German Society for Fat Science (DGF)
- Division Analysis and Quality Assurance -

Statement on the applicability of methods for the determination of pyropheophytin A and isomeric diacylglycerols in virgin olive oils

In 2001, A. *Serani* et al [1,2,3] published two methods to detect thermal treated oils. Meanwhile, these methods have been examined in three ring tests in Italy without success. Nevertheless, the calculation of the *Cold-Index* using the pyropheophytin A content and the isomerization degree of *1,2*-diacylglycerols have been used by many companies to check the quality of virgin olive oils during the last years. The so-called *TMP*-Value shall characterize the isomerization time of the *1,2*-diacylglycerols.

Ch. Gertz from the German Official Institute for Food Analysis in Hagen/Germany modified these methods to improve their precision, the applicability and examined the calculations of the proposed parameters like *TMP* or *Cold-Index*. During two workshops, organized by the DGF in May and September 2005 with over 80 experts from Italy, Spain, France, Belgium and Germany, he explained the reasons why the *Serani*-Methods does not give reliable results.

1. The presence of fat during the determination by HPLC or GC worsen the integration in both methods.

2. The calculations proposed by *Serani* for the *TMP* value or the *diffReal* (difference between real diacylglycerols and their theoretical content due to the formed free fatty acids) are very strongly influenced by the accuracy of the determination of acidity. A very small change of 0.05 % in the free fatty acids content (FFA), which is within the repeatability of the method, increases the TMP value from 0 to 300 (from a non, to a totally neutralised oil) because the calculation uses an exponential/logarithmic function.

3. The calculation of the *Cold-Index* is influenced by the chlorophyll content of the olive oil and by the quantification of the amounts of pyropheophytin A and pheophytin A, because chlorophyll is extremely unstable and therefore no calibration standard is available.

After the proposed modifications, the methods seemed to give reliable results. Therefore the participants decided during the first workshop in May 2005 to organize a ring test with all methods and calculations. The statistical evaluation of the data delivered by 22 laboratories from 5 countries in accordance with ISO 5725 shows that only the modified methods are appropriate and reliable methods to analyse olive oils in an accurate, simple and quick manner with good precision

[1] A. Serani, D.Piacenti (2001) Sistema analytico per l'dentificazione di oli deodorati in oli vergini di oliva – Nota 1 – Analisi dei pigmenti clorofilliani in oli vergini di oliva Riv Ital Sost. Grasse LXXVIII, 459-463

[2] A. Serani, D-Piacenti, G. Staiano (2001) Sistema analytico per l'dentificazione di oli deodorati in oli vergini di oliva – Nota 2 – Cinetica di isomerizzazione dei diglyceridi in oli vergini di oliva Riv Ital Sost. Grasse LXXVIII, 525—528

[3] A. Serani, D.Piacenti (2001) Sistema analytico per l'dentificazione di oli deodorati in oli vergini di oliva – Nota 1 – Analisi HPLC/VIS delle feofitini e GC/FID dei diglyceridi in oli vergini di oliva, Riv Ital Sost. Grasse LXXVIII, 567-570

data. The methods of *Serani* were refused by the participants of the 2nd Workshop in September 2005 due the bad precision (reproducibility):

"The cited analytical parameters *Cold-Index*, determination of isomeric diglycerides in ppm and the calculation of *TMP*, and *diff-Real* proposed by *Serani* have been proven in the DGF-Ringtest 2005 as difficult to reproduce (bad repeatability and bad reproducibility)."

The implementation of the new modified methods for the analysis of more than 250 different olive oils samples from different countries was also demonstrated by *Gertz* during the 2nd DGF workshop. The multivariate analysis of the collected data showed that the *1,2*-diacylglycerol content depends on the storage life of the olive oils but is not an indicator for a thermal treated or neutralised olive oil as proposed by *Serani* before. This observation has recently been confirmed by other authors[4]. Nevertheless, the amount of *1,2*-diacylglycerols helps to calculate and control the aging of olive oils. A fresh oil has more than 70 % of *1,2*-diacylglycerols. This amount is decreasing to about 60 % after one year, with approximately 1 % per month.

The statistical evaluation of approx. 250 different samples analysed in Hagen by *Gertz* shows that the pyropheophytin A content (calculated as the percentage of its precursors) does not increase significantly during storage using the expiry date for the calculation. This observation has recently been confirmed by laboratory tests with different olive oils[5], providing the data have been calculated in the same way as proposed by *Gertz (see figure)*. The pyropheophytin A content, measured as area % of all its precursors, does not increase significantly during storage (approximately 2-3 % per year).
 The descriptions of both methods and the statistical evaluation of all methods can be downloaded from www.dgfett.de.
Furthermore, during the workshop *it was* demonstrated that many blends of olive oil with other vegetable oils or with refined oils could not be detected using the official methods of the regulation (EEC) No 2568/91. Therefore, all participants of the workshops agreed on the following statement:
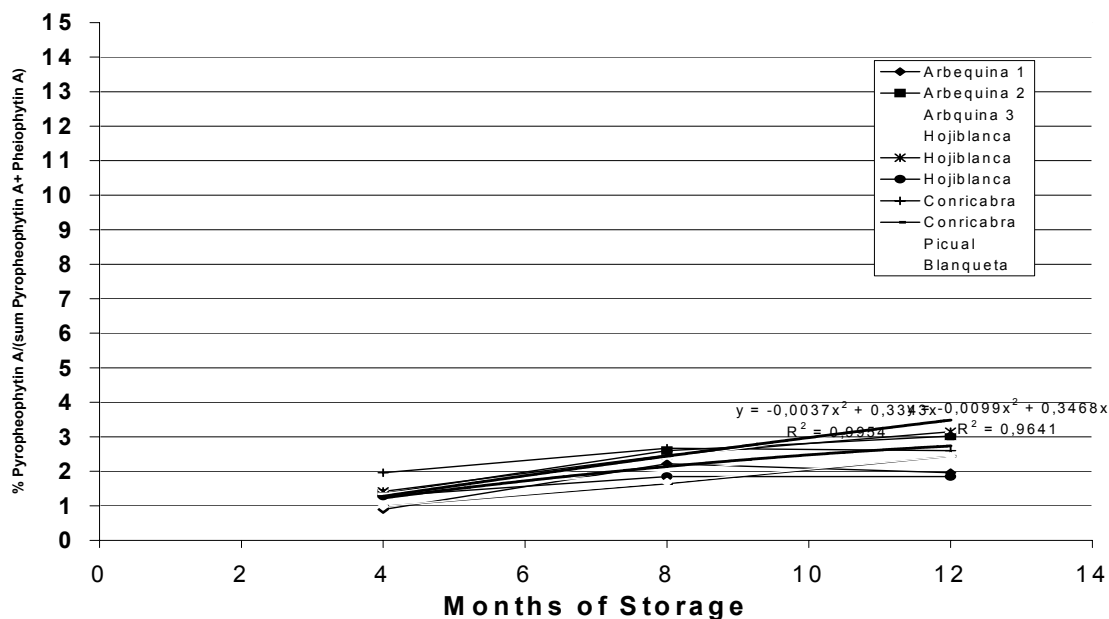
"The official analysis methods inserted in (EEC) No 2568/91 are not appropriate to verify the identity/authenticity or to detect thermal treatment <u>in any case.</u> They need an updating because of the development in the field of analytics and oil technology"

Nobody should wonder, that many consumer tests in the USA (1995), Canada (1995), Switzerland (1996) and Germany (1996, 1999, 2002, 2004, 2005) revealed that one third of all analysed olive oils were altered and wrongly labelled as "extra virgin olive oils". The detection of these manipulations succeeded because other modern, and more sensitive analytical methods were used than those listed in the official regulation (EEC) No 2568/91.

[4] F. Caponio, M.T.Bilancia, A.Pasqualone, E.Sikorska, T.Gomes (2005) Influence of the exposure to light on extra virgin olive oil quality during storage, Eur Food Res Technol 221:92-98
[5] L.Gallardo-Guerrero, B.Gandul-Rojas, M.Roca, M.Minguez-Mosquera,"Effects of storage on the original pigment profile of Spanish olive oils" J Amer.Oil Chem.Soc. 82, 33-39

Variation of Pyropheophytin A % during Storage (Calculation based on Data source of L.Gallardo-Guerro et al, Instituto de la Grasa, Sevilla, JAOCS 82, 32-37(2005))

Chart: Y-axis "% Pyropheophytin A/(sum Pyropheophytin A+ Pheliophytin A)" from 0 to 15. X-axis "Months of Storage" from 0 to 14.

Legend:
- Arbequina 1
- Arbequina 2
- Arbquina 3
- Hojiblanca
- Hojiblanca
- Hojiblanca
- Conricabra
- Conricabra
- Picual
- Blanqueta

Equations on chart:
$y = -0{,}0037x^2 + 0{,}3343x$
$R^2 = 0{,}9934$

$y = -0{,}0099x^2 + 0{,}3468x$
$R^2 = 0{,}9641$

The purpose of the regulations of the European food law [see reg. (EC) No. 178/2002] is to protect consumers from economic damages (misleading) and healthy risks. The actual regulation (EEC) No. 2568/91 for olive oil and olive pomace oils is limited in the detection of new frauds. Furthermore, the cited methods and limits integrated in the regulation do not fulfil the international standard (ISO, CEN, Codex Alimentarius) for analytical methods. There are no precision data given and some limits (for instance in case of the determination of *trans* fatty acids) cannot be controlled as the limits of determination for the method are too high. The cited analytical criteria do not allow the traceability as demanded in regulation (EC) No. 178/2002 or the detection of the addition of refined olive oils or olive oils which have been steam-washed at temperatures between 90 °C to 120 °C, using the new *Soft-Column* technology.

The proposed analytical parameter in the regulation (EEC) No. 2568/91, like the pattern of the individual sterols or fatty acids or the HPLC separation of some triacylglycerols, are limited in their possibility to control the authenticity or to recognize the addition of other vegetable oils. The methods are not validated, very time-consuming and expensive in the application and not specific enough to detect all kinds of fraud. Therefore, the participants of the 2nd Workshop recommended for the routine analysis a collection of parameters which allows an olive oil to be checked within a few hours (conclusion 6):

"In routine analysis for checking identity and quality it is recommended to analyse:

- ➢ **Sensory Test**
- ➢ **Peroxide Value (PV)**
- ➢ **Absorption at 232 and 270 nm (K232/K270)**
- ➢ **Acidity (FFA)**
- ➢ **Fatty Acid Composition**
- ➢ **Triacylglycerol Distribution by Capillary Gas-Chromatography**

> **Pyropheophytin A, in Area % (Pyropheophytin A*100/ total Pyropheophtyin A, Pheophytin A and A')**
> **1,2-Diacylglycerols Content, in Area % (*1,2*-Diacylglycerols *100/ total *1,2*- and *1,3*-Diacylglycerols)**

This list should be completed with the determination of the polyphenol content as this value represents the nutritional value of olive oils.

Conclusions from the DGF-Workshop on Thermally Treated Olive Oil, 19th of September 2005, Hagen

Conclusion 1
The official analysis methods inserted in (EEC) No. 2568/91 are not appropriate to verify the identity/authenticity or to detect thermal treatment <u>in any case.</u> They need an updating because of the development in the field of analytics and oil technology.

Conclusion 2
The list of the cited analytical methods in (EEC) No. 2568/91 cannot be regarded as final and does not exclude the application of other validated methods, according to ISO 17025.

Conclusion 3
The cited analytical parameters cold index, determination of isomeric diglycerides in ppm and the calculation of *TMP*, and *diffReal* proposed by *Serani* have proven in the DGF-Ringtest 2005 as difficult to reproduce (bad repeatability and bad reproduceability.

Conclusion 4
It is recommended to determine the pyropheophytin A contents and *1,2* and *1,3*-diglycerides using the tested modified methods (according to *Gertz*) calculating the area % (DGF-Ringtest 2005).

Conclusion 5
The *1,2*-Diglycerides (% Area) contents reflect the aging of olive oils during storage. A reduction of diglycerides may be caused also by a thermal treatment.

Conclusion 6
 In routine analysis for checking identity and quality it is recommended to analyse :
-Sensory Test
-Peroxide Value (PV)
-K232/K270
-Acidity (FFA)
-Fatty Acid Composition
-Triglyceride Distribution by GC
-Pyropheophytin A (Area % / total Pyropheophtyin A, Pheophytin A and A')
-*1,2*-Diglycerides Contents (Area % / total *1,2*- and *1,3*-Diglycerides (DG))

Conclusion 7
(not accepted by all participants)

Besides sensory test the following values could be suggested for good grade extra virgin olive oil during bottling:

1,2 DG	>	60% (Area)
1,2/1,3 DG Ratio	>	1,5

Conclusion 8
1,2-Diglycerides (% area/ sum of areas *1,2*- and *1,3*-diglycerides) contents less then 45% (Precision data included) may be an indicator of a low grade olive oil labelled as extra virgin.

Conclusion 9
Pyropheophytin A contents (area %) greater then 15% (precision data included) may be an indicator of thermal treatment.

Dr. Christian Gertz, Hagen
Dr. Hans-Jochen Fiebig, Münster