO)

- 9-cis: 17.08%
- 9,15-di-cis: 3.77%
- 13-cis: 22.46%
- 15-cis: 6.01%
- 13,15-di-cis: 2.55%
- Total: 46.60% (OO 150ºC 20 min)
Conclusions

1. Carotenoids naturally exist in the all-trans forms in VOO.

2. Carotenoid profile of an olive oil can be used as a parameter of quality and authenticity for this product ⇒ soft temperature process causes the isomerization of all-trans to cis forms.

3. The developed method, based on cold-saponification and liquid-phase extraction, is suitable for the isolation of β-carotene isomers in olive oils.

4. The use of C30 of 3μm column with HPLC technique for the separation of carotenoids leads to a good separation and short analysis times and results in sharp peaks which facilitate good detector response.
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mariae.escuderos@hotmail.com