Workshop on

olive oil authentication

Madrid, Spain

10 & 11 June 2013

Organised by
European Commission
Directorate General
Agriculture and Rural Development
&
European Commission
Joint Research Centre
Institute for Reference Materials and Measurements

With the participation of the
International Olive Council

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01 - Olive Oil: Trends in quality and product category shares

by J.L. Barjol

Olive oil: trends in quality and product category shares

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Although standardisation will be covered in another paper, the link between the concept of quality and standardisation and the data available on this subject will be reviewed briefly as an introduction to the trends in product quality and the shares of the different product categories. This introduction will outline the legal commitments of IOC Members to use IOC designations in their trade in olive products and the consequences for the world market. While IOC member countries account for 98 pc of world olive oil supply, the reverse occurs for demand, 80 pc of which comes from non-member countries, chiefly the United States, Brazil, Japan, China, Australia and Canada by descending order of importance. This complicates matters for business operators on the world market. However, the fact is that importing countries which are not members of the IOC cannot adopt compulsory international trade regulations contrary to IOC designations, which are used by the Codex Alimentarius; otherwise, they would be in contraction with World Trade Organisation rules.

Review of world market trends in terms of quality and product categories is therefore based on the statistics on hand. The only international harmonised statistics available are those of the World Customs Organisation, which only makes a distinction between three categories: virgin olive oil (customs heading 150910), olive oil (customs heading 150990) and olive pomace oil (customs heading 151000). It should be noted that IOC Members are not required to notify more detailed figures to the IOC. Nevertheless, more precise data are available for some countries. For instance, the European Union (EU) distinguishes the share of lampante virgin olive oil within its imports of virgin olive oil, and the United States and Canada itemise their imports of bulk and packed olive oil. North American business sources also give more detailed data on extra virgin olive oils but these are not verifiable.

Focusing on the U.S. market, in 2002/03 the category breakdown of imports was 56 pc virgin olive oil, 41 pc olive oil and 3 pc olive pomace oil. In 2011/12, the shares of these three categories were 65 pc, 30 pc and 5 pc, respectively. According to business data, in 2004 extra virgin olive oil accounted for 48 pc of U.S. imports; by 2012 this percentage had risen to 62 pc. In the same year, 59 pc of the bottles sold by U.S. distributors were labelled extra virgin olive oil. Another striking feature of the U.S. market is the expansion of bulk imports of virgin olive oils, which represented almost 40 pc of import volume in 2012. This phenomenon is not seen in Canada.

On the EU market, imports by category went from 63 pc for virgin olive oil and 37 pc for olive oil in 2002/03 to 91 pc and 2 pc, respectively, in 2011/12. Notably, lampante virgin olive oil’s share of imports of virgin olive oils (150910) dropped from 62 pc in 2001 to below 38 pc in 2012. In Japan, virgin olive oil’s share of imports climbed from 55 to 67 pc between 2002/03 and 2011/12 while olive oil saw its share fall from 44 to 28 pc; the remainder was olive pomace oil. In China, virgin olive oil’s share of imports shot up from 35 to 84 pc while olive oil slumped from 41 to 5 pc; the rest of imports was olive pomace oil. The category breakdown is different in Australia, with the share of the virgin grade going up from 29 to 53 pc and that of olive oil going down from 71 to 46 pc. Lastly, during the same period, virgin olive oil’s share of Canadian imports increased from 60 to 74 pc, olive oil narrowed from 39 to 24 pc and olive pomace oil accounted for the rest. Overall, it is estimated that virgin olive oil’s (150910) slice of the world market expanded from 54 pc in 2002/03 to 65 pc in 2011/12; at the same time the share of non-virgin olive oil (150990) narrowed from 43 pc to 25 pc; again the remaining percentage is olive pomace oil. In a nutshell, virgin olive oil is gaining increasing prominence over the other categories and it is highly probable that in some markets extra virgin olive oil is predominant under the virgin category.

OLIVE OIL MARKET

WHAT ARE WE TALKING ABOUT ?

IOC STANDARD : VIRGIN OLIVE OILS

oils obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions, particularly thermal conditions, that do not lead to alterations in the oil, and which have not undergone any treatment other than washing, decantation, centrifugation and filtration

1. **EXTRA VIRGIN OLIVE OIL** -
2. **VIRGIN OLIVE OIL -**
3. **ORDINARY VIRGIN OLIVE OIL**
4. **LAMPANTE VIRGIN OLIVE OIL (DOES NOT FIT FOR DIRECT CONSUMPTION)**

Each of them fulfils various specific characteristics fixed in the IOC trade standard.

IOC STANDARD : OLIVE OILS:

•**OLIVE OIL -** oil consisting of a blend of refined olive oil and virgin olive oils fit for consumption.

•**REFINED OLIVE OIL -** olive oil obtained from virgin olive oils by refining methods which do not lead to alterations in the initial glyceridic structure.

Each of them fulfils various characteristics fixed in the IOC trade standard.

IOC STANDARD : OLIVE POMACE OILS:

REFINED OLIVE-POMACE OIL - oil obtained from crude olive pomace oil by refining methods which do not lead to alterations in the initial glyceridic structure.

OLIVE-POMACE OIL - oil comprising the blend of refined olive pomace oil and virgin olive oils fit for consumption.

CRUDE OLIVE POMACE OIL - It is intended for refining for use for human consumption, or for technical use.

Each of them fulfils various characteristics fixed in the IOC trade standard.

WHAT ARE WE TALKING ABOUT ?

4 different grades for virgin olive oils
2 different grades for olive oils
3 different grades for pomace olive oils

UNITED NATIONS CONFERENCE ON TRADE AND DEVELOPMENT

INTERNATIONAL AGREEMENT ON OLIVE OIL AND TABLE OLIVES, 2005

UNITED NATIONS

Article 22

Undertakings by Members

1. The Members of the International Olive Council **undertake to apply the designations** prescribed in Annexes B and C in their international trade **and shall encourage** their application in their internal trade.

UNITED NATIONS CONFERENCE ON TRADE AND DEVELOPMENT

INTERNATIONAL AGREEMENT ON OLIVE OIL AND TABLE OLIVES, 2005

UNITED NATIONS

Article 24

Examination of the situation of and developments in the market for olive oil, olive-pomace oil and table olives

1. Within the framework of the general objectives set forth in article 1, and with a view to contributing towards the standardization of the market for olive oil, olive-pomace oil and table olives and correcting any imbalance between international supply and demand due to irregularity of harvests or to other factors, **Members shall make available and furnish to the International Olive Council all the necessary data**, statistics and documentation on olive oil, olive-pomace oil and table olives.

UNITED NATIONS CONFERENCE ON TRADE AND DEVELOPMENT

INTERNATIONAL AGREEMENT ON OLIVE OIL AND TABLE OLIVES, 2005

UNITED NATIONS

17 IOC MEMBERS REPRESENT 96% OF WORLD EXPORTS

BUT

LESS THAN 20% OF THE WORLD IMPORTS

DATA PROVIDED AND AVAILABLE

WHAT ARE WE TALKING ABOUT ?

INTERNATIONAL HARMONISED SYSTEM

WORLD CUSTOMS ORGANIZATION	IOC STANDARD
VIRGIN OLIVE OILS	
150910	**EXTRA VIRGIN OLIVE OIL**
	VIRGIN OLIVE OIL
	ORDINARY VIRGIN OLIVE OIL
	LAMPANTE VIRGIN OLIVE OIL
OLIVE OILS	
150990	**REFINED OLIVE OIL**
	OLIVE OIL
OLIVE POMACE OILS	
151000	**REFINED OLIVE-POMACE OIL**
	OLIVE-POMACE OIL
	CRUDE OLIVE POMACE OIL

EUROPEAN UNION /27 **HARMONISED SYSTEM**

15. 09. 10. 10 : VIRGIN LAMPANTE OLIVE OIL OBTAINED FROM THE FRUIT OF THE OLIVE TREE SOLELY BY MECHANICAL OR OTHER PHYSICAL MEANS UNDER CONDITIONS THAT DO NOT LEAD TO DETERIORATION OF THE OIL and

15. 09. 10. 90: OLIVE OIL OBTAINED FROM THE FRUIT OF THE OLIVE TREE SOLELY BY MECHANICAL OR OTHER PHYSICAL MEANS UNDER CONDITIONS THAT DO NOT LEAD TO DETERIORATION OF THE OIL, UNTREATED (EXCL. VIRGIN LAMPANTE OIL)

15. 09. 90: OLIVE OIL AND FRACTIONS OBTAINED FROM THE FRUIT OF THE OLIVE TREE SOLELY BY MECHANICAL OR OTHER PHYSICAL MEANS UNDER CONDITIONS THAT DO NOT LEAD TO DETERIORATION OF THE OIL (EXCL. VIRGIN AND CHEMICALLY MODIFIED)

15. 10. 00. 10: CRUDE OLIVE OILS AND BLENDS, INCL. BLENDS WITH THOSE OF HEADING 1509

15. 10. 00. 90: OTHER OILS AND THEIR FRACTIONS, OBTAINED SOLELY FROM OLIVES, WHETHER OR NOT REFINED, BUT NOT CHEMICALLY MODIFIED, INCL. BLENDS OF THESE OILS OR FRACTIONS WITH OILS OR FRACTIONS OF HEADING 1509 (EXCL. CRUDE)

USA HARMONISED SYSTEM

15091020: Virgin olive oil and its fractions, whether or not refined, not chemically modified, **weighing with the immediate container under 18 kg**

15091040: Virgin olive oil and its fractions, whether or not refined, not chemically modified, **weighing with the immediate container 18 kg or over**

15099020: Olive oil, other than virgin olive oil, and its fractions, not chemically modified, weighing with the immediate container under 18 kg

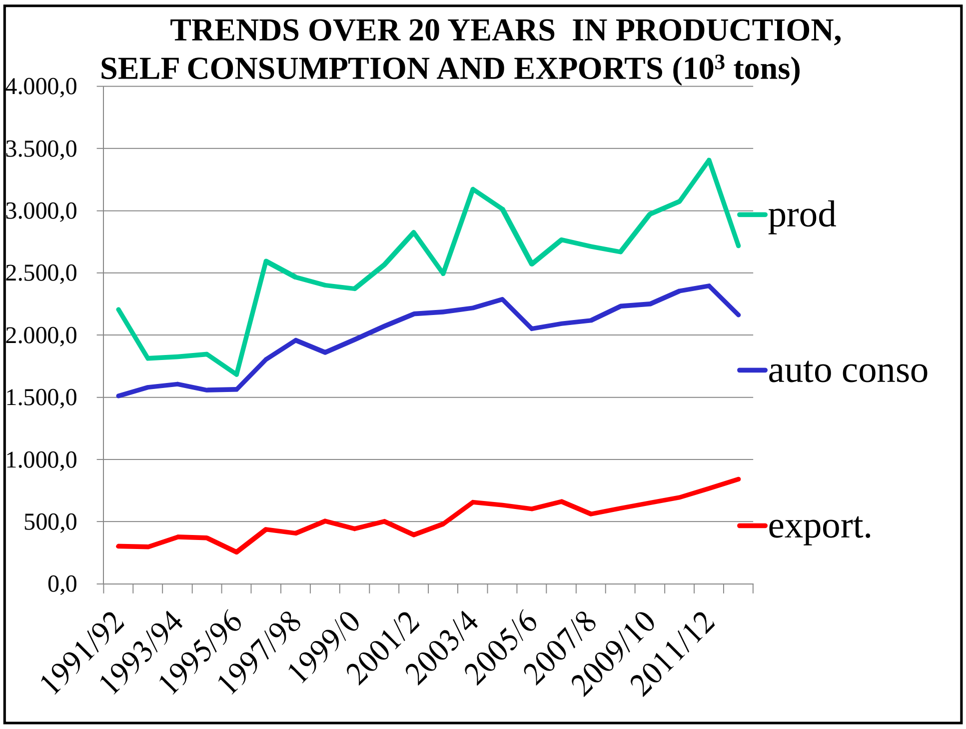
15099040: Olive oil, other than virgin olive oil, and its fractions, not chemically modified, weighing with the immediate container 18 kg or over

15100020: Olive oil, including blends, and their fractions, not chemically modified, rendered unfit for use as food

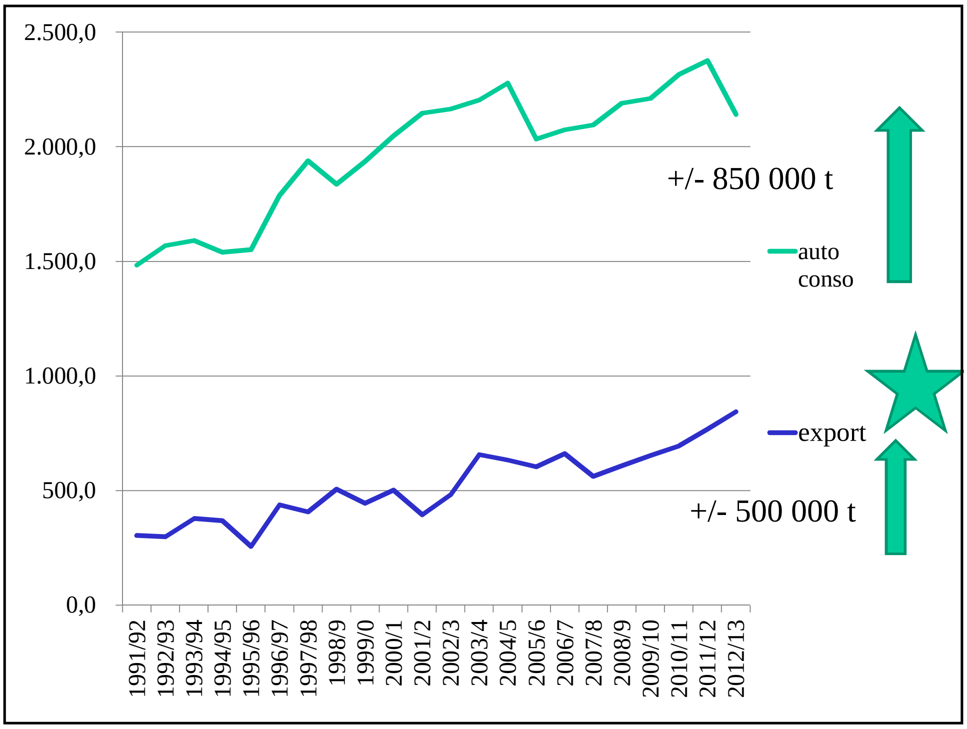
15100040: Edible oil including blends, and their fractions, not chemically modified, weighing under 18 kg

15100060: Edible oil including blends, and their fractions, not chemically modified, weighing 18 kg or over

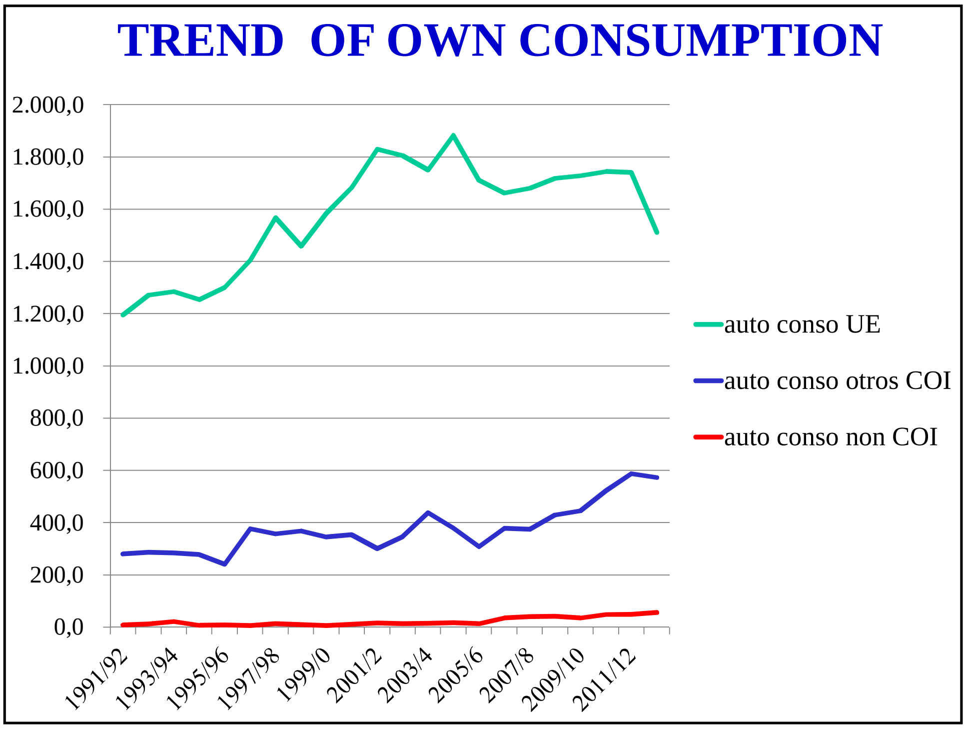
SITUATION OF THE OLIVE OIL WORLD MARKET



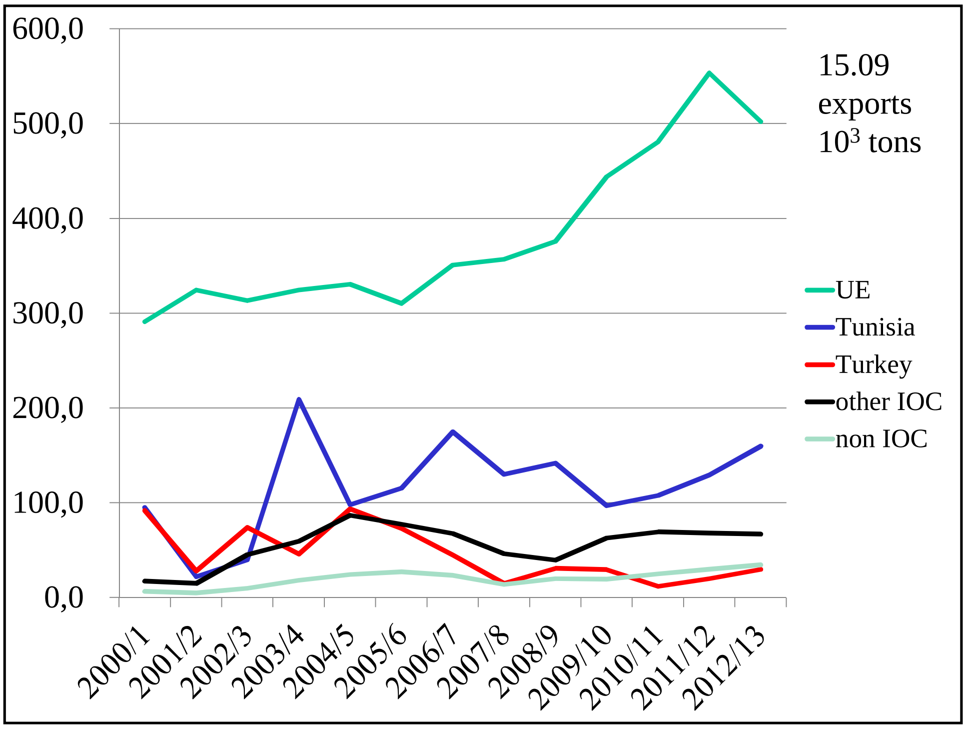
TRENDS OVER 20 YEARS IN PRODUCTION, SELF CONSUMPTION AND EXPORTS (10^3 tons)
4.000,0
3.500,0
3.000,0
2.500,0
2.000,0
1.500,0
1.000,0
500,0
0,0
1991/92
1993/94
1995/96
1997/98
1999/0
2001/2
2003/4
2005/6
2007/8
2009/10
2011/12
prod
auto conso
export.

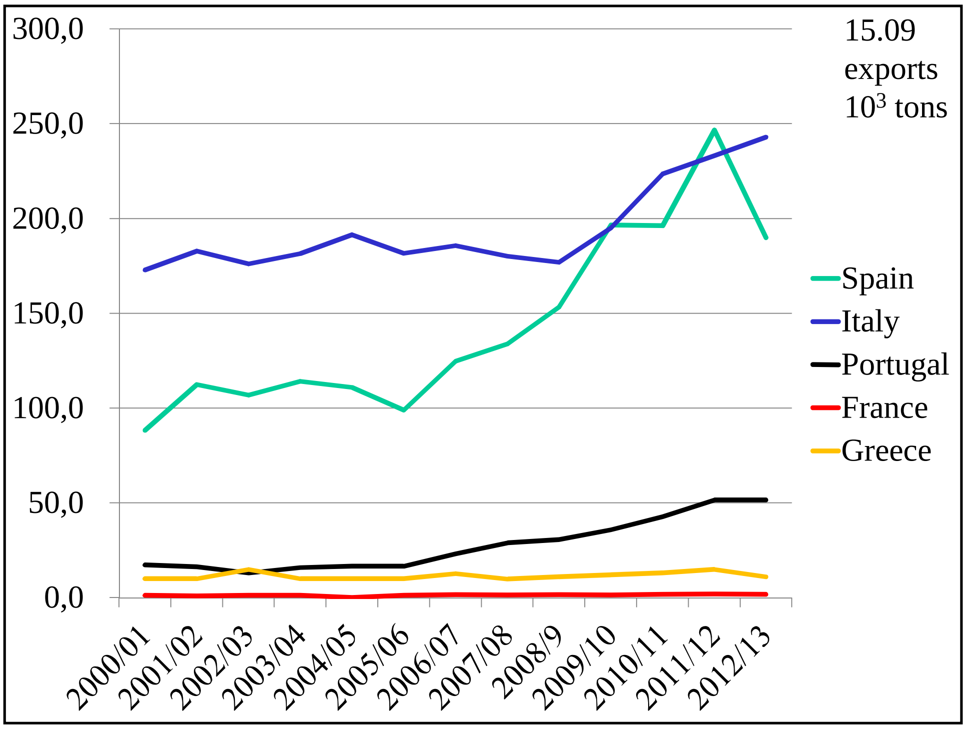


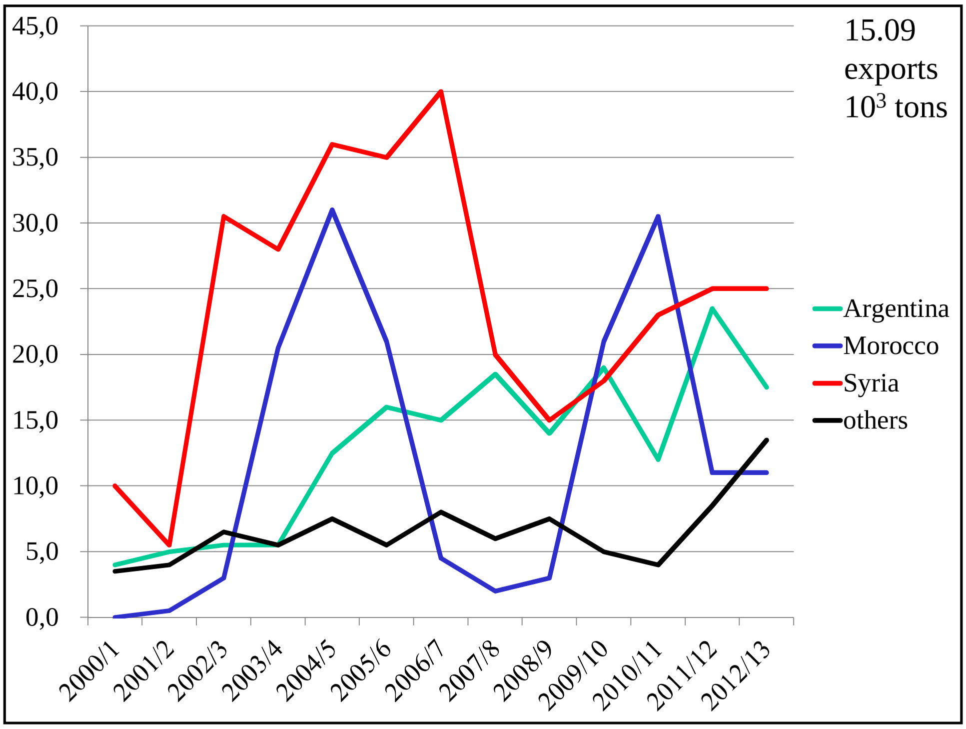
2.500,0
2.000,0
1.500,0
1.000,0
500,0
0,0
1991/92
1992/93
1993/94
1994/95
1995/96
1996/97
1997/98
1998/9
1999/0
2000/1
2001/2
2002/3
2003/4
2004/5
2005/6
2006/7
2007/8
2008/9
2009/10
2010/11
2011/12
2012/13
+/- 850 000 t
auto conso
export
+/- 500 000 t

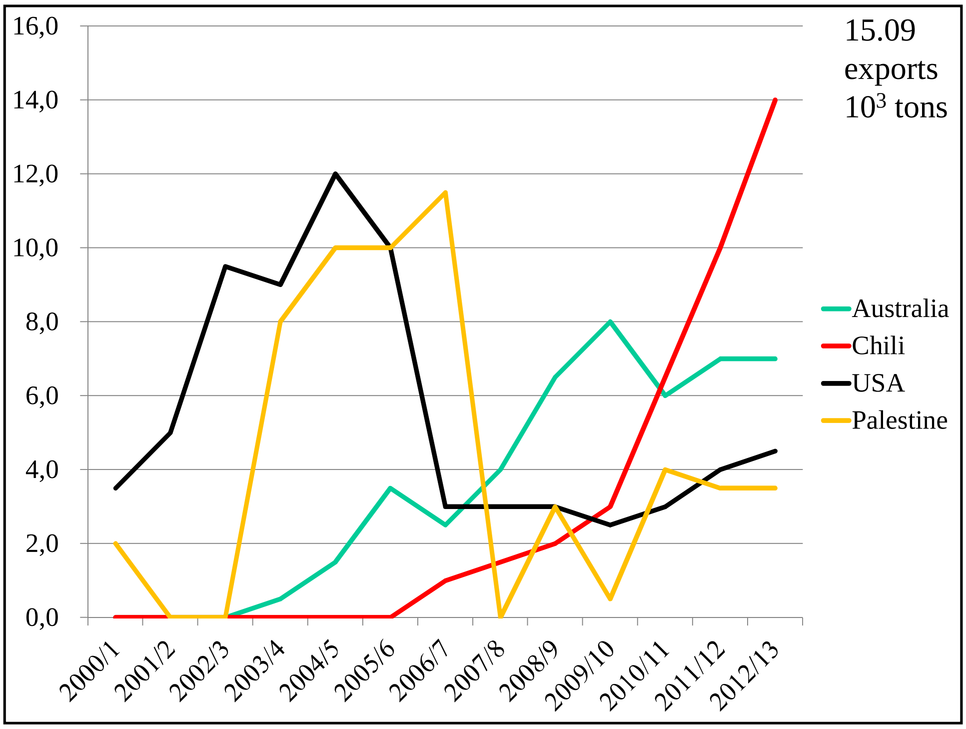


WHO ARE THE EXPORTERS ?


600,0
500,0
400,0
300,0
200,0
100,0
0,0
2000/1
2001/2
2002/3
2003/4
2004/5
2005/6
2006/7
2007/8
2008/9
2009/10
2010/11
2011/12
2012/13
15.09
exports
10^3 tons
UE
Tunisia
Turkey
other IOC
non IOC

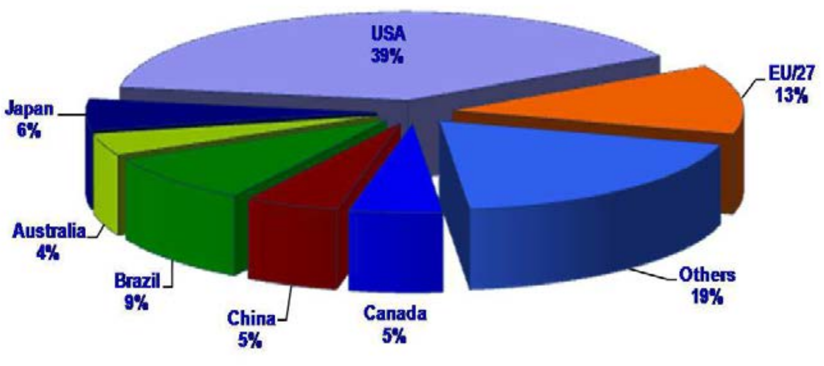

300,0
250,0
200,0
150,0
100,0
50,0
0,0
2000/01
2001/02
2002/03
2003/04
2004/05
2005/06
2006/07
2007/08
2008/9
2009/10
2010/11
2011/12
2012/13
15.09
exports
10^3 tons
Spain
Italy
Portugal
France
Greece


45,0
40,0
35,0
30,0
25,0
20,0
15,0
10,0
5,0
0,0
2000/1
2001/2
2002/3
2003/4
2004/5
2005/6
2006/7
2007/8
2008/9
2009/10
2010/11
2011/12
2012/13
15.09
exports
10³ tons
Argentina
Morocco
Syria
others


16,0
14,0
12,0
10,0
8,0
6,0
4,0
2,0
0,0
2000/1
2001/2
2002/3
2003/4
2004/5
2005/6
2006/7
2007/8
2008/9
2009/10
2010/11
2011/12
2012/13
15.09
exports
10³ tons
Australia
Chili
USA
Palestine

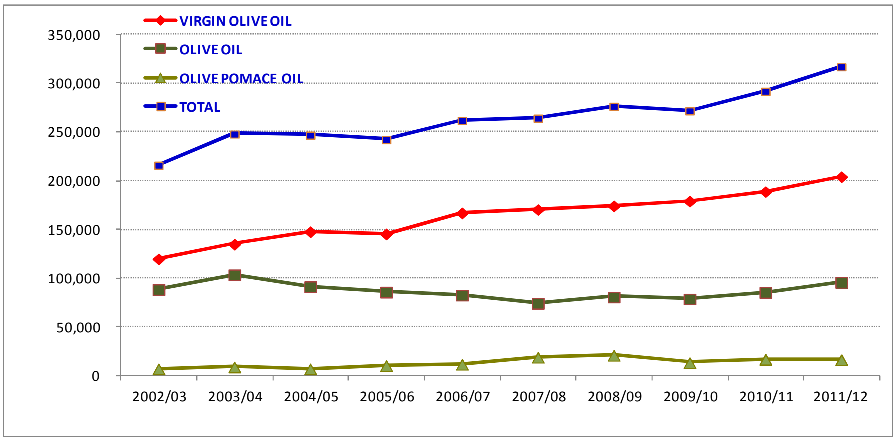
WHO ARE THE IMPORTERS ?

OLIVE OIL IMPORTS
2011/12

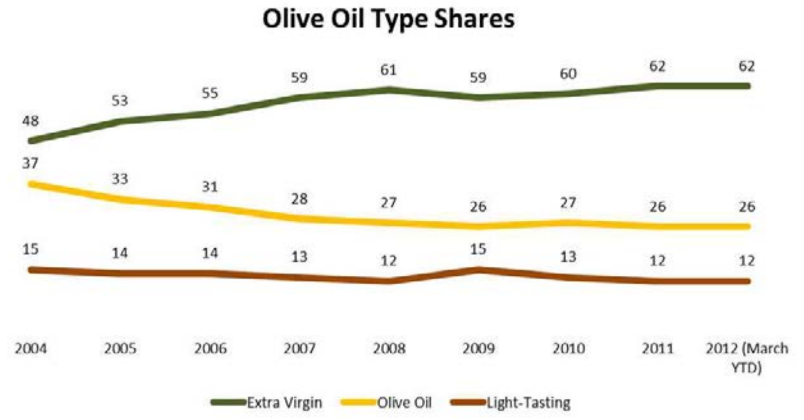


TRENDS IN QUALITY/GRADES CATEGORY SHARES ON WORLD MARKET

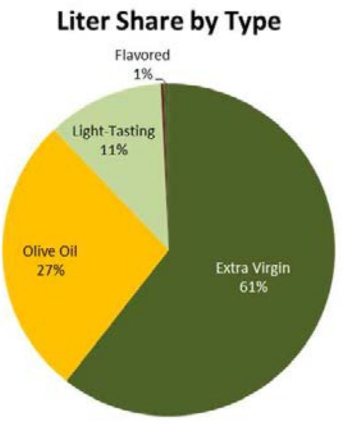
USA - OLIVE OIL IMPORTS BY CATEGORY (t)



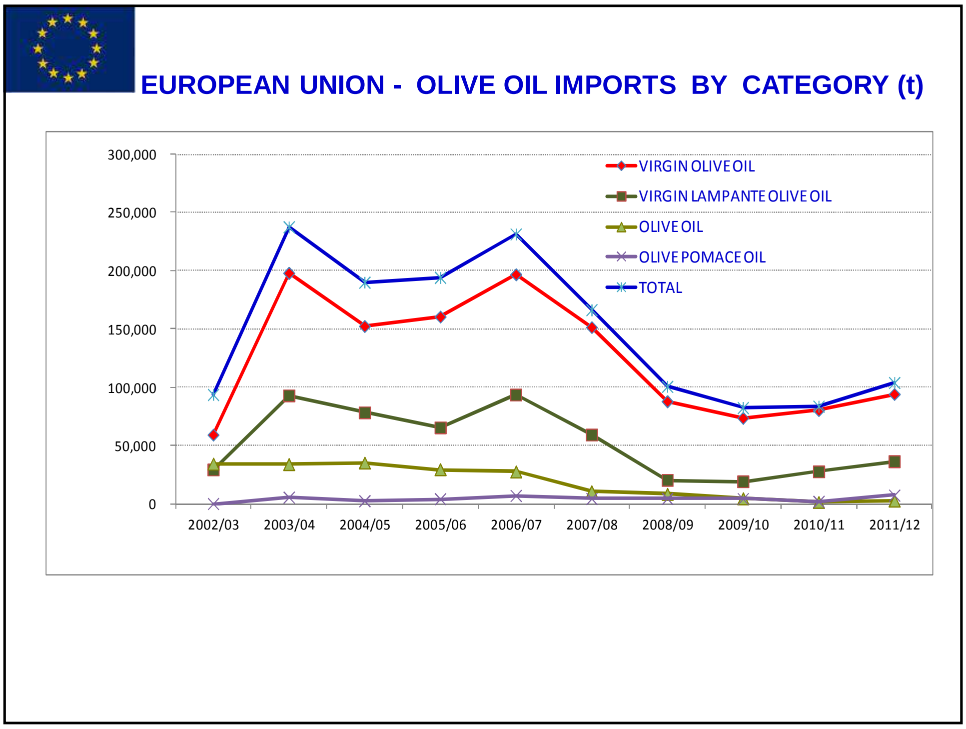
Olive Oil Type History



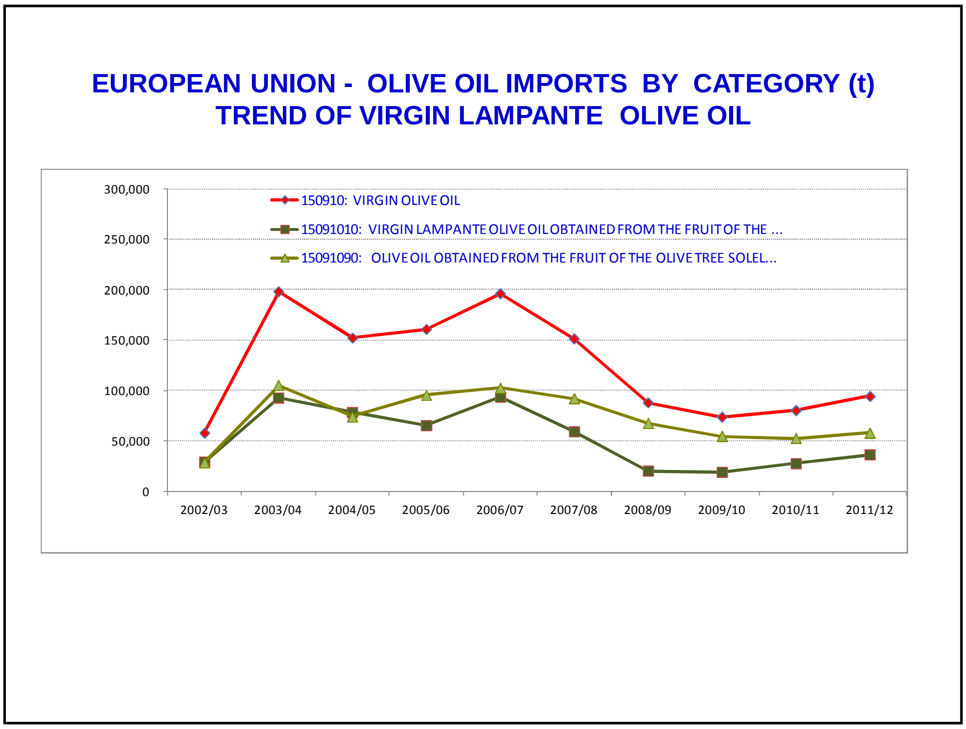
Total US Olive Oil Retail Market



Source: Nielsen 52 Weeks Ending April 14, 2012

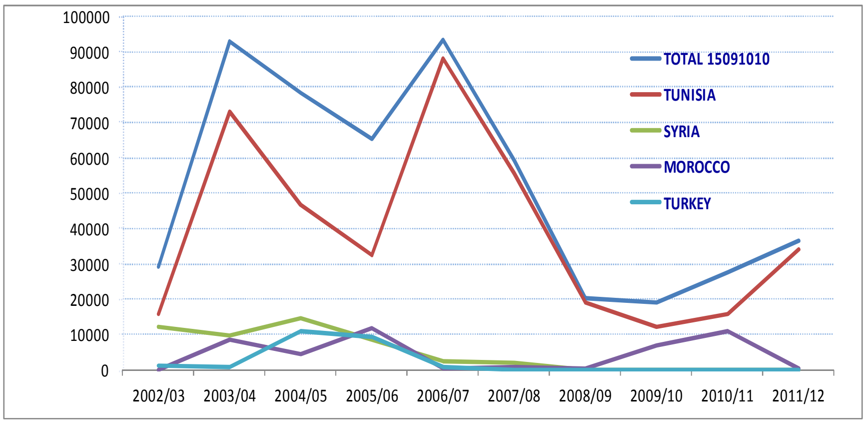


EUROPEAN UNION - OLIVE OIL IMPORTS BY CATEGORY (t)
300,000
250,000
200,000
150,000
100,000
50,000
0
2002/03
2003/04
2004/05
2005/06
2006/07
2007/08
2008/09
2009/10
2010/11
2011/12
VIRGIN OLIVE OIL
VIRGIN LAMPANTE OLIVE OIL
OLIVE OIL
OLIVE POMACE OIL
TOTAL



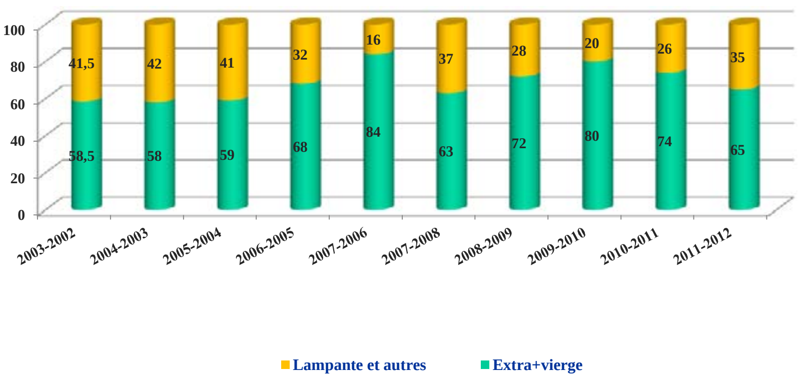
EUROPEAN UNION - OLIVE OIL IMPORTS BY CATEGORY (t)
TREND OF VIRGIN LAMPANTE OLIVE OIL
300,000
250,000
200,000
150,000
100,000
50,000
0
2002/03
2003/04
2004/05
2005/06
2006/07
2007/08
2008/09
2009/10
2010/11
2011/12
150910: VIRGIN OLIVE OIL
15091010: VIRGIN LAMPANTE OLIVE OIL OBTAINED FROM THE FRUIT OF THE ...
15091090: OLIVE OIL OBTAINED FROM THE FRUIT OF THE OLIVE TREE SOLEL...

EUROPEAN UNION - VIRGIN LAMPANTE OLIVE OIL IMPORTS BY COUNTRY (t)

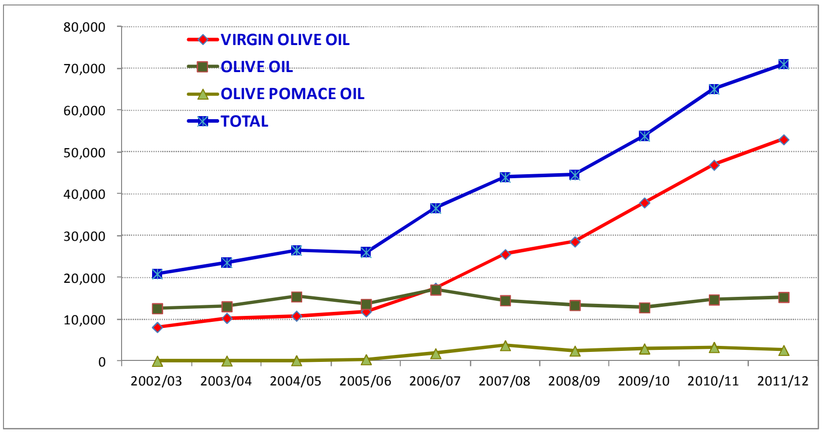


TUNISIE - Evolution de la part des huiles de qualités exportées durant les dernières années

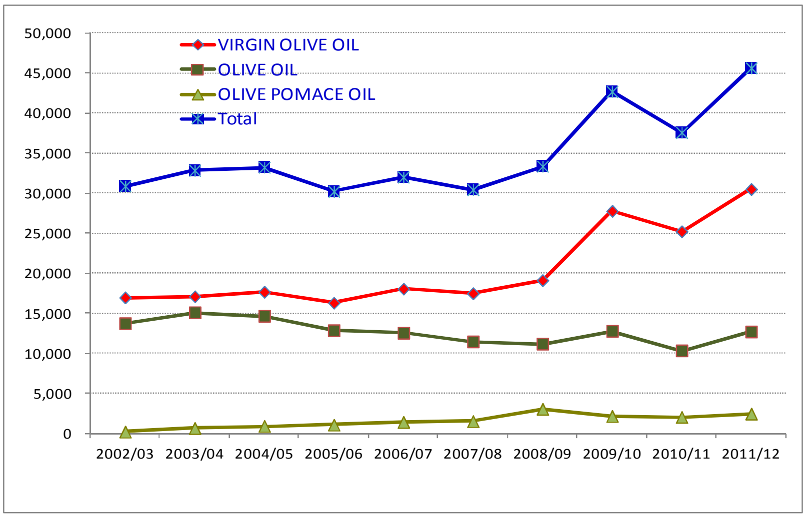
- ✓ **Evolution nette de la part des huiles de qualité exportées.**
- ✓ **La part de la lampante s'est réduite a environ 40 % durant les dernières années contre 70 % au début des années 80.**



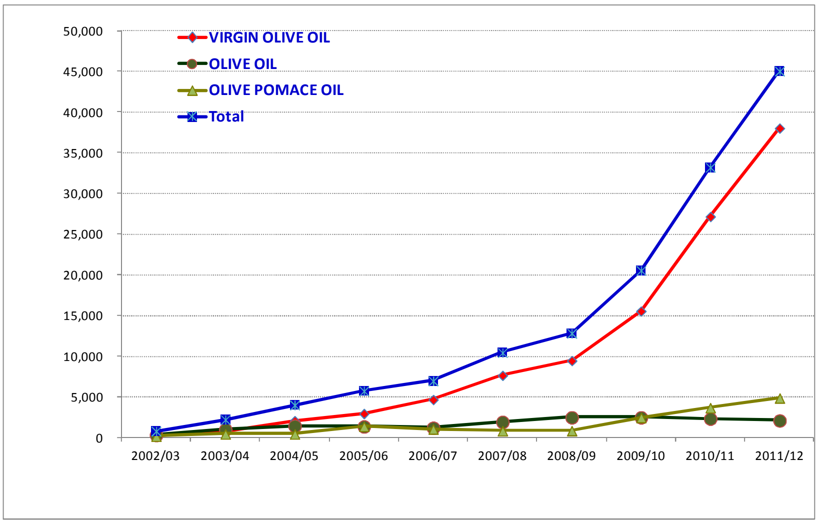
BRAZIL - OLIVE OIL IMPORTS BY CATEGORY (t)



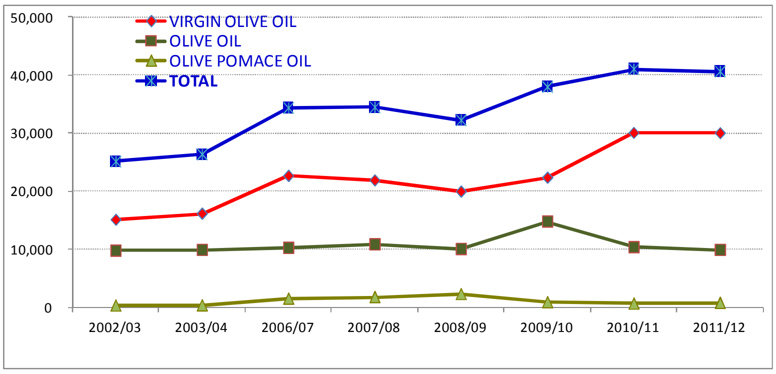
JAPAN- VIRGIN OLIVE OIL IMPORTS BY COUNTRY (t)

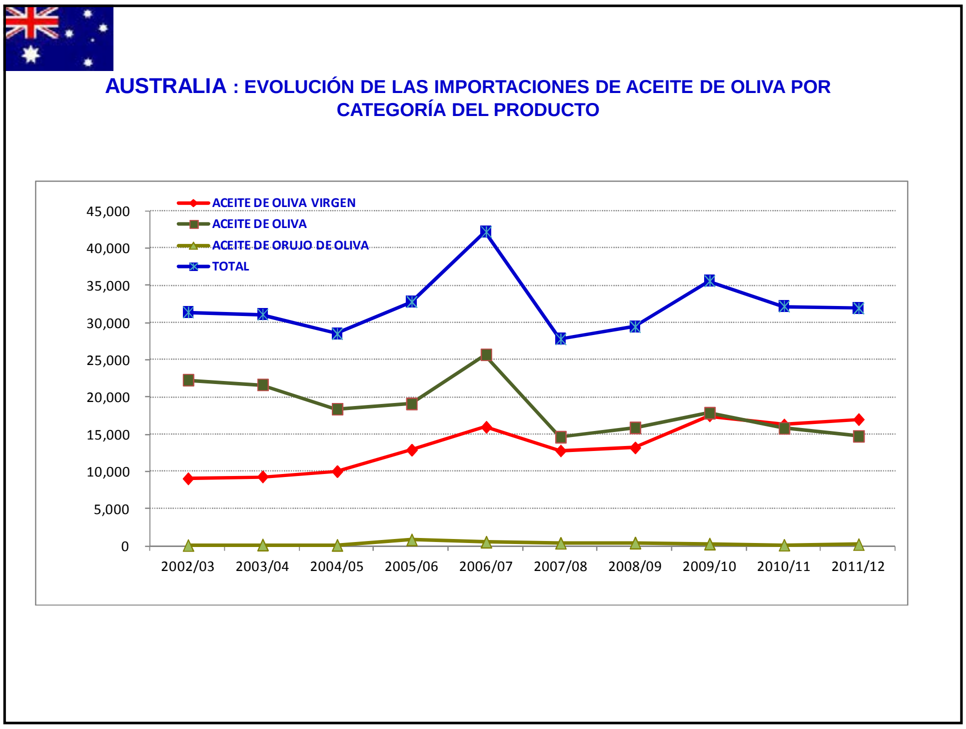


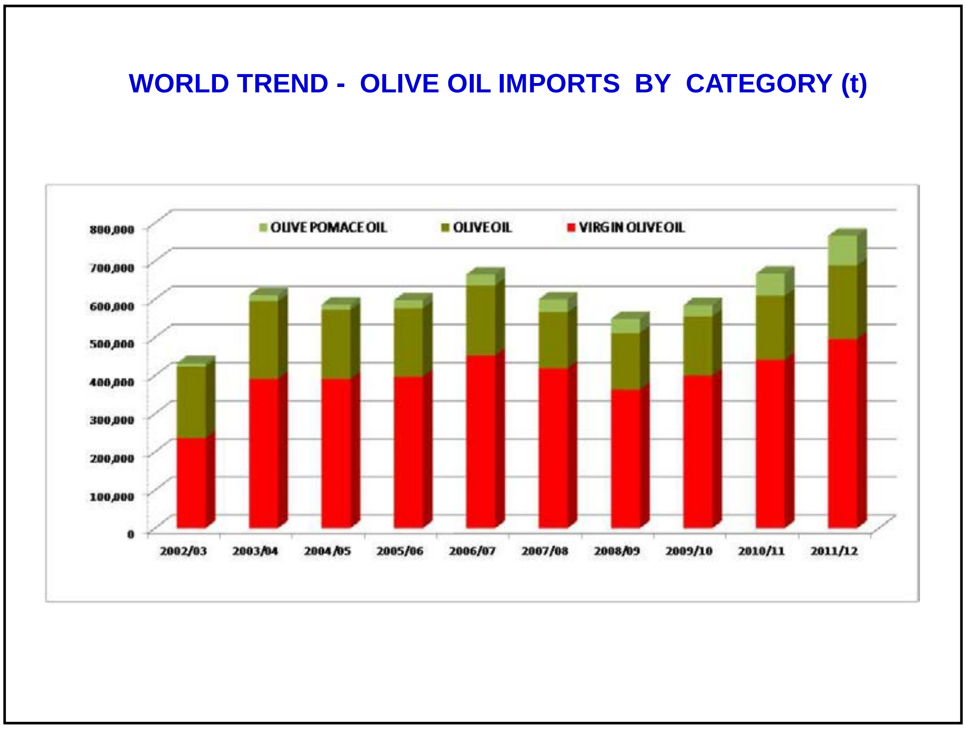
CHINA - VIRGIN OLIVE OIL IMPORTS BY COUNTRY (t)



CANADA - VIRGIN OLIVE OIL IMPORTS BY COUNTRY (t)




AUSTRALIA : EVOLUCIÓN DE LAS IMPORTACIONES DE ACEITE DE OLIVA POR CATEGORÍA DEL PRODUCTO
ACEITE DE OLIVA VIRGEN
ACEITE DE OLIVA
ACEITE DE ORUJO DE OLIVA
TOTAL
45,000
40,000
35,000
30,000
25,000
20,000
15,000
10,000
5,000
0
2002/03
2003/04
2004/05
2005/06
2006/07
2007/08
2008/09
2009/10
2010/11
2011/12


WORLD TREND - OLIVE OIL IMPORTS BY CATEGORY (t)
OLIVE POMACE OIL
OLIVE OIL
VIRGIN OLIVE OIL
800,000
700,000
600,000
500,000
400,000
300,000
200,000
100,000
0
2002/03
2003/04
2004/05
2005/06
2006/07
2007/08
2008/09
2009/10
2010/11
2011/12

CONCLUSION

- 9 different grades (IOC standard) are used by the 17 IOC members who account for 98% of world production
- 80-85% production used to be consumed locally (now 70-75%)
- 25-30% of world production goes for export, compared with 15-20% before
- World data are only available for headings15.09.10, 15.09.10 and 15.10
- 96% of world exports are by IOC counties (Italy, Spain, Tunisia, Portugal, Turkey, Syria, Argentina, Morocco) then Chile
- 80% world imports are by non IOC members (USA, Brazil, Japan, China, Canada, …) ➡ Standards ?
- 15.09.10 share is increasing, but EVOO or VOO ? ➡ analysis

02 - Trade standards for olive oil and olive-pomace oil in the world

By S. Valentin

Trade standards for olive oil and olive-pomace oil in the world

Sandrine Valentin

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Olive oil and olive-pomace oil trade standards aims to protect the consumer's health, to guarantee the quality of the olive oil and olive-pomace oil and ensure fair trade practices.

Many olive oils standards exist in the world. Some of them have been established by a single country and are therefore considered to be national standards (Australia/NZ, USA, Israel, Iran…). Others established by an international body (Codex Alimentarius, International Olive Council) are international standards. The European Union has put in place a mandatory standard[1] for olive oil and olive-pomace oil, based on the International Olive Council (IOC) standard. In addition, according to the agreement on technical barriers to trade reached in the framework of the World Trade Organization, countries are strongly encouraged to use the international standards that exist. The same principle applies for members of the IOC.

The basic format for almost all olive oil standards consists of categories of oils (description), purity criteria (chemical composition), quality criteria (including organoleptic characteristics), food additives, contaminants, hygiene, methods of analysis and sampling. For this last issue, references are made to internationally recognised testing methods provided by international bodies (ISO, IUPAC, CEN, AOCS…). The EU standard for olive oil and olive-pomace oil has no provisions related to food additives, contaminants or hygiene because these are already covered by the horizontal legislation. Moreover, the AUST/NZ standard introduced two additional parameters that can be considered as freshness parameters: pyropheophytin a and 1,2 diacyglycerols.

A comparison of five standards (IOC, EU, Codex, USA and AUST/NZ) shows some differences in the chemical parameters limits (oleic, linolenic acid, campesterol, stigmasterol, apparent β-sitosterol, stigmastadiene) and quality criteria (median of the defect). These differences may be explained by the spread of olive oil production beyond its historical home and the influence of geography on the development of olives varieties and products. As an example, AUST/NZ and US standards establish a limit for campesterol of respectively 4,5 and 4,8 which is much higher than the limit fixed by the IOC/EU/Codex standard (4,0).

All the provisions of the olive and olive-pomace oils standards are regularly revised to adapt to any change in the composition of the olive oil and/or variety and to technical and scientific progress.

References

Codex standard for olive oils and olive pomace oils (*CODEX STAN 33-1981).*
Commission Regulation (EEC) No 2568/91 of 11 July 1991 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis.
Commission implementing Regulation (EU) No 29/2012 of 13 January 2012 on marketing standards for olive oil.
Trade standard applying to olive oils and olive-pomace oils (IOC, last revised 2011).
Australian/New Zealand Standard, Olive oils and olive-pomace oils (2011, draft version).
United States Standards for grades of olive oil and olive-pomace oil (2010).

[1] Regulation (EEC) N°2568/1991 & Regulation (EU) N°29/2012

Trade standards for olive oil and olive-pomace oil in the world

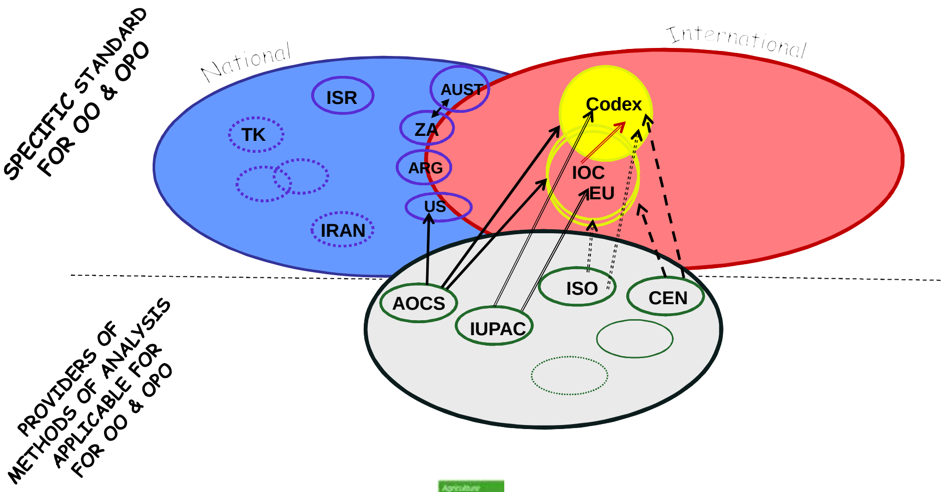
Workshop on olive oil authentication
Madrid, 10-11 June 2013

Sandrine Valentin
European Commission – Agriculture and rural development

Why a standard for olive oil and olive-pomace oil?

- To protect the consumer's health
- To avoid confusing and/or misleading labelling pratices
- To guarantee the quality of olive oil (OO) and olive-pomace oil (OPO)
- To ensure fair trade pratices

A single olive oil and olive-pomace oil standard?



How do they work?

- **"Voluntary"** basis:
  Codex
  IOC
  US
  AUST
  ...

  The most widely used

- **"Compulsory"** basis:
  The EU regulation applies to produced and imported olive oil and olive-pomace oil

What does the standard of olive oil and olive-pomace oil contain?

1. Categories of olive oils (definitions)
2. Purity criteria
3. Quality criteria (including organoleptic characteristics)
4. Methods of analysis (+ sampling)
5. Food additives
6. Contaminants
7. Hygiene
8. Labelling & packaging

IOC, EU, Codex, US, AUST-NZ standard (I)

	IOC	EU	Codex	US	AUST-NZ
Designations/definitions/descriptions/ grades	*	*	*	*	*
Purity criteria/chemical composition	*	*	*	*	*
Quality criteria (including organoleptic characteristics)	*	*	*	*	* + PPP/DAG
Methods of analysis (incl. Sampling)	24	20	24	21	19 + 2

[1]Regulation (EEC) No 2568/1991

IOC, EU, Codex, US, AUST-NZ standard (II)

	IOC	EU	Codex	US	AUST-NZ
Food additives	*		*	no	*
Contaminants	*		*	*	*
Hygiene	*		*	no	*
Packaging	*		no	no	*
Labelling	*	*[1]	*	no	*
including "best before date"	*		no	no	*[2]

covered by the horizontal legislation

[1] Regulation (EU) No 29/2012

[2] not greater than 2 years from the date of packaging

Types/Categories of olive oil

U.S.

		IOC	Codex	EU	AUST/NZ	US
1	Extra virgin olive oil (EVOO)	*	*	*	*	*
2	Virgin olive oil (VOO)	*	*	*	*	*
3	Ordinary virgin olive oil	*	*	no	no	no
4	Lampante virgin olive oil	*	no	*	*	*
5	Refined olive oil	*	*	*	*	*
6	Olive oil (blend of refined olive oil & virgin olive oils)	*	*	*	*	*

Types/Categories of olive-pomace oil

		IOC	Codex	EU	AUST/NZ	US
7	Crude olive-pomace oil	*	no	*	*	*
8	Refined olive-pomace oil	*	*	*	*	*
9	Olive pomace oil (blend of refined olive-pomace oil & virgin olive oils)	*	*	*	*	*

Purity criteria (EVOO, VOO) – main differences

	EU/IOC	Codex	US	AUST/NZ
FAME + FAEE	75 mg/kg	no limit		
Oleic acid (C18:1)	55,0-83,0			53,0-85,0
Linolenic acid (C18:3)	≤ 1,0	no limit	≤ 1,5	
Campesterol	≤ 4,0		≤ 4,5	≤ 4,8
Stigmasterol	< campesterol			≤ 1,9
Apparent β-sitosterol	≥ 93,0			≥ 92,5
Stigmastadiene (mg/kg)	≤ 0,10	0,15	≤ 0,15	≤ 0,10

Quality criteria of EVOO & VOO

- Organoleptic characteristics (assessed by a Panel)

	IOC/EU/Codex/US/AUST/NZ	
	Median of the defect	Median of the fruity attribute
EVOO	Md = 0	Mf > 0
VOO	0 < Md ≤ 2,5 0 < Md ≤ 3,5 (IOC/EU)	Mf > 0

- Additional parameters in AUST/NZ standard for EVOO
  - Pyropheophytin a (PPP) ≤ 17%
  - 1,2 Diacylglycerols (DAGs) ≥ 35%

Conclusions

- Standards which characterize olive oil and olive-pomace oil are **numerous**
- ***A few differences*** between standards were observed
- Provisions of standards are **regularly revised** to adapt to any change in olive oil and to technical progress


European Commission
Thank you
for
your attention
Agriculture and Rural Development

03 - Olive oil authenticity: a Canadian perspective

by A. Sheridan

Olive oil Authenticity: A Canadian Perspective

Angela Sheridan, Maude Gunville-Vachon, Christine Gibeault

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Abstract:

In the mid 1990's, the Government of Canada adopted the International Olive Council (IOC) standards for olive oil composition and quality along with the prescribed methods of analysis. Since that time, the Canadian Food Inspection Agency's Ottawa Laboratory Carling has further refined its testing program by obtaining ISO guide 17025 accreditation, Approved Chemist status from the American Oil Chemist Society (AOCS) and the International Olive Council's recognition for proficiency in olive oil chemistry testing. Along with the improvements in the testing program, the Agency's integrated inspection surveillance program paid stricter attention to the olive oil market in response to allegations from Canadian importers that a large amount of fraudulent olive oil was being sold in Canada. Consequences for the sale of adulterated olive oil have included fines, removal from the market or re-labelling of the product. A summary of laboratory testing results will be presented to illustrate the occurrence of fraudulent olive oil in the Canadian market. Technical issues encountered by the laboratory testing program will also be discussed.

Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

Canadian Food Inspection Agency

Our vision:
To excel as a science-based regulator, trusted and respected by Canadians and the international community.

Our mission:
Dedicated to safeguarding food, animals and plants, which enhances the health and well-being of Canada's people, environment and economy.

Olive oil Authenticity: A Canadian Perspective

A. Sheridan, M. Gunville-Vachon, C. Gibeault
Canadian Food Inspection Agency
Ottawa, Canada

Canada

Food Fraud

- Gaining worldwide attention
  - Numerous news articles
  - Dr. Oz food fraud episodes
- United States Pharmacopeial Convention (USP) report
  - Outlined the top frequently adulterated foods
  - Top of the list: olive oil
  - Fraud up 60%

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Canada 2

Canadian Food Inspection Agency

- Safeguards food, animal and plants which enhances the well-being of Canada’s people, environment and economy
- Regulatory Agency
  - Enforces standards and regulations
- Key activities include
  - Fair labelling of products and consumer protection

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Legislative and Regulatory Base

- The *Food and Drugs Act (FDA*) is the primary legislation that applies to all food sold in Canada, whether produced domestically or imported.

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Food and Drug Act

- Key sections outlining requirements for the labelling of food:

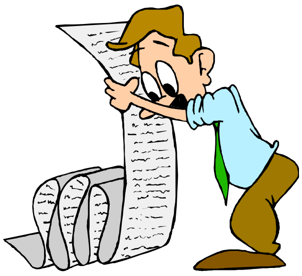
4. (1) No person shall sell an article of food that
(d) is adulterated

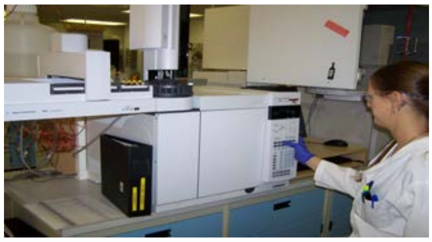
5. (1) No person shall label, package, treat, process, sell or advertise any food in a manner that is false, misleading or deceptive or is likely to create an erroneous impression regarding its character, value, quantity, composition, merit or safety

Activities

- CFIA undertakes activities to protect consumers against product misrepresentation and fraud
- National strategies, policies, and programs in support of consumer protection

Strategies





Food and Drug Regulation

- Outdated fatty acid composition
- No other detailed compositional data
- No provisions to distinguish between virgin and refined oils

- International Olive Council (IOC) standard adopted to augment the Canadian regulations

- Olive oil industry
  - Letter campaign and website posting

Ottawa Laboratory (Carling)

- ISO 17025
- Recognized by IOC proficiency testing program for chemical analysis of olive oil
- Approved Chemist by AOCS for the Gas Chromatography Series
- Participate in the SSOG ring tests

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Laboratory Program

- Fatty acid composition
- Trans fatty acid content
- Sterol composition (including total sterol content)
- Erythrodiol and uvaol content
- Wax content
- ECN42 difference
- Stigmastadiene
- 2-glyceryl monopalmitate
- Aliphatic alcohol
- Free fatty acidity
- Peroxide value
- Absorbency in ultra-violet
- Fatty acid methyl esters (FAMEs) and Fatty acid ethyl esters (FAEEs)

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Assessment

- Every analysis includes controls (when possible) in order to ensure instrumentation and method are performing
- All the results are examined together to make an overall assessment
- Samples assessed as “Unsatisfactory” have a high certainty of adulteration

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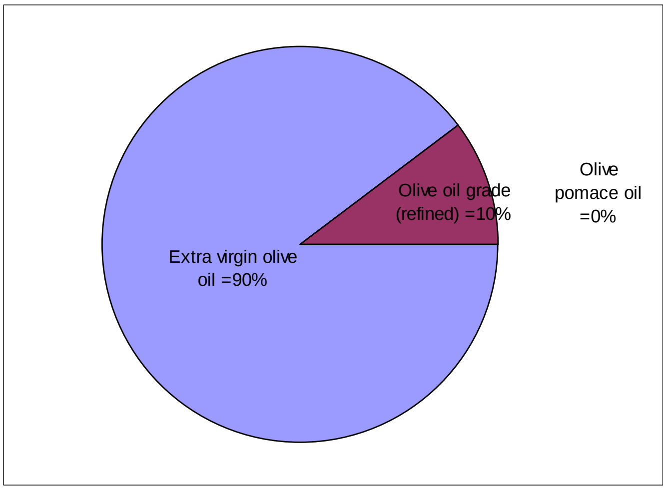
Type of oil analysed (2006-2012)



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Canada

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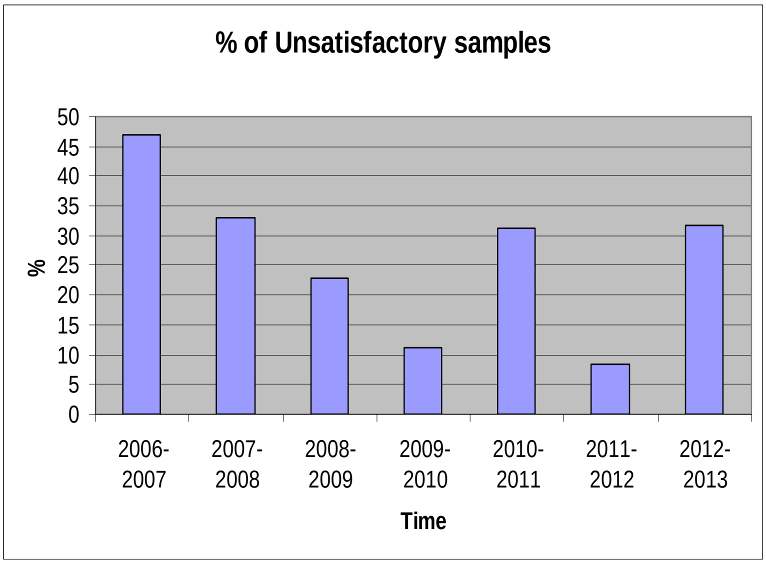

	>IOC limit (%)				
Methods	**2008-2009**	**2009-2010**	**2010-2011**	**2011-2012**	**2012-2013**
K232	**60**	**25**	**50**	**100**	**50**
K268	**60**	**25**	**70**	**100**	**29**
delta K	**30**	**13**	**20**	**100**	**7**
FFA	**25**	**38**	**50**	**0**	**0**
Fatty acid profile	**50**	**13**	**10**	**0**	**21**
MUFA	**40**	**13**	**20**	**100**	**21**
PUFA	**60**	**25**	**10**	**100**	**29**
Stig	**90**	**38**	**40**	**100**	**50**
Sterols	**75**	**75**	**20**	**100**	**36**
Alkyl esters	**n/a**	**n/a**	**n/a**	**n/a**	**36**

Common adulterants found

- Vegetable oil
  - Canola
  - Sunflower oil
  - Soybean oil
- Refined olive oil
- Olive pomace oil

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Non-compliance Consequences

- Parties may be prosecuted
- Voluntary removal of product
  - Product may be re-labelled
  - Product may be returned to exporter
- Product may be seized and/or put under detention

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Statistics

Since 2007:

- $250K in fines
- > $500K worth of oil was ordered disposed
- Up to 3 year probation
  - Cannot be involved in any type of food related import and export, manufacture or sale

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Available tools

- Detailed standard
- Variety of established methods to be used to detect adulteration
- Proficiency testing availability

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Technical issues

- Methods of analysis (e.g. Sterols)
  - Length of analysis
  - Cost
- Training
  - Familiarization with the methods can take time

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Recommendations/Conclusions

- Successful olive oil program
  - Constant surveillance
  - Dedicated project
- Future
  - Faster, shorter methods of analysis
  - Application and availability of newer methods

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Acknowledgements

- Staff in the Food Chemistry Section at the Ottawa Laboratory Carling

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Canada

04 - Frauds in olive oil Sector in Spain

by J.R. Izquierdo

FRAUDS IN OLIVE OIL SECTOR IN SPAIN

Juan Ramón Izquierdo

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Inspection of frauds in the Kingdom of Spain was transferred to the Regional Governments and these are responsible for the control performed, with the Ministry of Agriculture, Food and Environment in charge of the coordination between the different services.

Official Control is regulated by the Spanish Royal Decree 1945/1983 of 22 June, regulating the infractions and penalties relating to consumer protection and agro-food, which are all typified in it as well as the embodiment, from the administrative point of view, of the whole process.

Obviously all the measures that are reflected in the **COMMISSION REGULATION (EEC) No 2568/91 of 11 July 1991 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis** are applied with its all subsequent amendments in regard to certain procedures related to the organoleptic assessment of virgin olive oils, the sampling procedures if lots and analytical methods to be used by the official control laboratories. These are appointed by the Regional Governments and also there is a working group of experts in analytical methods of Oils and Fats in the Ministry of Agriculture, Food and Environment, which is used to coordinate the official laboratories in all matters concerning the technical issues.

According to data supplied by the inspection services of the Regional Governments, in 2012 there were a total of 770 inspections of which approximately 23% were non compliant. The violations found are related in 47.5% with quality and purity, 32.7% in labelling the products, by 4% to traceability and 15.7% of others than those listed above. Those regarding quality are basically related to the organoleptic quality of virgin olive oils. Basically they consist of packing lower quality oils as EXTRA VIRGIN oils, but also quality LAMPANTE oils were found.

Also, as noted, defects have been found in the product labelling, mainly due to the misuse of legal designations listed in the **COMMISSION IMPLEMENTING REGULATION (EU) No 29/2012 of 13 January 2012 on marketing standards for olive oil**, primarily focused on olive oil composed of refined olive oils and virgin olive oils, since this last underlined sentence is not used by the packer on many occasions.

In regard to testing procedures, official laboratories use the **COMMISSION REGULATION (EEC) No 2568/91** methods indicated by its annexes and in which they are accredited according to **ISO/IEC 17025 General requirements for the competence of testing and calibration laboratories**, as indicated by the **REGULATION (EC) No 882/2004 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 29 April 2004** in its Article 12.

Basically it is considered that all these methods are sufficient for detecting the more common frauds in olive oil purity. There are other types of frauds, including the use of deodorized oils for which the current methodology seems to be insufficient.

OFFICIAL CONTROL IN THE OLIVE OIL SECTOR IN SPAIN

Juan Ramón Izquierdo
Ministry for the Agriculture, Food and Environment Affairs

2012		
PRODUCTS	INSPECCTIONS	INSPECCTIONS WITH VIOLATIONS
Extra virgin olive oil	351	85
Virgin olive oil	296	67
Olive oil composed...	75	21
Olive pomace oil	48	9

2012				
PRODUCTS	VIOLATIONS DETECTED			
	Quality & purity	Labeling	Treazability	Others
Extra virgin olive oil	56	31	5	12
Virgin olive oil	38	33	2	16
Olive oil composed...	6	7	1	4
Olive pomace oils	6	2	1	3

INTERNATIONAL OLIVE COUNCIL

COI/T.15/NC No 3/Rev. 6
November 2011

ENGLISH
Original: FRENCH

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TRADE STANDARD APPLYING TO OLIVE OILS AND OLIVE-POMACE OILS

2. DESIGNATIONS AND DEFINITIONS

2.1. **Olive oil** is the oil obtained solely from the fruit of the olive tree *(Olea europaea* L.), to the exclusion of oils obtained using solvents or re-esterification processes and of any mixture with oils of other kinds. It is marketed in accordance with the following designations and definitions:

2.1.1. Virgin olive oils are the oils obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions, particularly thermal conditions, that do not lead to alterations in the oil, and which have not undergone any treatment other than washing, decantation, centrifugation and filtration.

2.1.2. Refined olive oil is the olive oil obtained from virgin olive oils by refining methods which do not lead to alterations in the initial glyceridic structure. It has a free acidity, expressed as oleic acid, of not more than 0.3 grams per 100 grams and its other characteristics correspond to those fixed for this category in this standard. [2/]

2.1.3. Olive oil is the oil consisting of a blend of refined olive oil and virgin olive oils fit for consumption as they are. It has a free acidity, expressed as oleic acid, of not more than 1 gram per 100 grams and its other characteristics correspond to those fixed for this category in this standard.[3/]

[2/] This designation may only be sold direct to the consumer if permitted in the country of retail sale.

[3/] The country of retail sale may require a more specific designation.

COUNCIL REGULATION (EC) No 1234/2007

of 22 October 2007

establishing a common organisation of agricultural markets and on specific provisions for certain agricultural products (Single CMO Regulation)

1. VIRGIN OLIVE OILS

Oils obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions that do not lead to alterations in the oil, which have not undergone any treatment other than washing, decantation, centrifugation or filtration, to the exclusion of oils obtained using solvents or using adjuvants having a chemical or biochemical action, or by re-esterification process and any mixture with oils of other kinds.

Virgin olive oils are exclusively classified and described as follows:

(a) *Extra virgin olive oil*

Virgin olive oil having a maximum free acidity, in terms of oleic acid, of 0,8 g per 100 g, the other characteristics of which comply with those laid down for this category.

(b) *Virgin olive oil*

Virgin olive oil having a maximum free acidity, in terms of oleic acid, of 2 g per 100 g, the other characteristics of which comply with those laid down for this category.

(c) *Lampante olive oil*

Virgin olive oil having a free acidity, in terms of oleic acid, of more than 2 g per 100 g, and/or the other characteristics of which comply with those laid down for this category.

2. REFINED OLIVE OIL

Olive oil obtained by refining virgin olive oil, having a free acidity content expressed as oleic acid, of not more than 0,3 g per 100 g, and the other characteristics of which comply with those laid down for this category.

3. OLIVE OIL — COMPOSED OF REFINED OLIVE OILS AND VIRGIN OLIVE OILS

Olive oil obtained by blending refined olive oil and virgin olive oil other than lampante olive oil, having a free acidity content expressed as oleic acid, of not more than 1 g per 100 g, and the other characteristics of which comply with those laid down for this category.

05 - Rapid assessment of quality parameters in olive oil using FTNIR Olive oil authenticity: a Canadian perspective and conventional standard methods

by C. Gertz

Rapid Assessment of Quality Parameters in Olive Oil using FTNIR and Conventional Standard Method

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Olive oil has a high price in comparison to other native vegetable oils. Therefore you find often adulterated olive oils and olive oils with a bad taste on the market. The international olive oil council (IOC) prescribes in Europe various methods and standards to define different olive oil qualities. The different categories of these official and trade standards are classified by a number of physico-chemical parameters and organoleptic characteristics. For each grade, minimum and/or maximum limits for most analytical parameters are prescribed in addition to a sensory test which has to be executed by a trained group of tasters. Sensory analysis alone may need to be repeated if faults are detected. It is however, easy to adulterate olive with low-grade olive oils or foreign oils such that physical and chemical properties still fall within the limits of the European standard of olive oils.

Quick objective chemical indices that correlate with sensory characteristics are needed to help to properly qualify and authenticate the world's olive oils.

Extra virgin olive oils from different countries, at different level age and qualities were checked by two officially assigned sensory panel. Simultaneously analytical parameters relevant for the quality such as fatty acid and TAG-composition, peroxide value, free fatty acid content, K-Values, pyropheophytin a and 1,2-diglyceride ratio have been determined. High calibration accuracy was obtained for the NIR determination of all these analytical parameters including the organoleptic tests. The NIR techniques have been also applied in combination isotope analysis to develop a statistical system to detect the geographical origin of olive oils. From these results, it is concluded that it is possible to design a simple and quick quality control system, which uses near-infrared technology.

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RAPID ASSESSMENT OF QUALITY PARAMETERS IN OLIVE OIL USING FT-NIR AND CONVENTIONAL STANDARD METHODS

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HAGEN (GERMANY)

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QUALITY OF OLIVE OIL

- **Organoleptic properties**
  - Flavor, Balance
  - Defects (**Age**, Oxidation, Harvesting)
- **Orgin of the product (PDO, Country)**
- **Identity – Adulteration -Traceability**
- **Agronomic practices (organic, conventional)**
- **Absence of contaminants (PAHs, Pestides)**
- **Nutrional Value**

OTHER METHODS TO CHECK THE QUALITY OF VIRGIN OLIVE OILS

Determination of 1.2 di- and 1.3-diacylglycerol
(DGF-Standard Method C-VI 16(06) (ISO 29822:2009)

Determination of degradation products of chlorophyll A in virgin olive oils
(DGF Standard Method C-VI 15(06) (ISO 29841:2009)

OFFICIAL ANALYTICAL METHODS TO VERIFY QUALITY AND AUTHENTICITY OF OLIVE OIL

Quality

- **Panel Test**
- Free Fatty acids
- K232, K270
- Peroxid Value

Authenticity testing

- Fatty acid profile
- Sterol composition
- Trans fatty acids
- Wax content
- 2-position palmitic acid
- Stigmastidiene content
- Equivalent carbon number (ECN)

HISTORY OF 1,2- AND 1,3-DIACYLGLYCEROLS

- **Growth**
  Intermediate formation of 1,2-**Diacylglycerols** during biosynthesis of triacylglycerols (Acyl-Transferase)

- **Period after harvesting until pressing:**
  Enzymatic lipolysis of triglycerols forming 1,3-Di-Glycerols and FFA catalysed by temperature

  Ratio 1,2 DiG /1,3 DiG decreases as a function of time and temperature

- **Storage after filtration (production):**
  Stop of Lipolysis

  Ratio 1,2 DiG /1,3 DiG decreases only slowly as a function of ffa and time

Note: FFA content changed only with 0,02 % per month during storage time after filtration

Christian Gertz-Olive OIl - Madrid 10-11 June 2013

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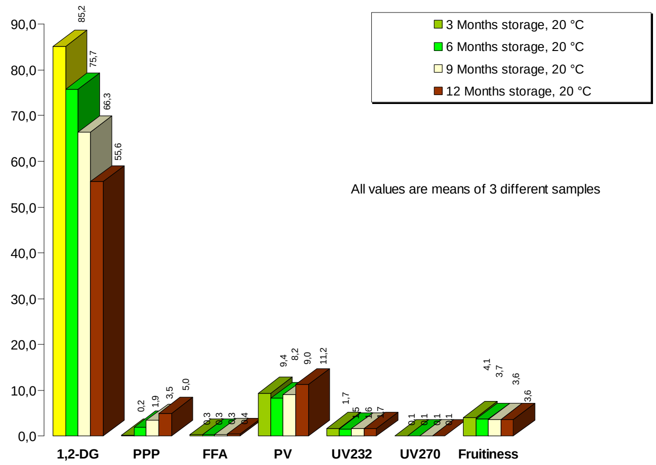
EVOLUTION OF SOME ANALYTICAL CRITERIA DURING STORAGE AT 20 °C



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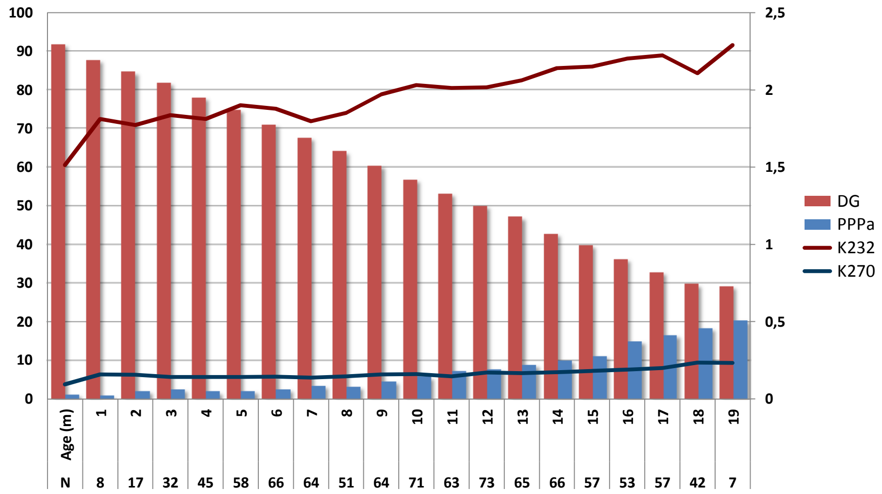
STATISTICAL EVALUATION OF 960 OLIVE OILS IN GERMANY (2005-2010)



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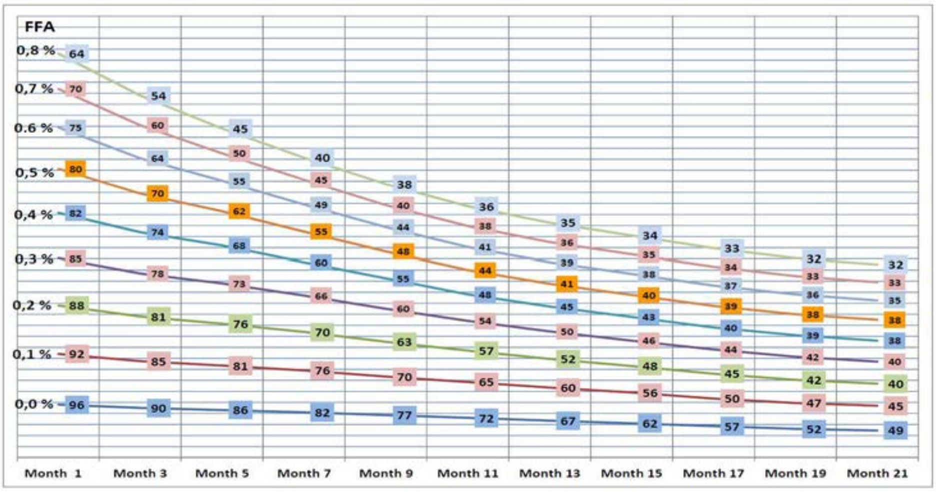
EVOLUTION OF 1,2 DG IN OLIVE OILS WITH DIFFERENT FFA CONTENT

(DATA SOURCE: C.GUILLAUME, CH.GERTZ,L.RAVETTI,2013 IN PRESS) .



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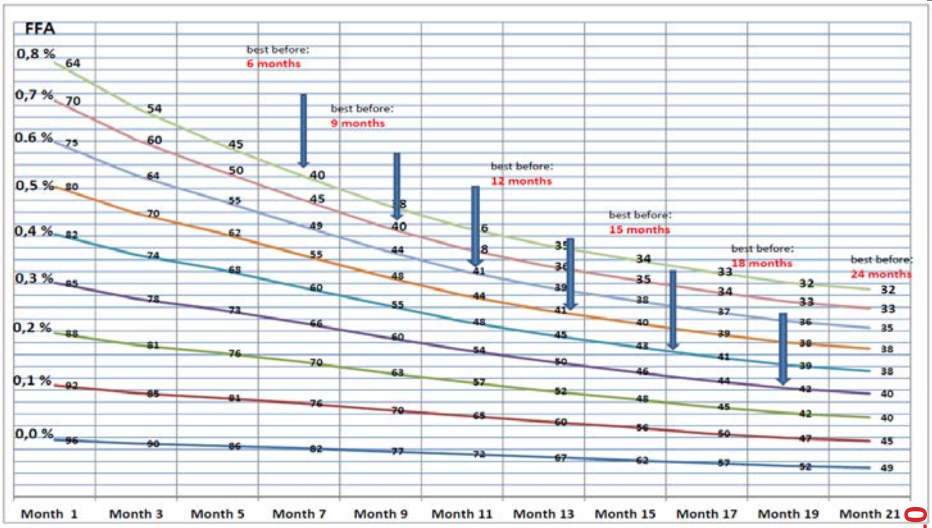
RELIABILITY OF SOME ANALYTICAL CRITERIA TO DETECT LOW QUALITY OLIVE OIL

(CALCULATED BY ROC-STATISTIC)

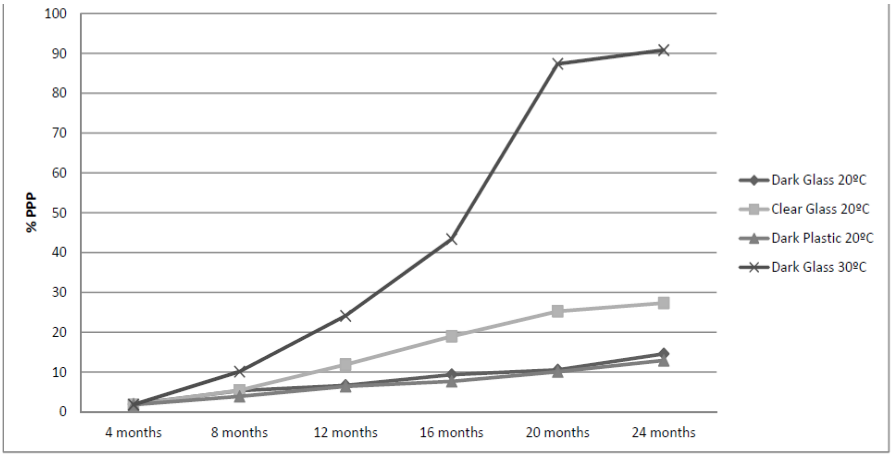
	Probability (80 %)	Probability (90 %)	Legal Limit /Proposed Limit
1,2-Diglycerides	<40,9 %	<35,1 %	<35,0 %/ 37,0 %
Pyropheophytin	>28,2 %	>33,5	>19 %
K232	>3,837	>4,284	>2,5
K270	>0,284	>0,316	>0,22
Peroxid Value	>41,079	>49,1	>20
FFA	>0,616	>0,715	>0,8 %

ESTIMATING THE EXPIRATION DATE



EVOLUTION OF PPP ACCORDING DIFFERENT STORAGE CONDITIONS

(C.GUILLAUME, CH.GERTZ, L.RAVETTI, 2013 IN PRESS)



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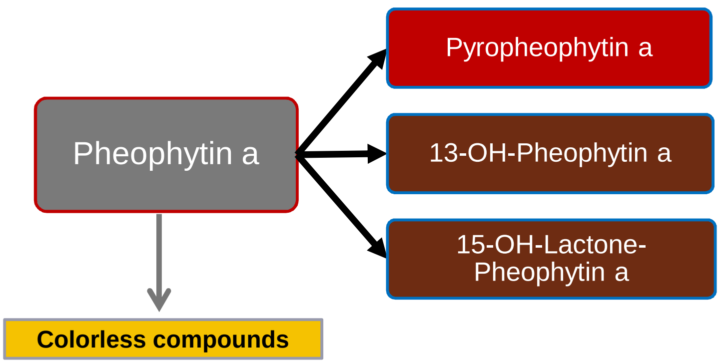
DEGRADATION OF CHLOROPHYLL



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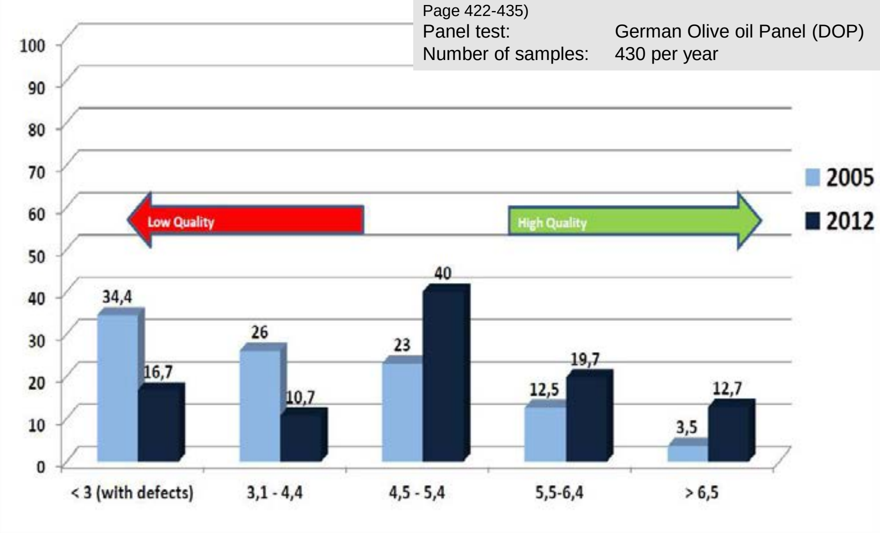
Development of the sensory quality of olive oil since 2005 on the German Market

Quality factor: Harmony(=balance)
(see A.Bongartz, D-G-Oberg (2011) J Agr Sci and Technol A1 Page 422-435)
Panel test: German Olive oil Panel (DOP)
Number of samples: 430 per year



SELECTED ANALYTICAL CRITERIA

- **Sensory Properties**
- **Quality of Harvesting and treatment before pressing (GMP)**
  - Free fatty acids (Titration)
  - 1,2-Diglycerides (GC)
- **Age of the Oil**
  - 1,2-Diglycerides (GC)
  - Pyropheophytin (HPLC)
  - K232 (Spectrometry)
- **Oxidation**
  - K232 (Spectrometry)
  - Peroxid Value (Titration)
- **Adulteration (Refining)**
  - K270 (Spectrometry)
  - Pyropheophytine (HPLC)
- **Authenticity (Origin, Identity)**
  - Fatty acid composition (GC)
  - Triacylglycerol Composition (GC)

BENEFITS OF NIR SPECTROSCOPY

Very fast analysis method (< 30 seconds)

No use of chemicals, solvents or gases

Untrained staff can carry out analyses

Operator independent results

Applicable in the production area

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FT-NIR MEASUREMENT OF OILS

- Sample preparation:
  fill in 8mm disposable vials
- Temperature control at 50^{o}C
- Measurement time: 20 sec
- Display of results on the screen, as PDF or print out

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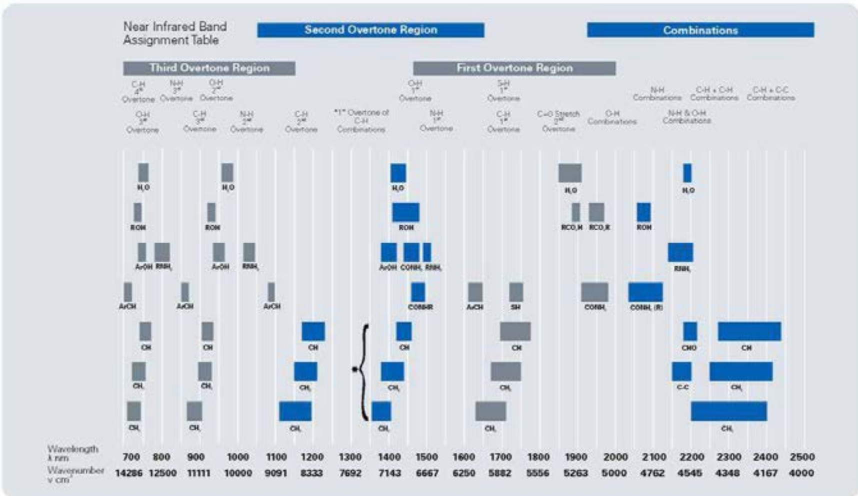
NIR ASSIGNMENT TABLE
(SOURCE: BRUKER OPTICS)



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LIMITS OF PREVIOUS STUDIES USING NIR

- **Small number of samples covering a low variability concerning**
  - **Origin**
  - **Cultivar of olives**
  - **Age of samples**
  - **Sensory qualities or defects**

Larger data sets with more variation required

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Samples: Olive Oils (N=1635) from 20 different countries (70 varieties)

Australia	21	Italy (without indicated region	319	Spain (without indicated region	181
California	12	Italy Abruzzo	20	Spain Andalusia	50
Chile	6	Italy Apulia	62	Spain Badajoz	6
Cypre	6	Italy Calabria	9	Spain Catalonia	10
France	50	Italy Campania	14	Spain Cordoba	45
Greece (without indicated region)	110	Italy Lake Garda	8	Spain Mallorca	10
Greece Crete	70	Italy Latium	2	Spain Rioja/Aragon	25
Greece Crete Chania	8	Italy Le Marche	25	Tunesia	15
Greece Crete Heraclion	10	Italy Liguria	23	Turkey	50
Greece Crete Sitia	60	Italy Sicilia	145		
Greece Kalamata	30	Italy Toscany	98		
Greece Lakonia	12	Italy Toskany Chianti	13		
Greece Lesbos	10	Italy Umbria	23		
Greece Messini	5	Italy Venetia	5		
Greece Peloponnes	19	North Africa /Joradan	6		
Greece Thassos	3	Portugal	26		
Istria (Crotia/Slovenia)	10	South Africa	3		

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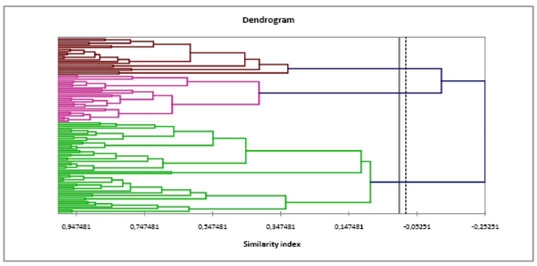
DENDROGRAMS OF AGGLOMERATIVE CLUSTERING TO FIND THE BEST VARIABLES TO DIFFERENCIATE THE ORIGIN OF OLIVE OILS

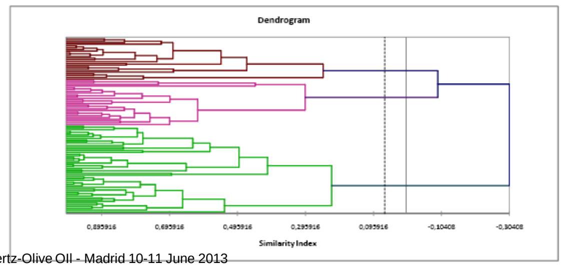
Note: To detect foreign oils:

C16:0
C18:1
C18:2

POP
PLP
PLO
OOO
OLL
LLL



C16
C16:1
C18:1
C18:1 (11c)
C18:2
POP
POO
PLO
OLO



C16
C16:1
C18:1
C18:1 (11c)
C18:2
POP
PLP
POO
PLO
OOO
OLO
OLL

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DISCRIMINANT ANALYSIS OF OLIVE OIL

(ANALYSIS OF ISOTOPES: M.BONER, AGROISOLAB, JÜLICH GERMANY)

Discriminant analysis-Confusion matrix — Number of Varieties: 70 — Number of Countries: 17 — Number of Samples: 349

from/To	Australia	Chile	Croatia	France	Greece	Israel	Italy	Maroc	Portugal	Slovenia	Spain	Syre	Tunesien	Turkey	USA	Uruguay	Total	% well classified	13C/12C [‰]	% well classified	
Australia	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	100	D/H [‰]	100	
Chile	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	100	18O/16O [‰]	66,7	
Croatia	0	0	6	0	0	0	2	0	0	0	0	0	0	0	0	0	8	75	K232	75	K232
France	0	1	0	10	0	0	0	0	0	0	1	0	0	0	0	0	12	83,3	C16:0	83,3	C16:0
Greece	0	2	0	0	41	0	3	0	0	0	0	0	0	0	0	0	46	89,2	C16:1	60,1	C16:1
Israel	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	6	100	C18:0	66,6	C18:0
Italy	0	0	0	0	6	1	107	0	3	2	1	0	0	0	0	0	120	89,2	C18:1 (9c)	77,5	C18:1 (9c)
Maroc	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	2	100	C18:1 (11c)	100	C18:1 (11c)
Portugal	0	0	0	0	0	0	0	0	28	0	7	0	0	0	0	0	35	80	C18:2	74,3	C18:2
Slovenia	0	0	0	0	0	0	1	0	0	3	0	0	0	0	0	0	4	75	C18:3	25	C18:3
Spain	0	3	0	0	1	0	4	0	3	0	76	0	0	0	0	0	87	87,4	PLO	78,1	PLO
Syre	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	2	100	PLP	100	PLP
Tunesien	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2	100	POO	100	POO
Turkey	0	0	0	0	1	0	0	0	1	0	0	0	0	6	0	0	8	75	OOO	75	OOO
USA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	10	100	OLO	50	OLO
Uruguay	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	100	OLL	100	OLL
Sum	2	9	6	10	49	7	117	2	35	5	85	2	2	6	10	2	349	87,68%	LLL	74,21%	LLL

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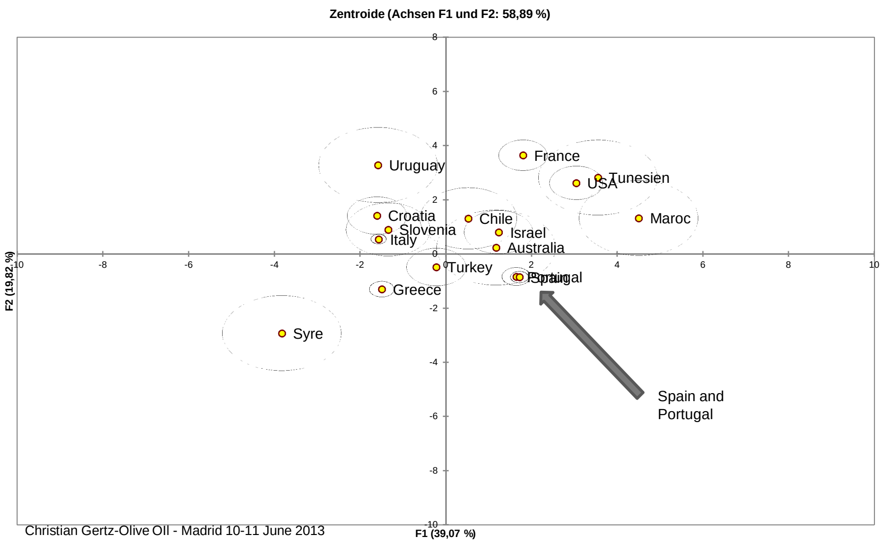
DISCRIMINANT ANALYSIS OF 349 OLIVE OILS FROM 17 COUNTRIES



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NIR REPORT
OLIVE OIL REFINED - POMACE OIL

Olive Oil refined (150°C,30 min)

Name	Wert	
Fruityness	4.2	✓
Bitterness	1.6	✓
FFA	0.33 %	✓
POZ	10.36 %	✓
AV	4.57	✓
C16:0	14.1 %	✓
C16-1	1.7 %	✓
C18:0	2.2 %	✓
C18-1 9c	57.3 %	✓
C18:1 11c	2.9 %	✓
C18-2	19.1 %	✓
C18-3	0.63 %	✓
POP	6.7 %	✓
POO	25.7 %	✓
PLP	3.9 %	✓
PLO	16.4 %	✓
OOO	25.1 %	✓
OLO	16.0 %	✓
OLL	6.1 %	✓
LLL	1.5 %	✓
IV	87.7	✓
K232	2.7104	✓
K270	0.21897	✓
1,2 Digly	32.51	✓
PPP	15.57	✓

Pomace Oil

Wert
-1.5
3.8
0.52 %
5.77 %
19.06
11.5 %
0.6 %
2.2 %
65.3 %
3.2 %
10.5 %
0.55 %
3.3 %
31.5 %
2.3 %
8.6 %
44.0 %
12.8 %
3.1 %
-1.2 %
80.9
3.3412
1.4433
21.35
31.799

MDI = 9.7	✗
MDI = 12.0	✗
MDI = 0.9	✓
MDI = 16.5	✗
MDI = 8.6	✗
MDI = 26.5	✗
MDI = 1.9	✗
MDI = 1.7	✗
MDI = 0.8	✓
MDI = 1.7	✗
MDI = 0.1	✓
MDI = 0.7	✓
MDI = 2.0	✗
MDI = 0.6	✓
MDI = 1.1	✗
MDI = 1.5	✗
MDI = 1.2	✗
MDI = 0.2	✓
MDI = 0.6	✓
MDI = 0.2	✓
MDI = 0.2	✓
MDI = 4.9	✗
MDI = 6.0	✗
MDI = 3.0	✗
MDI = 7.4	✗

NIR DATA –SENSORY – QUALITY

REFERENCE: GERMAN OLIVE OIL PANEL

Property	Data Set (Calibration)				Data Set (Validation with independent Oil)				Calibration Error	Prediction Error
Name	N	Min	Max	R^2	N	Min	Max	R^2	RMSEE	RMSEP
Fruitiness	262	0,9	5,2	73,4	221	3,2	5,1	63,8	0.28	0,20
Bitterness	219	0,9	3,7	71,6	203	1,6	3,5	60,9	0,23	0,22
Pungency	207	0,8	4,2	66,8	195	0,8	4,2	62,3	0,29	0,32
Harmony	228	3,8	7,1	76,7	148	3,8	6,8	61,5	0,34	0,41
FFA	174	0,1	8,9	99,8	168	0,1	7,6	99,8	0,1	0,1
1,2-Diglycerides (%)	284	29,6	96,5	96,5	111	30,1	95	98,4	2,2	2,2

NIR DATA: OXIDATION- THERMALLY TREATMENT - AGE

Property	Data Set (Calibration)				Data Set (Validation)				Calibration Error	Prediction Error
Name	N	Min	Max	R^2	N	Min	Max	R^2	RMSEE	RMSEP
Peroxid Value (meq O2/kg)	308	2,4	16,7	94,0	122	3,2	16,6	95,8	0,584	0,538
K232	133	1,554	3,516	92,1	95	1,562	2,415	90,3	0,088	0,063
K270	101	0,100	0,230	92,4	97	0,10	0,253	90,6	0,009	0,009
Anisidine Value	100	0,2	8,7	96,5	95	0,2	8,6	92,4	0,34	0,42
Pyropheophytin (%)	124	0	17,2	94,6	132	0	14,1	90,7	0,872	1,360
1,2-Diglycerides (%)	277	29,6	96,5	98,7	111	30,1	95	98,5	1,85	2,18

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NIR DATA: TRIACYLGLYCEROL IDENTITY

Property	Data Set (Calibration)				Data Set (Validation)				Calibration Error	Prediction Error
Name	N	Min	Max	R^2	N	Min	Max	R^2	RMSEE	RMSEP
POP	417	1,2	36,5	99,6	396	2,2	35,5	98,9	0,53	0,75
PLO	411	4,4	16,3	98,83	399	4,4	16,3	98,9	0,28	0,35
OLO	405	4	28,3	98,1	398	0	36,5	97,3	0,61	0,63
OOO	418	5,5	55,6	99,4	432	5,5	55,6	99,11	0,89	0,98
OLL	411	0	36,3	99,3	409	0	36,0	99,3	0,48	0,64
LLL	402	0	30,4	99,5	399	0	30,4	99,3	0,32	0,39

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ANALYTICAL METHODS TO DESCRIBE THE QUALITY OF OLIVE OILS

Method	Quality	AGE	Treatment	Identity	Geographical Origin
Fatty Acid Distribution (GC) (NIR)				X	X
Triacylglycerides (GC) (NIR)				X	X
FFA (Titrat) (NIR)	X	X			
K270 (Spectrometry) (NIR)			X		
K232 (Spectrom) (NIR)	X	X			X
1,2 Diglycerides (GC) (NIR)	X	X			
Pyropheophytin (HPLC) (NIR)		X	X		

CONCLUSION

NIR Spectroscopy :

- Is a quick easy cheap effective, simple and safe method
- It is a less sophisticated method of evaluating olive oil quality
- All relevant analytical criteria can be determine with a sufficient precision to describe the quality, the authenticity (including identity, orgin, and age)
- The determination of some isotopes helps to verifiy the determination of origin
- NIRS will become a dominant analytical tool for routine and real-time food safety and quality controls

THANK YOU !

Questions ?

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06 - Instrumental approaches to understand the sensory quality

by R. Aparicio

Instrumental Approaches To Understand The Sensory Quality Of Virgin Olive Oil.

Diego L. García-González and Ramón Aparicio

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The classification of olive oils into quality categories lays on the panellists' opinion -panel test is the only official method for quality assessment- , together with other chemical indexes that do not inform about aroma or taste (1). The alternative to these methods may come from the volatile analysis since there is a general agreement that these compounds are the responsible for virgin olive oil (VOO) aroma (2), and some researchers have pointed out some plausible relationships between sensory descriptors and volatile concentrations (3). However, the extensive knowledge on volatile and phenolic composition fails in reproducing the results of the panellists because the misinterpretation of the chemical results from a sensory standpoint, or *vice versa* (4). Recent researches have been carried out to save the gap between instrumental analysis and VOO sensory assessment by studying not only the etiology of the defects but also the chemical markers of VOO sensory defects together with their odour activity values, limits of detection and quantification, the synergy and masking effects between compounds among other aspects. The mapping of the brain response to the selected compounds in relation to the perception of sensory defects is giving support to these studies to join chemical and sensory information (5). The aim of developing new methodologies based on volatile compounds can be carried out by implementing an array of sensors - alone or in-tandem with gas chromatography (GC) (6) - or a method based on SPME-GC, which have resulted in the Defects Wheel® for Olive Oil (7).

References

(1) International Olive Council (IOC). (2011). *COI/T.15/NC No 3/Rev. 6.Trade standards applying to olive oils and olive-pomace oils*. Madrid, Spain.

(2) Harwood, J., & Aparicio, R. (2013). *Handbook of Olive Oil: Analysis and Properties* 2nd edition. New York: Springer.

(3) Aparicio, R., Morales, M.T., & García-González, D.L. (2012). Towards new analyses of aroma and volatiles to understand sensory perception of olive oil. *European Journal of Lipid Science and Technology*, 114, 1114-1125**.**

(4) García-González, D.L., & Aparicio, R. (2010). Research in olive oil: Challenges for near future. *Journal of Agricultural and Food Chemistry*, 58, 12569–12577.

(5) García-González, D.L., Vivancos, J., & Aparicio, R. (2011). Mapping brain activity induced by olfaction of virgin olive oil aroma. *Journal of Agricultural and Food Chemistry*, 59, 10200-10210.

(6) García-González, D.L., & Aparicio, R. (2010). Coupling MOS sensors and gas chromatography to interpret the sensor responses to complex food aroma: Application to virgin olive oil. *Food Chemistry*, 120, 572-579.

(7) The Defects Wheel® for Olive Oil, http://appliedsensory.com/Defects_Wheels.html

Workshop on

Authentication of Olive Oil

Instrumental Approaches To Understand The Sensory Quality Of Virgin Olive Oil.

CSIC
Spanish National Research Council

Diego L. García-González and Ramón Aparicio
dluisg@cica.es aparicio@cica.es
CSIC

IOC, Madrid, June/2013

Sensory Quality in Perspective: Stating the Problem

Objective aspects
(chemical compounds)

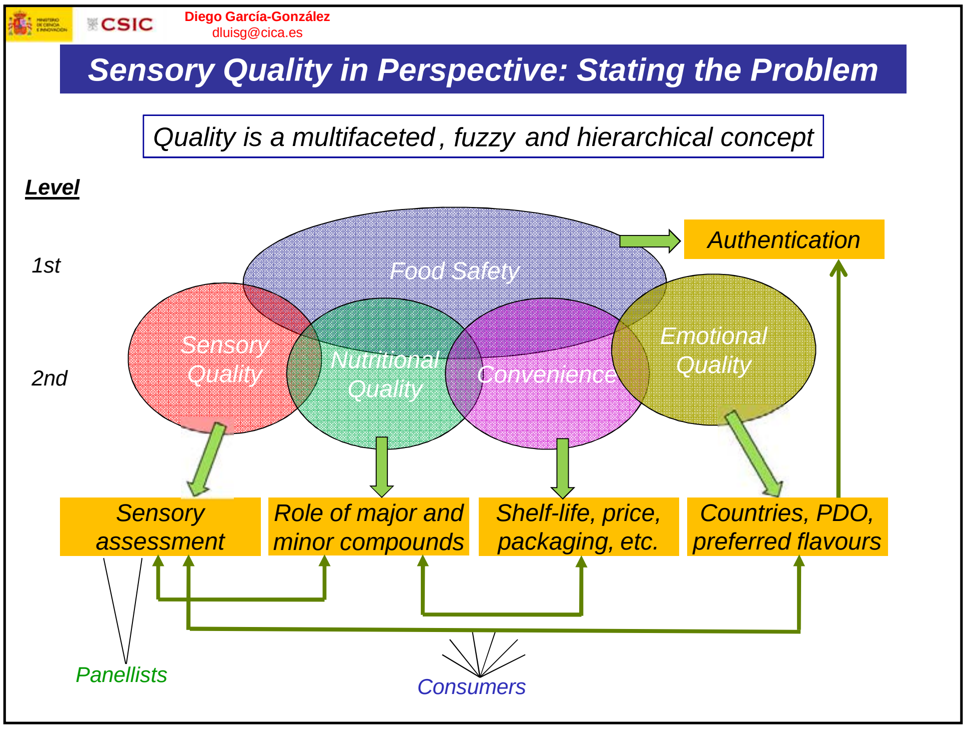
QUALITY

The ability of a set of intrinsic characteristics to satisfy requirements
(ISO 9000:2000)

Subjective aspects
(Fashionable attributes)

Cultivar: Arbequina

	Eighties	Nineties	Today
Colour	Yellow	Pale Green	Green
Aroma	Ripe Fruity	Green Fruity	Cut Green Lawn
Odour	Slight oily	Apple	Tomato
Taste	Sweet	Slightly Bitter	Medium Bitter



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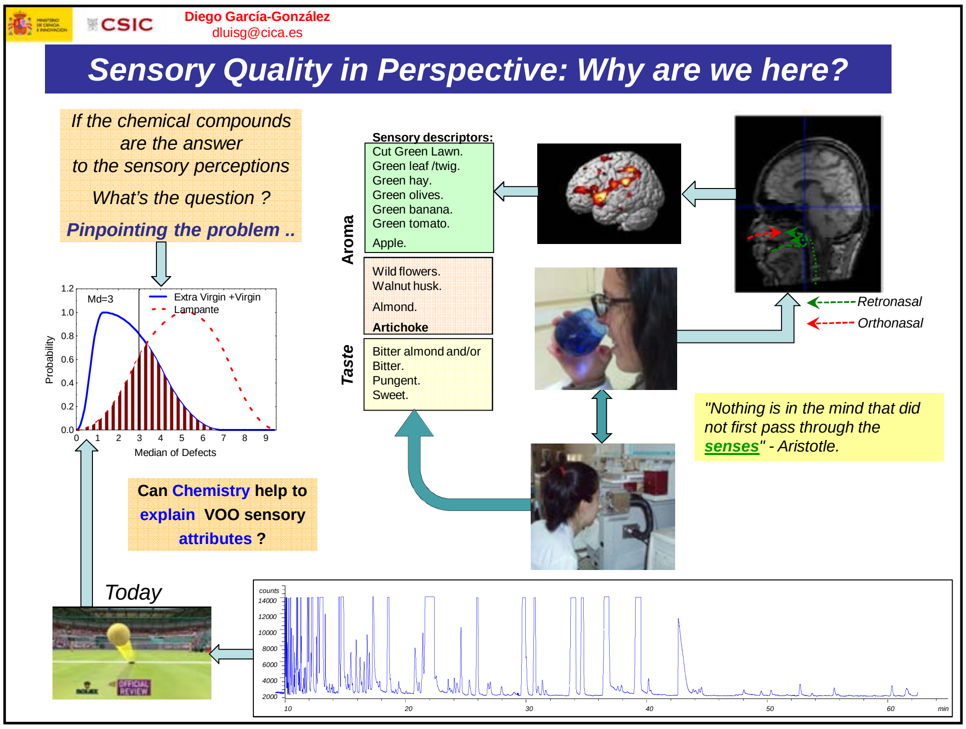
Sensory Quality in Perspective: Stating the Problem
Quality is a multifaceted, fuzzy and hierarchical concept
Level
1st
2nd
Food Safety
Authentication
Sensory Quality
Nutritional Quality
Convenience
Emotional Quality
Sensory assessment
Role of major and minor compounds
Shelf-life, price, packaging, etc.
Countries, PDO, preferred flavours
Panellists
Consumers



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Sensory Quality in Perspective: Why are we here?
If the chemical compounds are the answer to the sensory perceptions
What's the question ?
Pinpointing the problem ..
Md=3
Extra Virgin +Virgin
Lampante
Probability
Median of Defects
Can Chemistry help to explain VOO sensory attributes ?
Today
Sensory descriptors:
Cut Green Lawn.
Green leaf /twig.
Green hay.
Green olives.
Green banana.
Green tomato.
Apple.
Wild flowers.
Walnut husk.
Almond.
Artichoke
Bitter almond and/or Bitter.
Pungent.
Sweet.
Aroma
Taste
Retronasal
Orthonasal
"Nothing is in the mind that did not first pass through the senses" - Aristotle.

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Sensory Quality in Perspective: Looking for solutions

Are all the Chemical Compounds responsible for Virgin Olive Oil Flavor ?

Researchers usually have three key questions to consider :

Is there an underlying casual relationship ? *Do they smell/taste ?*

Is the association merely a spurious correlation ? *Partial explanation?*

Is the association statistically significant ? *Strict validation?*

Association that can be:

Causal** or **Casual

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Sensory Quality in Perspective: Looking for solutions

What's a causal relationship? *I think, therefore I exist.*

And in Chemistry ?: *I smell, therefore there are volatiles.*

How can Causality be detected in VOOs?

There are four criteria:

Consistency of association
If detected by all the assessors/consumers

Strength of association
If ther are differences with and without aroma

Temporal relationship
If compounds vary over time as aroma does

Mechanism
If there are physiological and biochemical explanations

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Sensory Quality in Perspective: Looking for solutions

"To the man who only has a hammer in the toolkit,
every problem looks like a nail."

Abraham Maslow
(American Philosopher and Psychologist, 1908-1970)

*The **Man-with-a-hammer** Syndrome:*

You think of an idea and then, pretty soon, it becomes the idea.

You start seeing how the idea can apply to anything and everything, it's the universal explanation for how the universe works.

Suddenly, everything you've ever thought of before must be reinterpreted through the lens of the idea and you're on an intellectual high.

For example: Utilitarianism.

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Sensory Quality in Perspective: Looking for solutions

Objective: Analysis of Aroma

Gas Chromatography	Panel test	Sensor systems
Individual identification and quantification of compounds		*Determination of the whole set of compounds*

Causal

Causal

✓ DHS-GC (TENAX trap)	✓ Standardized method	✓ MOS sensors
✓ SPME-GC (SPME fiber)	✓ Large experience.	✓ SAW sensors
✓ GC-Olfactometry	❑ Model to follow by other food products	❑ DHS-MS

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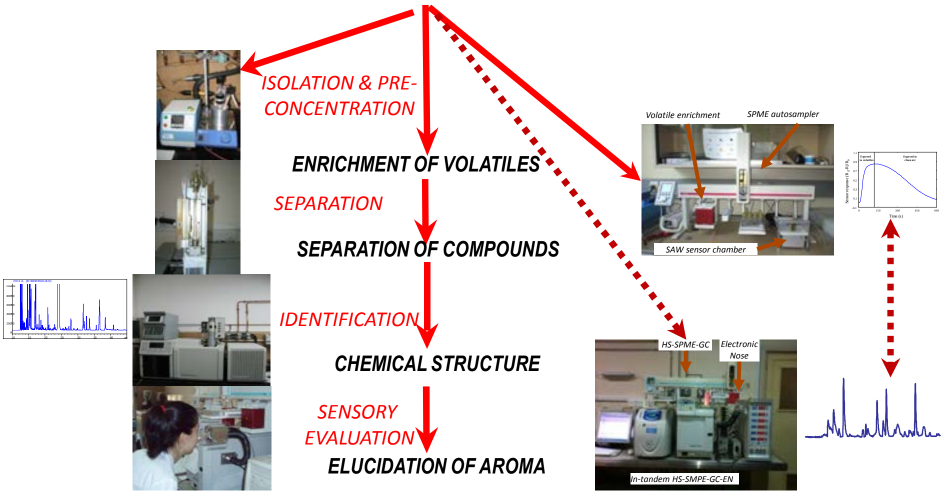
Sensory Quality in Perspective: The ROAD MAP

Steps in the analysis of volatiles



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Sensory Quality in Perspective: The ROAD MAP

Classification of the analytical methods

1. Not involving pre-concentration

- *Direct injection*
- *Static headspace*

2. Involving pre-concentration

- *Dynamic headspace*
  - *Thermal Desorption Cold Trap Inyector with Tenax TA trap: Chrompack.*
  - *Thermal Desorption with Tenax TA/Silica gel/Charcoal: Teledyne-Tekmar.*
- *Static headspace*
  - *Solid phase microextraction traps (DVB/CAR/PDMS): SPME*
  - *Tenax TA/Silica gel/Charcoal traps: Teledyne-Tekmar.*

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Sensory Quality in Perspective: The ROAD MAP

Classification of the analytical methods

Technique	Description	Advantages	Disadvantages
Dynamic headspace	An inert **gas sweeps** the sample **headspace** that is stirred or bubbled. Volatiles are trapped. Trap is desorbed by GC	High adsorption capacity. Useful for almost all kind of volatiles. Good recovery factors. No artifacts	Less sensitive to some acids. Temperature & flow-rate must be controlled. An analysis per sample. Expensive.

Teledyne-Tekmar technology

Thermal desorption cold trap inyector

Purge and Trap, with automatic sampler

Purge and Trap, and mass spectrometry

Our instrumentation for the analysis of volatiles with DHS-GC/MS

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Sensory Quality in Perspective: The ROAD MAP

Classification of the analytical methods

Technique	Description	Advantages	Disadvantages
Dynamic headspace.	An inert gas sweeps the sample headspace that is stirred or bubbled. Volatiles are trapped in Tenax. Trap is desorbed by GC	High adsorption *capacity*. Useful for almost all kind of volatiles. Good *recovery factors*. No artifacts *Automatic* (new designs)	Expensive. Less sensitive to some acids. Many variables to control

Thermal desorption cold trap inyector

Purge and Trap

Purge and Trap

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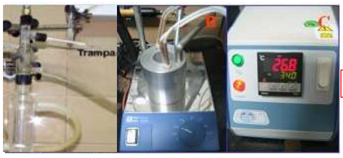
Sensory Quality in Perspective: The ROAD MAP

Classification of the analytical methods



Pre-concentration	
Sample amount (g)	0.5
Tª (ºC)	40
t (min)	15
Gas / Flow-rate (ml/min)	N_2 / 200
Stirring	Yes
Tenax TA trap	20-35 mesh
Desorption Tª (ºC) / t (min)	220 / 5
Cold trap	Fused silica
Crio-Trapping Tª (ºC)	-110
Thermal desorption Tª (ºC)	170
Standard	Isobutyl acetate

Analytical instrumentation:
- GC-MS
- Adsorbent trap: Tenax TA

Chromatographic conditions			
Tª injector	175 ºC		
Tª detector	275 ºC		
Carrier-gas H_2	1 ml/min		
Column	DB-WAX (60m, 0,25mm, 0,25µm)		
Detector	FID		
Oven temperature programe			
Tª (ºC)	Ratio (ºC/min)	t isotherm (min)	t total (min)
40		4	4
201	1	10	175

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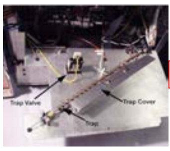
Sensory Quality in Perspective: The ROAD MAP

Classification of the analytical methods



Pre-concentration	
Sample amount (g)	1.0
Tª (ºC)	40
t (min)	15
Gas / Flow-rate (ml/min)	He/ 200
Stirring	Yes
Tenax TA trap	20-35 mesh
Thermal desorption Tª (ºC)	220
Standard	Isobutyl acetate

Analytical instrumentation:
- GC
- Adsorbent trap: Tenax TA

Chromatographic conditions			
Tª injector	175 ºC		
Tª detector	275 ºC		
Carrier-gas H_2	1 ml/min		
Column	DB-WAX (60m, 0,25mm, 0,25µm)		
Detector	FID		
Oven temperature programe			
Tª (ºC)	Ratio (ºC/min)	t isotherm (min)	t total (min)
40		4	4
201	1	10	175

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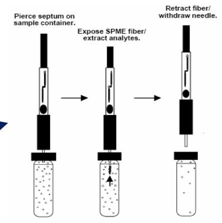
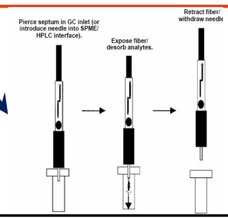
Sensory Quality in Perspective: The ROAD MAP

Classification of the analytical methods

Technique	Description	Advantages	Disadvantages
Static Headspace with SPME	A SPME fiber is exposed to sample vapor phase. Volatiles adsorbed on the fiber are desorbed in the GC injection port.		





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Sensory Quality in Perspective: The ROAD MAP

Classification of the analytical methods

Technique	Description	Advantages	Disadvantages
Static Headspace with SPME	A SPME fiber is exposed to sample vapor phase. Volatiles adsorbed on the fiber are desorbed in the GC injection port.	**Automatic.** Rapid. **Cheap.** Easy to use. Diverse **fibers**.	Differences in quantification of low weight molecules. Less number volatiles at low concentrations. **Repeatability** isn't fine enough.

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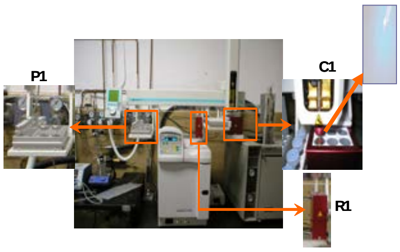
Sensory Quality in Perspective: The ROAD MAP

Classification of the analytical methods



Pre-concentration	
Sample amount (g)	1
Tª (ºC)	40
Time equilibrium (min)	10
Time adsortion (min)	40
Stirring	Si
Tª desorption (ºC)	260
Time desorption (min)	5
Standard	4-methyl-2-pentanol

Analytical instrumentation:

- GC Varian 3900 + CombiPAL
- Triple fiber SPME: DVB/CAR/PDMS

Chromatographic conditions			
Tª injector	260 ºC		
Tª detector	280 ºC		
Carrier-gas H_2	1 ml/min		
Column	DB-WAX (60m, 0,25mm, 0,25µm)		
Injector	FID		
Oven temperature programe			
Tª (ºC)	Ratio (ºC/min)	t isotherm (min)	t total (min)
40		4	4
200	3	10	67

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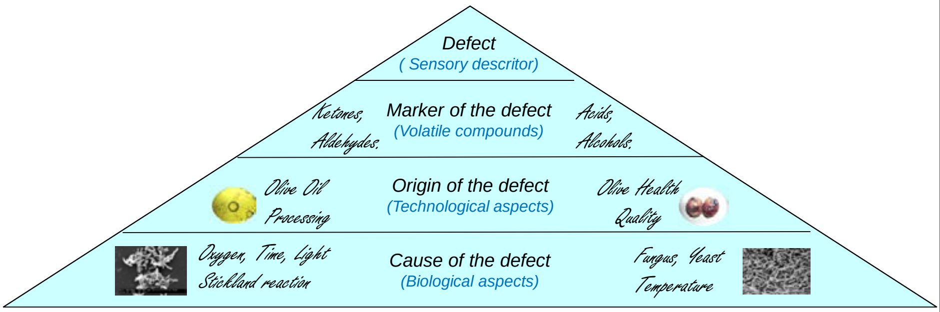
Sensory Quality in Perspective: Backward-tracing

Explaining origin of olive oil defects
by Olive Oil Aroma Markers



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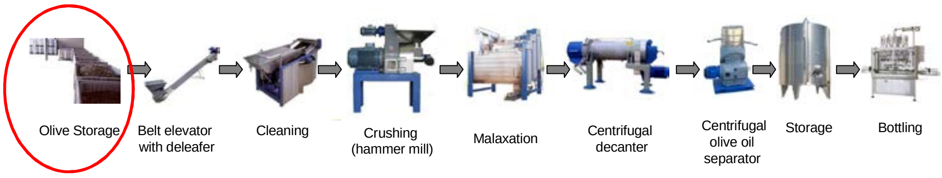
Backward-tracing: Explaining origin of olive oil defects



Origin:
- Inadequate Storage of Olives in Transportation and/or prior to processing

Defect	Cause	Volatiles	Threshold
Fusty	Olives in an advanced stage of anaerobic fermentation	2-Methyl-1-propanol	1.02
		3-Methyl -1-butanol	0.10
		Ethyl butanoate	0.03
		Propanoic acid	0.72
		Butanoic acid	0.65

Values of Odor Threshold in mg/kg

Aparicio & García-González; Olive Oil. In: Gourmet and Health-Promoting Oils. Edited by A. Kamal-Erdin, R.A. Moreau AOCS Press, 2009

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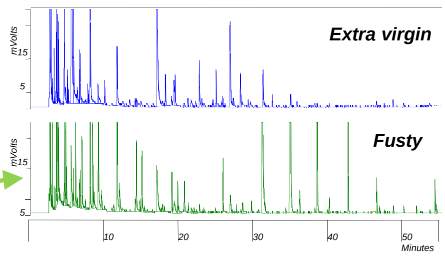
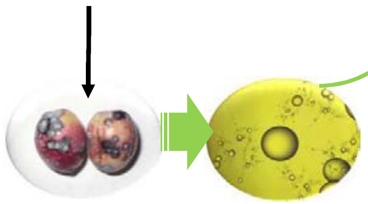
Ethiology & Olive Oil Aroma Markers

"Alcoholic fermentation of paste or damaged olives"

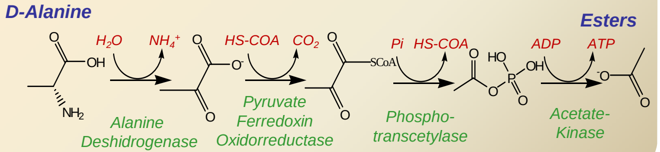
- *Clostridium*
- *Pseudomonas*
- *Enterobacter*

+ ↑Temperature and ↑time, and without Oxygen





Stickland reaction



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Sensory defects: origin and volatile compounds

Mustiness-humidity

Infestation by Aspergillus or Penicilium
↓ *Temp.* & ↑ *Humidity*

2-heptanone
1-octen-3-ol
1-octen-3-one
3-methyl-1-butanol
6-methyl-5-hepten-2-one

Winey-vinegary

*Aerobic fermentation by **Acetobacterias***
↑ *Temp.*

Acetic acid
Ethyl acetate
Ethanol

Fusty

Anaerobic fermentation
↑ *Temp* & ↑ *time*

Octane
2-Methyl-1-propanol
3-Methyl-1-butanol
Ethyl butanoate
Propanoic acid
Butanoic acid

Rancid

Oil autoxidation
↑ *Temp.*, ↑ *Light*, ↑ *Air*, ↑ *metals*

Pentanal, Hexanal, Heptanal, E-2-heptenal
Octanal, Nonanal, Acetic acid, Propanoic acid,
Butanoic acid, Hexanoic acid

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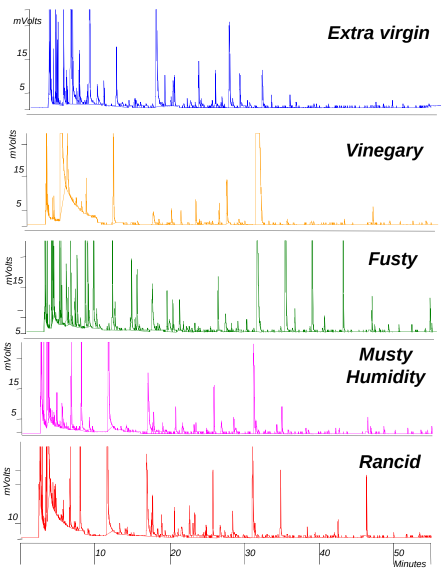
Etiology & Olive Oil Aroma Markers

SUMMARIZING:

1. *The volatile profiles of VOOs qualified without and with diverse defects are completely different.*
2. *The sensory descriptors are different because the volatile profiles are also different.*
3. *The simultaneous detection of various defects in a sensory evaluation results in a sum of volatile markers with an already well-known effect of synergy among them.*

An example of profiles:



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Sensory Quality in Perspective: OPEN ROAD

The results

Sensory defect	Chemical compound	OT (mg/kg)	Sensory descriptor
Fusty	n-Octane	0.94	Alkane
	Ethyl butanoate	0.03	Fruity
	Butanoic acid	0.65	Fusty
	Propanoic acid	0.72	Sour, Mould
	3-Methyl-1-butanol	0.10	Winey
	2-Methyl-1-propanol	1.00	Irritant, Fishy
Musty, Humidity	1-Octen-3-ol	0.05	Mould, Earthy
	1-Octen-3-one	0.01	Mushroom, Mould
	Ethyl acetate	0.94	Sticky
	Heptan-2-ol	0.01	Earthy
	Acetic acid	0.50	Vinegary, Sour
	Heptan-2-ol	0.01	Earthy
	E-2-Heptenal	0.042	Tallowy, Oxidized
	Propanoic acid	0.72	Sour, Mould

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Sensory Quality in Perspective: OPEN ROAD

The results

Sensory defect	Chemical compound	OT (mg/kg)	Sensory descriptor
Winey-Vinegary	Acetic acid	0.50	Vinegary, Sour
	Ethyl acetate	0.94	Sticky
	3-Methyl butan-1-ol	0.10	Whiskey
Muddy sediment	Heptan-2-ol	0.01	Earthy
	6-Methyl-5-hepten-2-one	1.00	Oily
	1-Pentene-3-one	0.004	Mustard
Rancid	Pentanal	0.24	Oily
	Hexanal	0.08	Oily, Fatty
	Heptanal	0.50	Oily, Fatty
	E-2-Heptenal	0.042	Tallowy, Oxidized
	Octanal	0.32	Fatty
	Nonanal	0.15	Waxy, Fatty
	E-2-Decenal	0.01	Fishy, Fatty
	Hexanoic acid	0.70	Rancid

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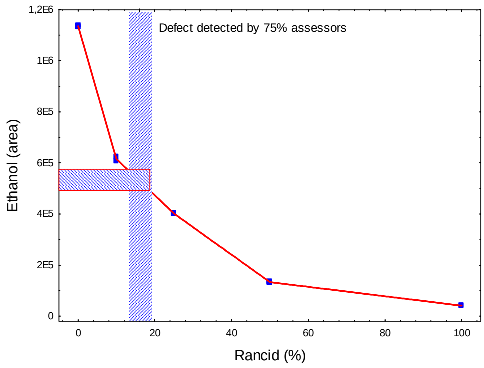
Volatile Compounds: Analytical Issues

NEEDED IMPROVEMENTS:

- *To understand the competitive effect between volatiles.*
- *Response factors may be variable in different matrix due to competitive effect/saturation.*



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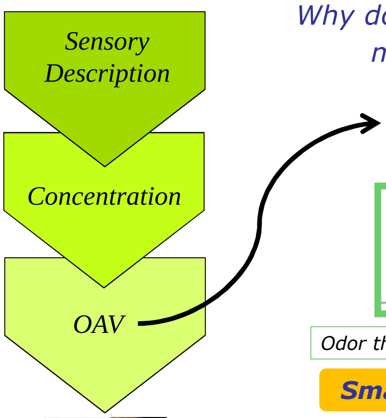
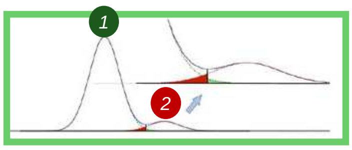
Volatile Compounds: Analytical Issues



Why do minor flavour compounds have major impact in perception?

$$\textbf{OAV} = \frac{\textit{Concentration in Sample}}{\textit{Odor Threshold}}$$



Odor threshold 1 = 1000 x Odor Threshold 2

***Small** differences in concentration*

***Major** impact on sensory perception*

Drawback:
There is not a consensus about odor thresholds

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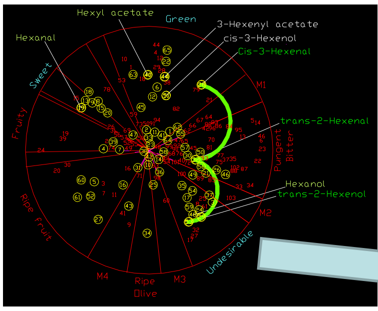
Sensory Quality in Perspective: OPEN ROAD

More than a decade of research



1996 Statistical Sensory Wheel (SSW)

A practical guide for flavour chemists, producers and consumers:

- *Origin of sensory defects*
- *Chemical markers (volatile compounds)*
- *Odour threshold*

2011

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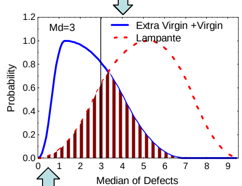
Sensory Quality in Perspective: Why are we here?

If the chemical compounds are the answer to the sensory perceptions

What's the question ?

Pinpointing the problem ..



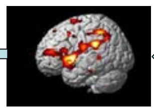
Can Chemistry help to explain VOO sensory attributes ?

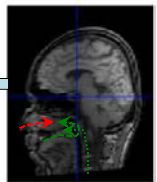
Sensory descriptors:

Aroma:
Cut Green Lawn.
Green leaf /twig.
Green hay.
Green olives.
Green banana.
Green tomato.
Apple.

Wild flowers.
Walnut husk.
Almond.
Artichoke

Taste:
Bitter almond and/or Bitter.
Pungent.
Sweet.

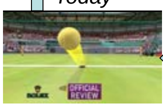


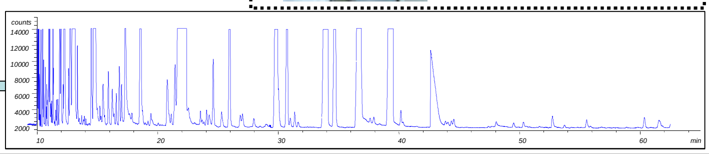


Retronasal
Orthonasal

"Nothing is in the mind that did not first pass through the senses" - Aristotle.

Today





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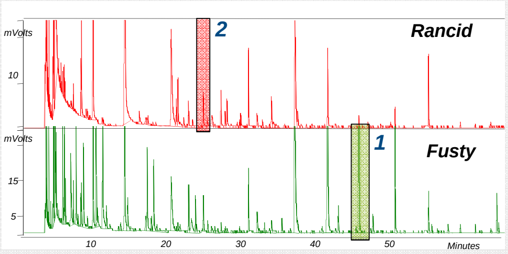
Why are consumers good at odour detection?

What would happen if we presented volatiles to consumers?

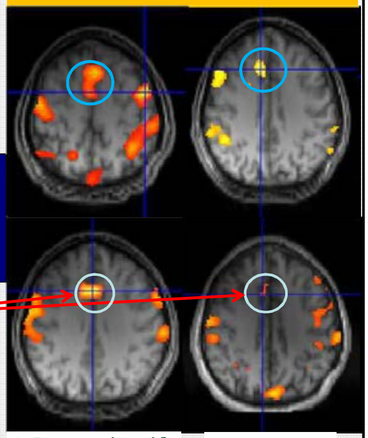


Code	Volatile	OT	Sniffing (mg/kg)	Concentrations (mg/kg)
1	Propanoic acid	0.72	Sour, strong	15.6
2	Heptanal	0.50	Oily, fatty	3.8

- Anterior cingulate (Brodman area 32)

Negative emotions

Rancid | Fusty



1. Propanoic acid | **2. Heptanal**

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The ROAD MAP: Current Results and Challenges

- To find markers explaining less studied defects (e.g., frozen).

-To identify minor peaks (e.g., GC × GC hexanal peaks, isomers).

- To establish concentration limits for markers responsible for defects.

-To develop certified materials of markers in lipid matrices for GC validation and panellist training and proficiency testing

Concluding remarks

- ***Volatile compounds analysis has proven to be a reliable*** ***alternative to routine sensory assessement*** *and it is based on a* ***casual relationship*** *with quality.*

- The headspace of virgin olive oil is complex and diverse and further studies are needed to know the **interaction between volatile compounds** in highly odorant and slightly odorant matrices.

- The analysis of volatile compounds could be used as
  - **Internal/external** control analysis,
  - **Contrast analysis** when contradictory results are obtained.
  - **Routine analysis,** leaving panel test only for particular cases.

07 - Methods of detection and analyses of deodorised olive and vegetable oils

by E. Frankel

Methods of Detection and Analyses of Deodorised Olive and Vegetable Oils

Edwin Frankel

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E-mail: enfrankel@ucdavis.edu

Much analytical work has been published on the chemistry of extra virgin olive oil (EVOO) to detect the type and amount of adulteration with deodorized olive oils. Common adulteration practices consist of blending EVOO with low-quality and cheaper olive oils. These oils are generally deodorized at lower temperatures than conventionally practiced with vegetable oils to remove undesirable flavour volatile lipid oxidation compounds.
The following methods used for detection of deodorized oils include (Frankel, E. (2010). Chemistry of Extra Virgin Olive Oil: Adulteration, Oxidative Stability, and Antioxidants. *Journal of Agricultural and Food Chemistry, 58*, 5991–6006):

(1) Determination of dimer diacylglycerols (DAGs) and stigmastadiene formed during deodorization. A cleanup short silica gel column followed by size exclusion chromatography (SEC) with a refractive index detector is used starting at 90^{o}C and increasing at deodorization temperatures.

(2) Analyses of non-glyceride components in olive oils produced by deodorization and physical refining. Stigma-3,5-diene produced by thermal dehydration and β-sitosterol are detected and quantified in refined olive oil. Another approach is the determination of conjugated 9,11-18:2 fatty esters produced at deodorization temperatures. Although advanced GC-MS and GC-MS/MS were used to determine conjugated diene fatty acid esters, these isomers can be readily determined quantitatively by standard UV analyses.

(3) Determination of refined olive oil in EVOO on the basis of complex volatile and nonvolatile compounds in deodorized distillates. Samples are analysed by gas chromatography (GC) after separation by SEC into nonpolar compounds (hydrocarbons, alkyl esters triacylglycerols), and polar compounds including (monoacylglycerols, free fatty acids and sterols).

(4) The presence of fatty acid esters produced by soft deodorization are good markers of low quality olive oil. The FFAs, MAGs, DAGs and TAGs produced after storage of olive fruits before milling are converted into methyl and ethyl esters with methanol and ethanol, isolated with a silica gel solid phase cartridge and analysed by GC.

(5) To simulate home cooking or food catering, thermal treatments used were based on microwave and conventional heating at 180^{o}C for 90 min. Different mixtures of thermally stressed olive oils with EVOO were compared, but the results may be questionable because they were obtained under very artificial conditions of microwave and conventional heating.

(6) (Yang Yang, Ferro, M.D.,Cavaco, I. and Liang, Y. (2013). Detection and Identification of Extra Virgin Olive Oil Adulteration by GC-MS Combined with Chemometrics. *Journal of Agricultural and Food Chemistry 61*, 3693-3702): Adulteration of EVOO with corn, peanut, rapeseed and sunflower oils were evaluated on the basis of 22 fatty acids and 6 significant parameters (18:2/18:3, 18:1/18:2, total saturated fatty acids (SFAs), MUFAs/PUFAs. Statistical univariate analyses showed that higher levels of C20:0, C22:0, C24:0, and SFAs were unique of peanut oil adulteration, and higher levels of 18:3,11*c*-C20:1 erucic acid (13*c*-22:1), and nervonic acid (15-24:1) characterized rapeseed adulteration. Corn-olive adulteration was shown on the basis C16:0, C18:0, C18:1 and the ratio of 18:1/18:2 and MUFAs/PUFAs; sunflower-olive adulteration on the basis of 9-*cis* C16:1, C17:0, C18:1, C17:0, oleic acid, 11-C18:1, MUFAs, and the ratios of 18:1/18:2 and MUFAs/PUFAs.

Methods of Detection and Analyses of Deodorized Olive Oils

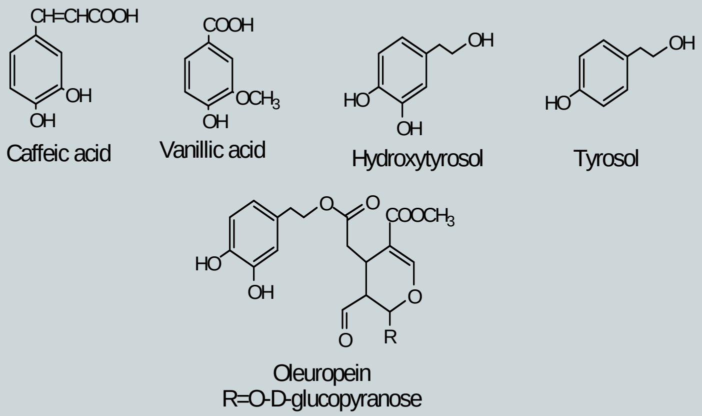
Edwin Frankel,
University of California, Olive Center
Davis, California, USA

Chemistry of Extra Virgin Olive Oil, Adulteration, Oxidative stability, and Antioxidants
(*J. Agric. Food Chem*. 2010, 58, 5991- 6006)

Methods of Detection and Analyses of Deodorised Olive Oils

- Although extra virgin olive oils (EVOO) are generally stable to oxidation due to:
- High oleic acid content and natural phenolic antioxidants,
- They are still susceptible to oxidation after prolonged storage,
- Due to their polyunsaturated fatty acid content (5-9%) and minor constituents (chlorophylls, carotenoids, and metal impurities),
- Much of the present literature on EVOO adulteration has depended on sophisticated statistical approaches that require analyses of large numbers of samples.

Main Phenolic Compounds in Extra Virgin Olive Oil



Common Adulteration Practices

- Blending EVOO with low quality and cheaper olive oils that have Sensory Defects:
- To remove undesirable flavor volatiles derived from lipid oxidation, these oils are generally subjected to mild deodorization at lower temperatures,
- The quality of stored commercial EVOOs can vary widely,
- Interactions between minor constituents in EVOO and trace metals can produce pro-oxidant effects,
- The choice of methods and conditions to evaluate oxidative stability and antioxidants is therefore critical.

Available Tools to Detect Adulteration of EVOO

- Determine level of dimer triacylglycerol (TAGs), polymers and stigmastadiene formed during deodorization,
- Use a cleanup short silica gel column followed by size exclusion chromatography with refractive index detector to show:
- Formation of dimer TAGs starting at 90◦C and increasing at deodorization temperatures.
  – Gertz *et al*, *Eur. J. Lipid Sci. Technol.* 2000, 329-336.

Available Tools to Detect Adulteration of EVOO

- Determine non-glyceride components in olive oils produced by deodorisation and physical refining,
- Stigma-3,5-diene produced by thermal dehydration of β-sitosterol detected and quantified in refined oils present in EVOO.
  – Léon-Gamachio *et al*, *Grasas Aceitas* 2004, 55, 227-232

Available Tools to Detect Adulteration of EVOO

- Statistical study of processing parameters (N_2 flow, temperature and oil load) for the formation of stigma-3,5-diene during deodorization,
- Based on determination of conjugated 9,11-18:2 fatty esters produced at high temperatures of deodorization using MS and GC-MS/MS,
- Actually both isomers diene-9,11-18:2 and 10,12-18:2 fatty esters isomers can be readily determined by standard spectrophotometeric UV analysis.
  - Saba *et al, J. Agr. Food Chem.* 2005, 53, 4867-4872.

Available Tools to Detect Adulteration of EVOO

- Presence of refined olive oil in EVOO based complex volatile and non-volatile compounds in deodorizer distillates,
- GC analyses improved by using size exclusion chromatography into nonpolar compounds (hydrocarbons and alkyl esters triacylglycerols) and polar compounds (mono- and di-acylglycerols, fatty acids and sterols).
  - Hafidi *et al, Food Chem.* 2005, 92, 607-613.

Available Tools to Detect Adulteration of EVOO

- Presence of fatty acid esters considered as good marker of low-quality olive oil subjected to soft deodorization,
- Free fatty acids, mono-, di- and tri-acylglycerols produced from olive fruits stored before milling and converted into methyl and ethyl esters with methanol and ethanol,
- These esters can be isolated by silica gel solid phase cartridge and analyzed by gas chromatography.
  – Ruiz-Mendez *et al, Food Chem.* 2007, 103, 1502-1507

Available Tools to Detect Adulteration of EVOO

- Effects of hydrolysis and oxidation determined by admixtures of mildly deodorized olive oil with EVOO by chromatographic and spectroscopic methods,
- Thermal treatments based on microwave and conventional heating at 180°C for 90 min.
- Results may be questionable under these very artificial conditions of microwave and conventional heating.
  – Bendini *et al, J. Agr. Food Chem. 2009, 57, 10055-10062*

Conclusions

- Several potential problems become apparent from the extensive literature published in the past several decades on different kinds of olive oils.
- Many studies on the adulteration of EVOO with cheaper vegetable oils were based on advanced sophisticated statistical methods that require the analyses of large numbers of samples.
- Powerful analytical methods are now available to provide more precise and accurate chemical information on olive oils that may obviate **too much dependence on statistics!**

08 - Possible markers of olive oil "soft" deodorization by physical stripping

by M. Gallina Toschi

Possible markers of olive oil "soft" deodorization by physical stripping

Tullia Gallina Toschi[1,2], Enrico Valli[2] and Alessandra Bendini[1,2]

[1]Dipartimento di Scienze e Tecnologie Agro-alimentari and [2]Centro Interdipartimentale di Ricerca Industriale Agroalimentare, Alma Mater Studiorum - Università di Bologna, P.zza Goidanich 60, 47521 - Cesena (FC), Italy.
E-mail: tullia.gallinatoschi@unibo.it

"Soft" deodorization can be reasonably achieved by two main methods, either separately or combined: physical stripping treatment and deodorizing "filtration". Both techniques can be considered refining phases, and are not allowed for extra virgin olive oils defined as "*oils obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions that do not lead to alteration in the oil, which have not undergone any treatment other than washing, decantation, centrifugation or filtration, to the exclusion of oils obtained using solvents or using adjuvants having a chemical or biochemical action, or by re-esterification process and any mixture with oils of other kinds.*" (EC Reg. No.1513/2001, Annex). The reason for their prohibition is because they are not aimed towards extraction ot to stabilization of the oil (such as a proper filtration procedure), but to the "correction" of a product, which otherwise would not be native, or no longer, extra virgin; they could, for example, be used to reduce defects of virgin or lampante oils, rejected for sensory scores from the extra virgin category.
Physical stripping "soft" deodorization, addressed here, can remove off-flavours but fatty acid alkyl esters (FAAEs), recently related to the main sensory defects such as fusty-muddy, winey, and mouldy (Gómez-Coca et al., 2012) can be permanent markers (Pérez-Camino et al., 2008) that reveal an oil of low quality. Their determination, performed together with the quantification of waxes (EU Reg. No. 61/2011) is lenghty and laborious and as a consequence, other preliminary screening methods have been proposed, or ara being studied, including:
a) FT-IR coupled by Partial Least Square (PLS) fast method (Valli et al., 2013a); b) Time Domain Reflectometry (TDR) and PLS multivariate statistical analysis (Valli et al., 2013b).
"Soft" physical stripping deodorization may produce other observed or theoretical effects, which are being investigated as possible markers, such as: *i*) the appearance of anomalous sensory attributes (e.g. the so called "cardboard like"); *ii*) the modification of volatile compounds, such as their relative ratios (e.g. ratio between ethanol and *E*-2-hexenal); *iii*) the lowering of the amount of water in oils, due to the stripping effect (Bendini et al., 2009); *iv*) the diacylglycerols content and the proportional amount of free fatty acids.

References

Gómez-Coca, R.B., Moreda, W., Pérez-Camino, M.C. (2012). Fatty acid alkyl esters presence in olive oil vs. organoleptic assessment. *Food Chemistry*, *135*, 1205-1209.

Pérez-Camino, M.C., Moreda, W., Cert, A., Romero-Segura, A. & Cert-Trujillo, R. (2008). Alkyl esters of fatty acids a useful tool to detect soft deodorized olive oils. *Journal of Agricultural and Food Chemistry*, *56*, 6740-6744.

Valli, E., Bendini, A., Maggio, R.M., Cerretani, L., Gallina Toschi, T., Casiraghi, E., Lercker, G. (2013a). Detection of low-quality extra virgin olive oils by fatty acid alkyl esters evaluation: a preliminary and fast mid-infrared, spectroscopy discrimination by a chemometric approach. *International Journal of Food Science and. Technology*, *48*, 548-555.

Valli, E., Berardinelli A., Cevoli C., Bendini, A., Gallina Toschi, T., Ragni, L. (2013b). Rapid assessment of fatty acids alkyl esters in extra virgin olive oils by Time Domain Reflectometry (TDR). *Poster submitted to 11th Euro Fed Lipid Congress, 2013.*

Bendini, A., Cerretani, L., Valli, E., Mazzini, C., Lercker, G. (2009). Metodi analitici per la determinazione di oli deodorati *mild* in oli extra vergini di oliva commerciali. *Industrie alimentari*, *496*, 46-51.

Possible markers of olive oil “soft” deodorization by physical stripping

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Madrid, June the 10th, 2013

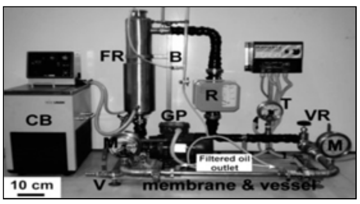
Soft deodorizing: two main methods

1)Soft deodorization column

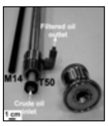
- Packed column (thickness: 0.2 mm)
- Inert gas or water steam
- High vacuum (2-5 Torr)
- T < 120-130°C
- Low time treatment (less than 4h)

Effects

- Stripping of volatile compounds responsible for off-flavours.
- No formation of markers of refining process (e.g. stigmastadienes, trans-isomers of fatty acids).



Pilot plant for micro and ultra filtrations of the oil



Membranes and steel components

2)Membrane filtration

- Micro and ultra tangential filtrations
- TiO_2-carbon, TiO_2-Al_2O_3 membranes.
- Decrease of volatile compounds, chlorophylls, phenolic compounds.

Why is "soft deodorization" a refining phase?

"*oils obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions that do not lead to alteration in the oil, which have not undergone any treatment other than washing, decantation, centrifugation or filtration, to the exclusion of oils obtained using solvents or using adjuvants having a chemical or biochemical action, or by re-esterification process and any mixture with oils of other kinds.*" (EC Reg. No.1513/2001, Annex).

AIM OF THE PROCESS

-**not the** extraction or to stabilization of the oil (such as a proper filtration procedure)

-**BUT** the "correction" of a product, which otherwise would not be native, or no longer, extra virgin

There are strict rules about producing virgin olive oils in Europe, while rules for other vegetable oils appear to be less strict for example for **many cold pressed oils a washing treatment with water vapour** (that indeed consists in a mild deodorization at around 120-150°C) is allowed (CODEX-STAN 210-1999, amended 2003, 2005; Grob et al., 1994).

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Agenda of the speech

Focus on analytical methods mainly able to detect

physical stripping soft deodorization

1) Fatty acid alkyl esters

a) Determination by LC-GC (Eu Reg. 61/2011)

b) Preliminary faster and cheaper screening methods

   a) PLS-FT-IR

   b) PLS-TDR

2) Volatile compounds (SPME/GC-MSD)

3) Sensory analysis

4) DAG content and FFA

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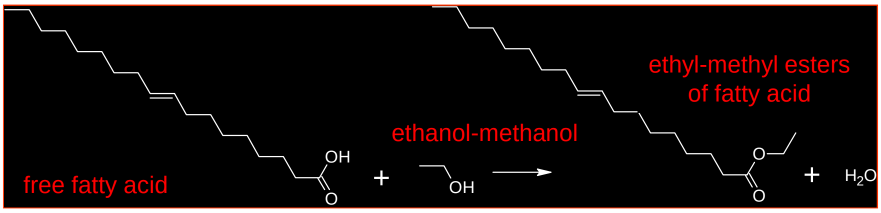
Fatty acid alkyl esters (FAAEs)

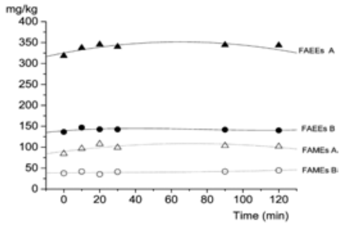
- The presence of FAAEs in extra virgin olive oils (Mariani et al., 1991) depends on the quality of the raw material (olives) (Perez-Camino et al., 2002).



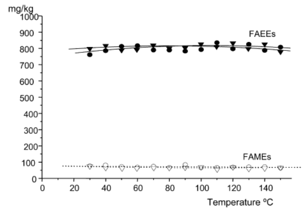
- *Overripening – cell damage of olives:*
  - increase of free fatty acids (hydrolysis of triacylglycerols by the enzyme lipase)
  - formation of methanol by the degradation of pectins (in the cells of the olives)
- *Storage of the olives in not optimal conditions:*
  - formation of ethanol due to fermentative processes

FAAEs and soft deodorization

Perez-Camino, M.C., Moreda, W., Cert, A., Romero-Segura, A. & Cert-Trujillo, R. (2008). Alkyl esters of fatty acids a useful tool to detect soft deodorized olive oils. Journal of Agricultural and Food Chemistry, 56, pp. 6740-6744.



Evolution of FAAEs when two low quality olive oils (Sample A ≈ 400 mg/kg FAAEs and sample B ≈ 170 mg/kg FAAEs) are submitted to a soft deodorization process (98 °C under nitrogen) for 2 h, under nitrogen (∆▲) and steam water (●○).



Evolution of FAAEs when an olive oil containing >800 mg/kg FAAEs is submitted to a deodorization process up to 150 °C for 4 h under nitrogen (∆▲) and steam water (●○).

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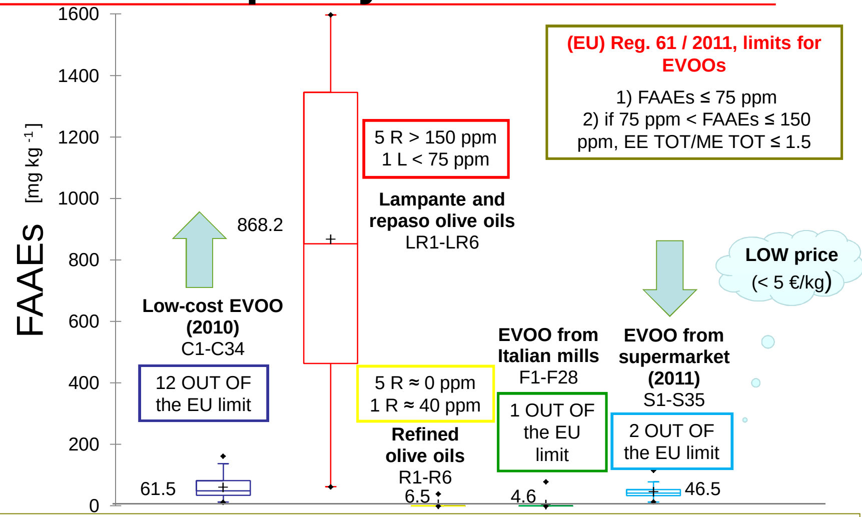
- The application of an illegal "soft" deodorization treatment does not cause a significant change in the total amount of FAAEs in the oil (Pérez-Camino et al., 2008)

Fatty acid alkyl esters (FAAES) as quality markers



Valli E., Bendini A., Gallina Toschi, T., Lercker. G. "***Qualità e purezza di oli extravergini di oliva: applicazione di idonei parametri analitici***" 113-138 (2012). In: Filiera olivicola. Monitoraggio di un campione di imprese e studi di settore, Unaprol.

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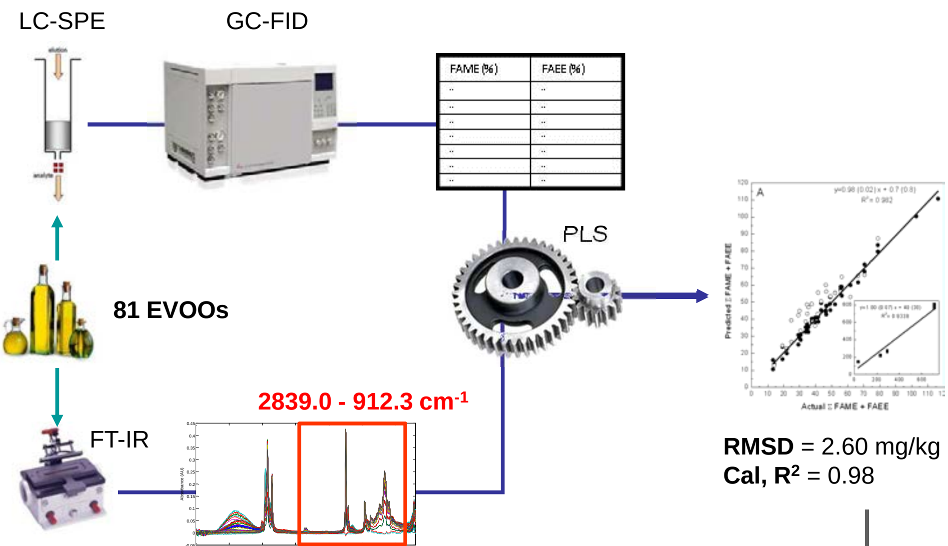
Detection of low quality extra virgin olive oils by fatty acid alkyl esters evaluation: a preliminary and fast mid infrared spectroscopy discrimination by a chemometric approach

Valli, E., Bendini, A., Maggio, R.M., Cerretani , L., Gallina Toschi, T., Casiraghi, E., Lercker, G.

International Journal of Food Science and Technology 2013, 48, 548–555



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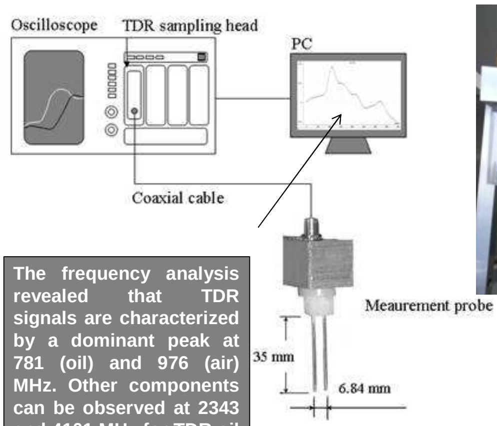
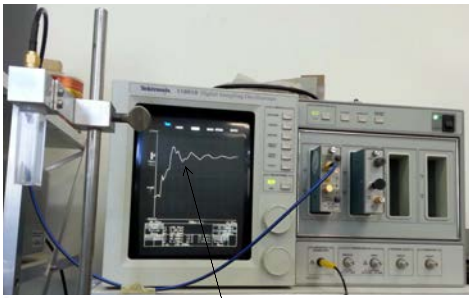
Rapid assessment of fatty acids alkyl esters in extra virgin olive oils by Time Domain Reflectometry (TDR).

Valli, E., Berardinelli A., Cevoli C., Bendini, A., Gallina Toschi, T., Ragni, L.

A poster will be presented at the 11th Euro Fed Lipid Congress, 2013.



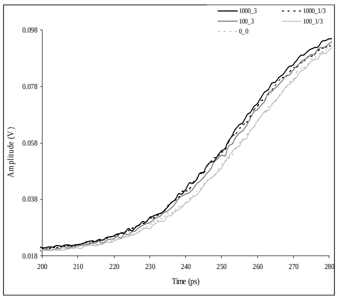


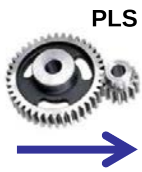
The frequency analysis revealed that TDR signals are characterized by a dominant peak at 781 (oil) and 976 (air) MHz. Other components can be observed at 2343 and 4101 MHz for TDR oil signals and 2734 and 4882 MHz for TDR air signals.

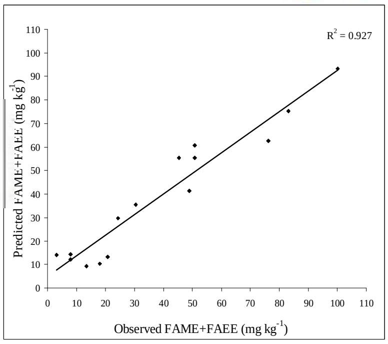
The dielectric behaviour is observed by analysing, in the time domain, the reflected signal from a generic load after the application of a fast rise time step, from picoseconds to fractions of microseconds (Sosa-Morales et al., 2010).

A rapid screening of fatty acid alkyl esters in olive oils by Time Domain Reflectometry (TDR)

A.Berardinelli, L. Ragni, A. Bendini, E. Valli, L. Conte, T. Gallina Toschi, paper submitted







In order to verify the influence of the FAEEs content on the TDR Signal one EVOO sample characterized by a content of FAEEs lower than LOQ value was used. The added standard concentrations were 1000 mg kg^{-1} and 100 mg kg^{-1} but with two different ratios, 3 to 1 and 1 to 3, between methyl oleate and ethyl oleate, respectively to identify in the waveforms the effects related to the FAAEs content (FAME + FAEE and different concentrations of FAME and FAE)E. Five replications were conducted to evaluate the repeatability of TDR signals.

Predicted versus observed values for FAME + FAEE (mg kg^{-1}) for test set validation.

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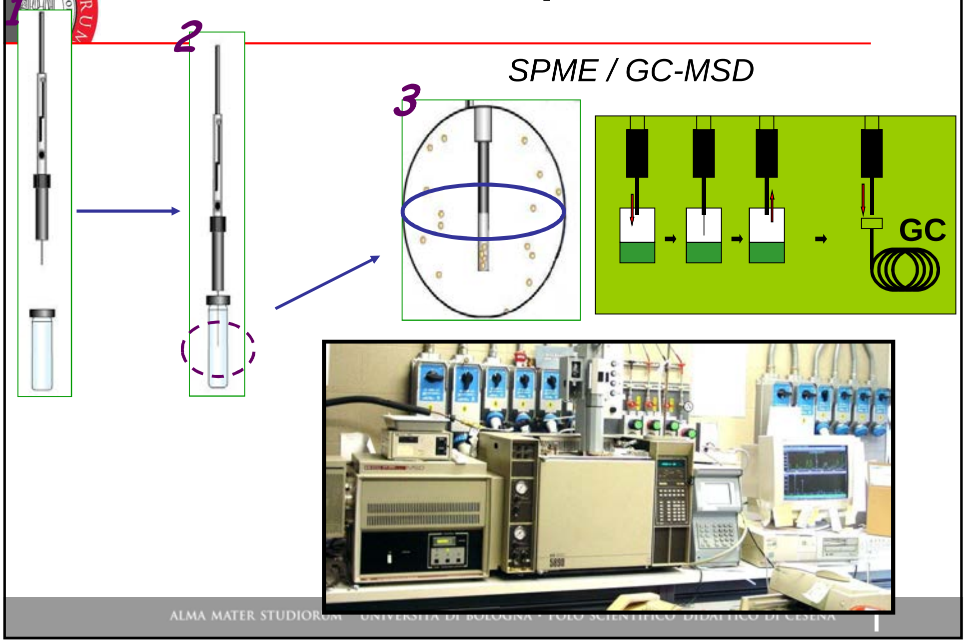
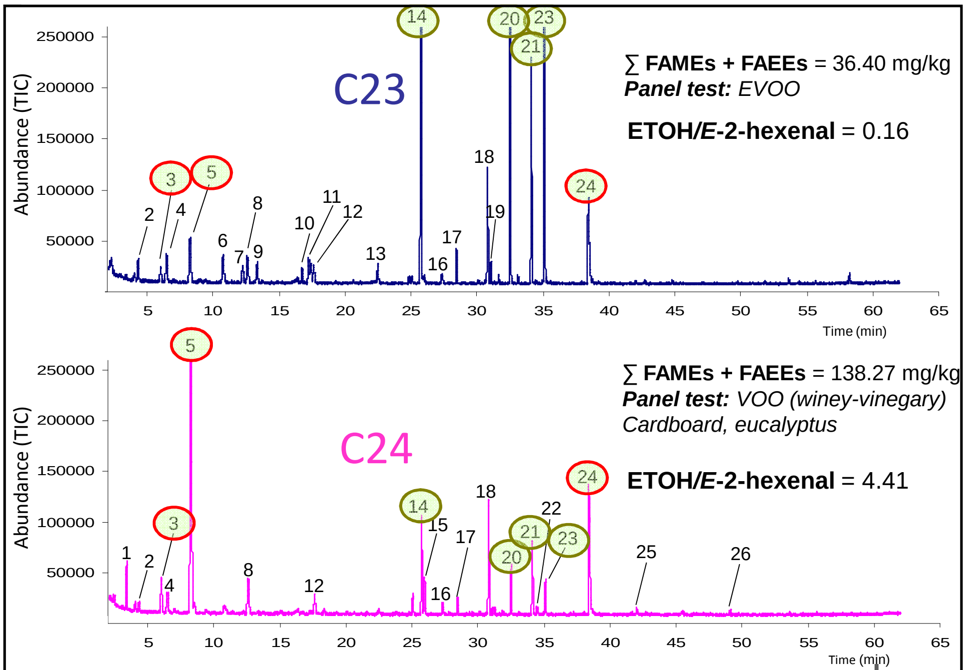
Volatile compounds





1, octane; 2, methyl acetate; 3, ethyl acetate; 4, methanol; 5, ethanol; 6, 3-pentanone; 7, penten dimer; 8, 4-methyl-2-pentanone (I.S.); 9, penten dimer; 10, (*Z*)-1,9-dodecadiene; 11, 4,8-dimethyl-1,7-nonadiene; 12, hexanal; 13, 1-penten-3-ol; 14, (*E*)-2-hexenal; 15, 2-tridecene; 16, α-pinene; 17, hexyl acetate; 18, (*Z*)-3- hexenyl acetate; 19, 2-penten-1-ol; 20, 1-hexanol; 21, (*Z*)-3-hexenol; 22, nonanal; 23, (*E*)-2-hexenol; 24, acetic acid; 25, propanoic acid; 26, α -farnesene.

SET of 34 extra virgin olive oils (C1-C34), all sold at medium-low price (2-5 €/kg) in the large scale retail trade (supermarkets and discount stores)

Samples	Fatty acid alkyl esters								Volatile compounds		Sensory analysis			
	C18:1EE		∑ FAMEs + FAEEs		RFF		Judjement according to Pérez-Camino et al., 2008	Classification according to EU Reg. 61/2011	ETOH/*E*-2-hexenal		Classification	Sensory defects	Other negative olfactory notes	Other peculiar descriptors
	mean	sd	mean	sd	mean	sd			mean	sd				
C1	7.26	0.13	26.70	3.92	1.19	0.05	G.	E.V.O.O.	4.07	0.38	E.V.O.O.			eucalyptus
C2	90.39	4.61	162.40	19.68	3.50	0.53	S.S.	NOT E.V.O.O.	2.99	0.17	V.O.O.	winey-vinegary	cardboard	eucalyptus
C3	16.87	0.87	42.39	12.00	1.38	0.14	G.	E.V.O.O.	11.79	1.38	V.O.O.	winey-vinegary		
C4	55.16	5.97	133.43	19.81	1.41	0.04	S.S.	E.V.O.O.	3.29	0.41	E.V.O.O.		cardboard	eucalyptus
C5	8.27	2.65	20.04	4.71	0.76	0.09	G.	E.V.O.O.	4.14	0.24	E.V.O.O.			eucalyptus
C6	36.13	5.33	59.70	15.57	0.79	0.11	G.	E.V.O.O.	3.17	0.43	E.V.O.O.			eucalyptus
C7	6.08	1.27	38.01	4.84	1.50	0.19	G.	E.V.O.O.	0.40	0.07	E.V.O.O.			
C8	4.28	0.77	15.07	2.46	0.56	0.02	G.	E.V.O.O.	0.07	0.00	E.V.O.O.			
C9	56.05	1.12	106.65	9.96	2.22	0.12	S.S.	NOT E.V.O.O.	2.25	0.11	E.V.O.O.			
C10	15.01	1.77	42.88	1.74	2.04	0.17	G.	E.V.O.O.	0.97	0.13	E.V.O.O.			
C11	33.11	0.75	78.14	6.55	4.05	0.63	S.S.	NOT E.V.O.O.	6.08	0.16	V.O.O.	winey-vinegary	cardboard	eucalyptus
C12	51.95	1.74	84.18	7.91	3.26	0.32	S.S.	NOT E.V.O.O.	3.36	0.31	E.V.O.O.		cardboard	eucalyptus
C13	45.20	1.46	79.44	1.53	3.95	0.43	S.S.	NOT E.V.O.O.	4.81	0.00	E.V.O.O.		cardboard	eucalyptus
C14	5.97	1.07	12.48	0.90	0.59	0.05	G.	E.V.O.O.	0.62	0.08	E.V.O.O.			
C15	4.38	0.38	14.22	0.29	0.70	0.15	G.	E.V.O.O.	0.60	0.05	E.V.O.O.			
C16	57.95	4.06	95.26	6.72	5.56	0.29	S.S.	NOT E.V.O.O.	1.45	0.03	E.V.O.O.			
C17	6.16	1.17	18.07	1.90	1.04	0.23	G.	E.V.O.O.	1.27	0.22	E.V.O.O.			eucalyptus
C18	7.46	0.31	19.78	1.34	1.01	0.07	G.	E.V.O.O.	0.29	0.03	E.V.O.O.			
C19	17.58	1.88	35.75	3.65	1.77	0.19	G.	E.V.O.O.	2.91	0.11	V.O.O.	winey-vinegary		
C20	15.81	2.60	31.06	3.64	2.32	0.34	G.	E.V.O.O.	0.39	0.04	E.V.O.O.			
C21	38.76	1.69	73.06	4.78	2.67	0.10	S.S.	E.V.O.O.	1.64	0.26	V.O.O.	rancid	cardboard	eucalyptus
C22	46.15	8.09	76.04	11.77	7.81	0.52	S.S.	NOT E.V.O.O.	1.03	0.09	E.V.O.O.		cardboard	eucalyptus
C23	1.76	0.14	36.40	9.76	0.43	0.09	G.	E.V.O.O.	0.16	0.00	E.V.O.O.			
C24	73.06	4.22	138.27	10.00	3.07	0.13	S.S.	NOT E.V.O.O.	4.41	0.56	V.O.O.	winey-vinegary	cardboard	eucalyptus
C25	10.91	0.71	52.70	2.62	2.02	0.27	G.	E.V.O.O.	0.65	0.10	E.V.O.O.			
C26	32.96	3.56	105.00	17.03	1.96	0.53	S.S.	NOT E.V.O.O.	0.24	0.02	E.V.O.O.		cardboard	
C27	26.02	3.80	76.03	10.50	2.00	0.58	S.S.	NOT E.V.O.O.	1.59	0.20	E.V.O.O.			eucalyptus
C28	3.43	0.12	35.45	1.19	1.40	0.16	G.	E.V.O.O.	0.16	0.02	E.V.O.O.			
C29	42.76	1.79	99.89	0.46	1.54	0.22	S.S.	NOT E.V.O.O.	19.21	2.11	V.O.O.	fusty-muddy	cardboard	
C30	15.58	0.73	45.48	5.47	1.06	0.08	G.	E.V.O.O.	3.30	0.51	E.V.O.O.		cardboard	
C31	16.04	0.60	69.78	9.53	1.41	0.20	G.	E.V.O.O.	0.35	0.02	E.V.O.O.			
C32	16.71	2.30	43.79	5.80	1.76	0.17	G.	E.V.O.O.	0.55	0.00	V.O.O.	fusty-muddy	cardboard	
C33	11.63	0.80	33.91	2.20	1.51	0.17	G.	E.V.O.O.	0.54	0.08	E.V.O.O.			eucalyptus
C34	34.24	0.12	88.59	2.40	1.57	0.26	S.S.	NOT E.V.O.O.	5.50	0.70	E.V.O.O.		cardboard	eucalyptus

Samples	Fatty acid alkyl esters					Volatile compounds	
	∑ FAMEs + FAEEs		RFF		Classification according to EU Reg. 61/2011	ETOH/*E*-2-hexenal	
	mean	sd	mean	sd		mean	sd
C2	162.40	19.68	3.50	0.53	NOT E.V.O.O.	2.99	0.17
C9	106.65	9.96	2.22	0.12	NOT E.V.O.O.	2.25	0.11
C11	78.14	6.55	4.05	0.63	NOT E.V.O.O.	6.08	0.16
C12	84.18	7.91	3.26	0.32	NOT E.V.O.O.	3.36	0.31
C13	79.44	1.53	3.95	0.43	NOT E.V.O.O.	4.81	0.00
C16	95.26	6.72	5.56	0.29	NOT E.V.O.O.	1.45	0.03
C22	76.04	11.77	7.81	0.52	NOT E.V.O.O.	1.03	0.09
C24	138.27	10.00	3.07	0.13	NOT E.V.O.O.	4.41	0.56
C27	76.03	10.50	2.00	0.58	NOT E.V.O.O.	1.59	0.20
C29	99.89	0.46	1.54	0.22	NOT E.V.O.O.	19.21	2.11
C34	88.59	2.40	1.57	0.26	NOT E.V.O.O.	5.50	0.70

ETOH/*E*-2-hexenal > 1

FAAEs: out of the EU legal limit for EVOOs

Samples	Volatile compounds		Sensory analysis			
	ETOH/*E*-2-hexenal		Classification	Sensory defects	Other negative olfactory notes	Other peculiar descriptors
	mean	sd				
C2	2.99	0.17	V.O.O.	winey-vinegary	cardboard	eucalyptus
C3	11.79	1.38	V.O.O.	winey-vinegary		
C11	6.08	0.16	V.O.O.	winey-vinegary	cardboard	eucalyptus
C19	2.91	0.11	V.O.O.	winey-vinegary		
C24	4.41	0.56	V.O.O.	winey-vinegary	cardboard	eucalyptus
C29	19.21	2.11	V.O.O.	fusty-muddy	cardboard	

Sensory analysis: VOO (winey-vinegary, fusty-muddy)

Samples	Volatile compounds		Sensory analysis			
	ETOH/*E*-2-hexenal		Classification	Sensory defects	Other negative olfactory notes	Other peculiar descriptors
	mean	sd				
C4	3.29	0.41	E.V.O.O.		cardboard	eucalyptus
C12	3.36	0.31	E.V.O.O.		cardboard	eucalyptus
C22	1.03	0.09	E.V.O.O.		cardboard	eucalyptus
C30	3.30	0.51	E.V.O.O.		cardboard	
C34	5.50	0.70	E.V.O.O.		cardboard	eucalyptus

Sensory analysis: EVOO, cardboard / eucalyptus

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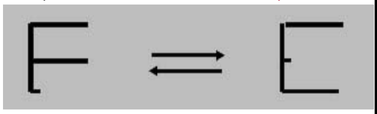
DAGs content and FFAs

DAGs isomerization (from 1,2- to 1,3-)

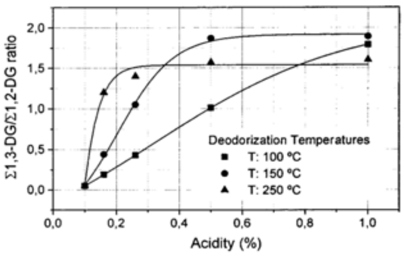
- ageing
- deodorization

1,2-DAG 1,3-DAG



Time and T afffect the DAG formation

DG theor.(mg/g) ~22 (% acidity - 0.10) + 10



Values of the 1,3-/1,2-DG ratio in virgin olive oils of various acidities deodorized at 100, 150, and 250 °C for 1.5 h under vacuum. The values corresponding to 0.5 and 1.0% acidities are the means of those obtained for the oils from three olive cultivars.

Pérez-Camino, M.C., Moreda, W. & Cert, A. (2001). Effects of olive fruit quality and oil storage practices on the diacylglycerol content of virgin olive oils. Journal of Agricultural and Food Chemistry, 49(2), 699-704

Water amount… an ambiguos value

Some olive oils with low amounts of water (< 700 mg/kg) were **strongly suspected** of "mild" deodorization (Cerretani et al., 2008).

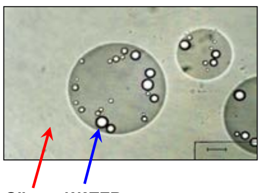
…but, at the time, **no significant correlation** (e.g. on a set of 24 samples, Bendini et al., 2008) was found between water amount and mild deodorized oils.

Physical stripping soft deodorization

- Water steam
- Nitrogen steam

Depletion of water

A microdispersed "technological" water (in virgin oils) is generally around 0,1%. But it is true that can be partially removed by filtration.



OIL WATER

Keywords for the future….

-Transformation of, at least, one promising lab-scale method (e.f. FT-IR, TDR) in a quick proceduresfor a **rapid and diagnostic screening of FAAEs**

-Deepening of the appearance of anomalous sensory attributes (cardboard like), due to a modified composition and distribution of volatile compounds.
→→ **conjoint volatile and sensory analyses**

-Study of the relative quantitative ratios between volatile compounds in authentic samples, and the changes produced by soft deodorization. → → **volatiles ratios**

- Study of the relative stripping ratios between free fatty acids and alkyl esters in different conditions of physical stripping. → → **FAAEs and FFAs**

-Study on diacylglycerols (isomers, composition) in relation with free fatty acids (% and composition) in different conditions of physical stripping.
→ →**DAGs and FFAs**

ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA · POLO SCIENTIFICO-DIDATTICO DI CESENA

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UNIVERSITÀ DI BOLOGNA
SEDE DI CESENA

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http://www.distal.unibo.it/it/ricerca/ambiti-di-ricerca/scienze-e-tecnologie-degli-alimenti/analisi-strumentali-e-sensoriali

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09 - Ten years of olive oil traceability: the se of "Food Genomics" to ensure the traceability of olive oil

by N. Marmiroli

Ten years of olive oil traceability: the use of "Food Genomics" to ensure the traceability of olive oil.

Nelson Marmiroli, Michelangelo Vietina, Caterina Agrimonti

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The capacity to identify the genetic components of foodstuffs has been exploited to obtain traceability and this is the concept of "Food Genomics".
Ten years featuring the start of OLIV-TRACK project (QLK1-CT-2002-02386) have led to development of methods based on DNA analyses for olive oil authentication. These methods have been successfully applied because of their high specificity, sensitivity and of their affordable costs.
The notion of an "Identity Card" of a prized olive oil needs to specify three items: *i*) authenticity or absence of adulterating oils; *ii*) geographical origin and *iii*) varietal composition. If determination of metabolite content and isotopic determination are the most used method to assess the geographical origin of oils, DNA may become the method of election to determine also their botanical composition. Methods for DNA extraction from olive oil have been developed and reviewed in Agrimonti et al., 2011. A range of molecular markers platforms has been developed that can be used both for the determination of plant genotype in fruits and in olive.
These platforms include randomly amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs; Pafundo et al., 2005), microsatellites (SSRs; Vietina et al., 2011), single-nucleotide polymorphisms (SNPs; Consolandi et al., 2008) and sequence characterised amplified regions (SCARs; Pafundo et al., 2007). All these markers can be useful to generate an "Identity Card" for the identification of a highly prized oil and for the adulteration of possible contaminants (alien oil; Vietina et al ., 2013).
The whole procedure is discussed, in the context of assembling an analytical platform suitable for the elaboration of an "Identity Card" for premium olive oils.

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Ten years of olive oil traceability: the use of Food Genomics to ensure the traceability of olive oil

Workshop
Authentication of ☼Olive Oil
10-11 June 2013, Madrid

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Ten years from the beginning of **OLIV-TRACK project (QLK1-CT-2002-02386)** have led to development of methods based on DNA for olive oil authentication.

DNA methods have been successfully applied because of their high specificity and sensitivity.

DNA is an invariant analyte that may be used to trace any cultivar contribution to an olive oil independently of the place of origin, leading to identity or authenticity determination.

The capacity to identify the genetic components of foodstuffs has been exploited to obtain traceability and this is the concept of **"Food Genomics".**

The notion of an **"Identity Card"** of a prized olive oil needs to specify three items:

i) authenticity or absence of adulterating oils;
ii) geographical origin;
iii) varietal composition.

THE USE OF FOOD GENOMICS TO ENSURE THE TRACEABILITY OF OLIVE OIL

- DNA extraction
- PCR amplification
- Genetic fingerprinting using molecular markers
- Identification of contaminants (alien oils)
- Platforms: PCR and Real-Time PCR
  Array
  HRM (High Resolution Melting) PCR


Trends in Food Science & Technology 22 (2011) 237–244

FOOD SCIENCE & TECHNOLOGY

Review

The use of food genomics to ensure the traceability of olive oil

Caterina Agrimonti, Michelangelo Vietina, Simona Pafundo and Nelson Marmiroli*

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THE EXTRACTION OF DNA FROM OLIVE OIL

Kinds of methods	Starting oil volume
CTAB methods	0,3<ml<400
DNA bindings	1<ml<500
Other methods	2<ml<250

With the purpose of scaling up for application at industrial level the methods were also evaluated for:

i) **cost of reagents;**
ii) **equipment required**;
iii) **training of personnel**;
iv) **flexibility and time needed for completing the extraction**.

Globally, this study demonstrated that DNA extraction from olive oils of different degrees of processing was indeed possible and that the DNA obtained can be amplified.

THE EXTRACTION OF DNA FROM OLIVE OIL: STORAGE-TIME

Food Chemistry 123 (2010) 787–793

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Storage-time effects on olive oil DNA assessed by Amplified Fragments Length Polymorphisms

Simona Pafundo[a], Matteo Busconi[b], Caterina Agrimonti[a], Corrado Fogher[b], Nelson Marmiroli[a,*]

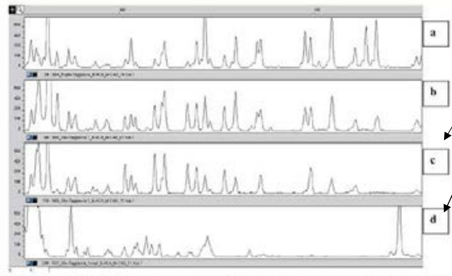


Fig. 1. (a) Electropherograms of AFLP profiles with the selective primers combination E-ACA/M-CAG: from leaves of cultivar Taggiasca, (b) from DNA extracted from monovarietal Taggiasca oil after a week, (c) from DNA extracted from monovarietal Taggiasca oil after three weeks, (d) from DNA extracted from monovarietal Taggiasca oil after one year.

AFLP profiles derived from Taggiasca leaves (Fig. 1a) and from the respective monovarietal oil, stored for:

1. one week (Fig. 1b);
2. three weeks (Fig. 1c);
3. one year (Fig. 1d).

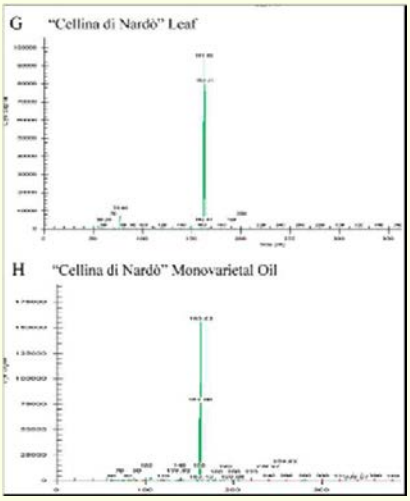
Although the AFLP profiles of leaf and of the oil DNA extracted after one and three weeks of storage were similar, the same profiles were quite different for DNA extracted in the one year stored oils.

MOLECULAR MARKER APPROACHES FOR ASSESSING VARIETAL COMPOSITION OF OIL

Molecular markers, already employed for the identification of olive varieties, are directly applicable to the analysis of DNA derived from oil.

	Reproducibility	Detection of polymorphims	High-throughput platform adaptability	Costs
RAPDs	Low	Low	Low	Low
AFLPs	High	High	Low	Medium
SCARs	High	High	Real-Time PCR Array	Medium
SSRs	High	High	Capillary electrophoresis HRM Real-Time PCR	Medium
SNPs	High	High	Real-Time PCR HRM Real-Time PCR Array	Medium

Identification of the plant contribute to olive oil with molecular markers: SCAR markers in olive oil



JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
J. Agric. Food Chem. 2005, 53, 6995–7002 6995

Traceability of Plant Contribution in Olive Oil by Amplified Fragment Length Polymorphisms

Simona Pafundo, Caterina Agrimonti, and Nelson Marmiroli*

6052 J. Agric. Food Chem. 2007, 55, 6052–6059
JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Applicability of SCAR Markers to Food Genomics: Olive Oil Traceability

Simona Pafundo, Caterina Agrimonti, Elena Maestri, and Nelson Marmiroli*

Electropherograms obtained in capillary electrophoresis with the primer labeled with the fluorescent dye Cy5.5.

(G) Cellina di Nardo` leaf

(H) Cellina di Nardo` monovarietal oil

Research Article
SCI
Received: 27 July 2010 Revised: 9 December 2010 Accepted: 20 December 2010 Published online in Wiley Online Library: 7 March 2011
(wileyonlinelibrary.com) DOI 10.1002/jsfa.4317

Applicability of SSR markers to the traceability of monovarietal olive oils

Michelangelo Vietina, Caterina Agrimonti, Marta Marmiroli, Urbana Bonas and Nelson Marmiroli*

Simple sequence repeat (SSR) markers have already proved useful in DNA fingerprinting because they:

(i) are characterised by a high level of polymorphism due to variations in the number of repeats;

(ii) are easily amplifiable by PCR;

(iii) can be rapidly analysed in a high-throughput genotyping platform.

Table 4. Behaviour of SSR markers on DNA extracted from different monovarietal oils. The parameters evaluated are complete correspondence, partial correspondence with leaf reference, total correspondence (amplificability) and reproducibility

SSR	Complete correspondence (%)	Partial correspondence (%)	Total correspondence (%)	Reproducibility (%)
GAPU89	6.12	43.20	49.32	24.34 ± 20.00
DCA5	18.52	22.22	40.74	71.43 ± 21.82
EMO30	10.58	26.46	37.04	57.67 ± 18.47
GAPU71B	7.93	28.96	36.89	31.75 ± 14.54
UDO099-09	8.67	23.12	31.79	23.02 ± 16.54
EMO90	0.00	30.98	30.98	46.30 ± 20.29
DCA18	6.17	8.64	14.81	Not determined
DCA17	6.35	5.56	11.91	29.89 ± 14.10
DCA8	0.00	5.26	5.26	Not determined

Total correspondence is the sum of complete and partial correspondences. All analyses were performed on a pool of 21 oils. Nine amplifications were conducted for each SSR locus for each oil. For each amplification set, two controls were introduced: an extraction blank and a sample with no DNA.

Table shows the differences in amplificability of SSR loci in the different oils (from Vietina et al., 2011)

The SSR **GAPU89** gave the highest value of amplificability (**49.32%**), followed by the loci **DCA5, EMO30, GAPU71B, UDO099-09** and **EMO90** with values ranging between **40.74 and 30.98%;** however, **DCA18, DCA17 and DCA8** performed poorly, with amplificability values **below 20%.**

Average **reproducibility of the SSRs** was calculated as the percentage of identical profiles in a typical 'three-replicate' experiment producing amplicons with sizes within the acceptable range.
The locus DCA5 gave the highest reproducibility (71.43±21.82%), while UDO099 09 gave the lowest (23.02 ± 16.54%).

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Journal of BIOTECHNOLOGY
www.elsevier.com/locate/jbiotec

Olive variety identification by ligation detection reaction in a universal array format

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Abstract
Molecular methodologies are increasingly being developed to assess the origin and authenticity of raw organic materials an
roducts. Here we describe the application of a microarray-based assay for single nucleotide polymorphisms (SNPs) identification
he assay distinguishes alleles in a ligation detection reaction (LDR), with subsequent fluorescent detection by hybridization on
UA). The LDR-UA approach was used to detect 17 SNPs in olive genomic sequences previously amplified by PCR from fi
enotype calls obtained with the LDR-UA were in full agreement with those determined by direct sequencing. The panel of 17 S
o discriminate 49 olive varieties selected among the most widely cultivated for olive oil production in the Mediterranean area.

Keywords: Olive; Single nucleotide polymorphisms; Genotyping array; Authenticity

LDR-Universal array

From olive genomics

To oil genomics

Eur Food Res Technol
DOI 10.1007/s00217-008-0863-5

ORIGINAL PAPER

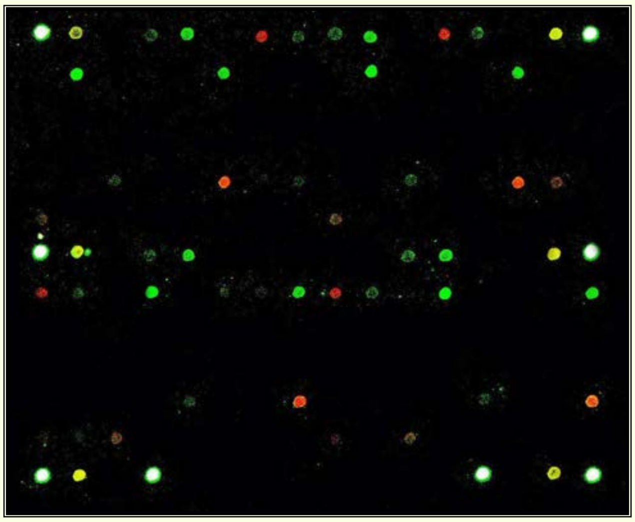
A procedure for olive oil traceability and authenticity: DNA extraction, multiplex PCR and LDR–universal array analysis

Clarissa Consolandi · Luisa Palmieri · Marco Severgnini · Elena Maestri · Nelson Marmiroli · Caterina Agrimonti · Luciana Baldoni · Paolo Donini · Gianluca De Bellis · Bianca Castiglioni

Received: 21 December 2007 / Revised: 6 March 2008 / Accepted: 10 March 2008

Abstract Traceability of olive oils is relevant not only in assessing their origin, but also in protecting against frauds. Here, we present an improvement of the assay previously developed for the genotyping of forty-nine frequently grown Mediterranean olive cultivars by ligation detection reaction (LDR)/universal array (UA), refining the entire procedure in order to address DNA extracted from monovarietal olive oils. Firstly, a simple and robust protocol to extract amplifiable DNA from olive oil was developed. Then, the SNP-containing DNA sequences were simultaneous amplified by multiplex PCR and used on a LDR-UA platform, which gave precise and accurate genotype results. Thirteen out of the seventeen investigated SNPs were amplifiable in multiplex PCR, and were sufficient to unequivocally discriminate the forty-nine cultivars. The availability of this semi-automated SNP genotyping assay should help food testing laboratories to verify the origin and authenticity of monovarietal extra-virgin olive oils.

Olive cultivar "Cellina di Nardò" analysed with microarrays based on SNP identification



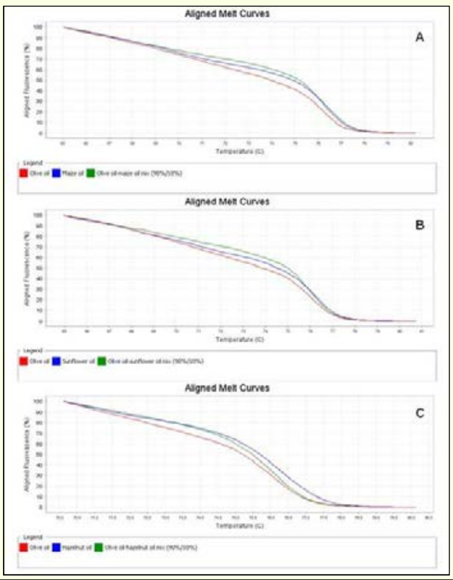
SNPs NAME
ACP.1
ACP.2
FAD2.1
FAD2.3
PAL70
PAL219
PAL223
PAL506
SNP G
SNP E
SNP J
SNP H
SNP F
SNP K
SNP I
SNP D

With one single analysis it is possible to genotype a sample for many different sequences at the same time.

Ligation Detection Reaction platform with universal ZIP code

Vietina, M., Agrimonti, C., Marmiroli., N. (2013). Detection of plant oil DNA using High Resolution Melting (HRM) post PCR analysis: a tool for disclosure of olive oil adulteration. *Food Chemistry, In press*.

High Resolution Melt curve analysis of PCR products obtained from oil's DNA

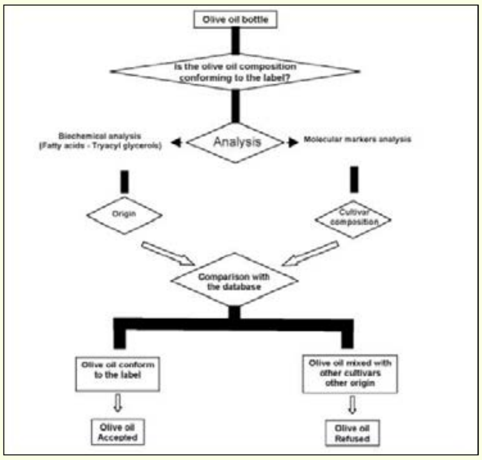


A. Red curve: HRM of DNA extracted from olive oil;
blue curve: HRM of DNA extracted from maize seed oil;
green curve: HRM of DNA extracted from an olive and maize oil mix (90%-10%).

B. Red curve: HRM of DNA extracted from olive oil;
blue curve: HRM of DNA extracted from sunflower seed oil;
green curve: HRM of DNA extracted from an olive and sunflower oils mix (90%-10%).

C. Red curve: HRM of DNA extracted from olive oil;
blue curve: HRM of DNA extracted from hazelnut seed oil;
green curve: HRM of DNA extracted from an olive and hazelnut oils mix (90%-10%).

Olive oil label validation flow chart



The flow chart, presented shows a procedure that can be adopted to accept or refuse a label used for an olive oil.

The olive oil database can be considered a reference system in evaluating data obtained from the analysis of unknown samples and in defining the origin and the composition of the olive oil.

Acknowledgement
s

Quality of Life and Management of Living Resources

QLK1-CT-2002-02386

Traceability of origin and authenticity of olive oil by combined genomic and metabolomic approaches

This study has been carried out with financial support from the Commission of the European Communities, specific RTD programme "Quality of Life and Management of Living Resources" project, QLK1-CT-2002-02386, "Traceability of origin and authenticity of olive oil by combined genomic and metabolomic approaches (OLIV-TRACK)" coordinated by N. Marmiroli. The content of this paper does not necessarily reflect the Commission of the European Communities views and in no way anticipates the Commission's future policy in this area. This paper had also the contribute of the Italian Minister of University and Research special program PRIN "Rintracciabilità della composizione e dell'origine di oli d'oliva DOP, IGP e 100% Italiani attraverso metodiche genomiche, proteomiche e metabolomiche" coordinated also by N. Marmiroli and a contribute from the University of Parma (fund FIL 2002, 2003, 2004, 2005, 2006). This work was also supported financially by Emilia-Romagna (IT) Regional project SIQUAL within the research framework PRRIITT, Misura 3.4.

10 - Replacing traditional, ineffectual limits with new and functional methods

by R. Mailer

Replacing Traditional, Ineffectual Limits with New and Functional Methods

Rodney Mailer

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Although olive trees were planted in Australia over 200 years ago, the industry is relatively young with commercial production becoming a reality over the last 30 years. There has however been a rapid expansion of the crop throughout appropriate growing areas. This development was assisted in early stages by the IOC who provided information on how to produce and test the product. Within this short time, we have seen the development of a sophisticated and modern process from propagation, growing, processing, quality control and marketing. Most importantly, Australian producers have been focused on producing a high quality product that meets international standards. The Australian Olive Association has developed a "Code of Conduct" product evaluation system to monitor Australian oil quality. The product is tested at several stages to ensure authenticity, quality and freshness.

Australia has been confronted by unnecessary restrictions on olive oil to meet traditional European standards initially developed around olive oil produced in Mediterranean countries. An example is fatty acids which are influenced by growing temperatures. IOC limits, developed for olive oil from Mediterranean regions, do not allow for the range of growing conditions of new world producers. Many years and vast sums of money have been invested in trying to prove that genuine olive oil may have 1.5% linolenic acid rather than 1.0% imposed by IOC (IOC 2011). Similarly, new cultivars are excluded because they have different sterol profiles to traditional cultivars. European limits have become barriers to innovation and to producers of the new world.

Despite this, poor quality olive oils continues to proliferate in Australian and US supermarkets, illustrated by numerous recent studies, with the majority of olive oils failing to meet IOC limits for EVOO. Some of these oils have been found to contain seed oil and others are old or refined oils. These findings have been reported to international standards authorities to no avail. In fact, Australia has been publicly chastised by the IOC for carrying out such studies and publicising the results.

With little support from the EU or IOC, Australia has worked closely with German scientists on new methods to detect fraud. Two methods in particular, DAGs and pyropheophytins, have shown considerable promise in determining oils which are old, have been poorly stored or possibly refined (Guillaume *et al* 2013). Considerable data have been acquired and published in peer reviewed journals illustrating good relationships between PPP, DAG and sensory analysis. There has however been resistance from some organisations to investigate these methods despite the evidence.

There is a strong argument toward having international standards for world trade. However, the continuing failure of Codex to recognise environmental variation within olive oil has severely hampered the process of international harmony. Although other primary industries are working together internationally, Codex has failed to reach a consensus on minor limits in olive oil. As a result there is a move towards national standards through which producers and buyers will need to negotiate. It is critical that the EU assist standards organisations to be more inclusive in setting trading standards.

References

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Guillaume, C., Gertz, Ch., Ravetti, L. (2013). Pyropheophytin a and 1,2 di-acyl-glycerols over time under different storage conditions in natural olive oils. *Journal American Oil Chemists 'Society*, in press.

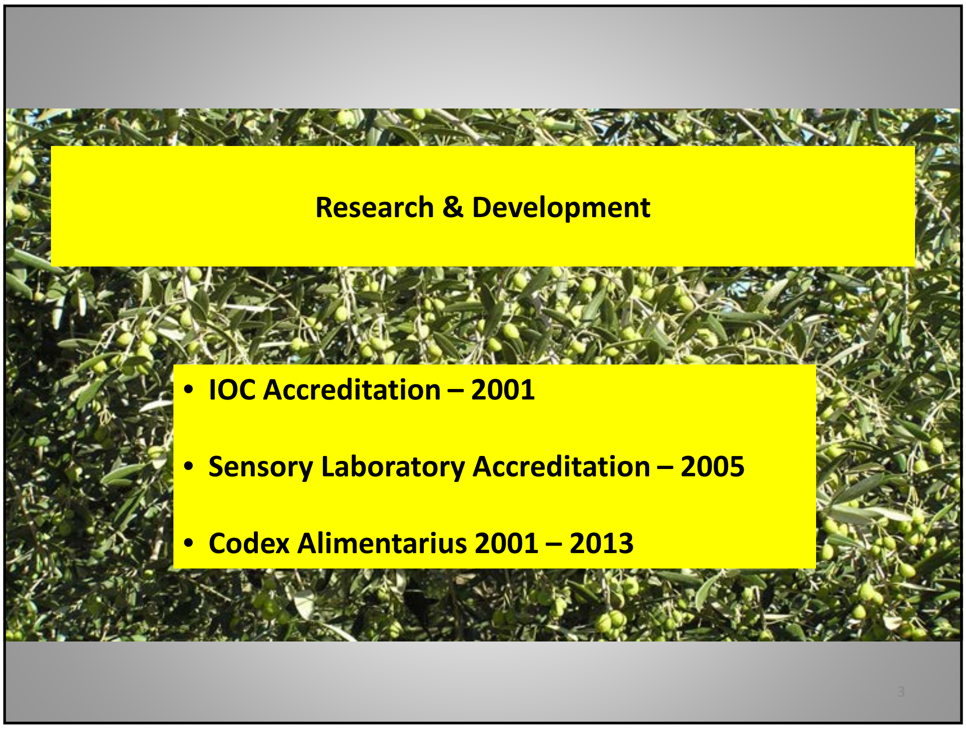
Authentication of Olive Oil Workshop
Madrid 10-11 June 2013

Replacing Traditional Limits with New & Functional Methods

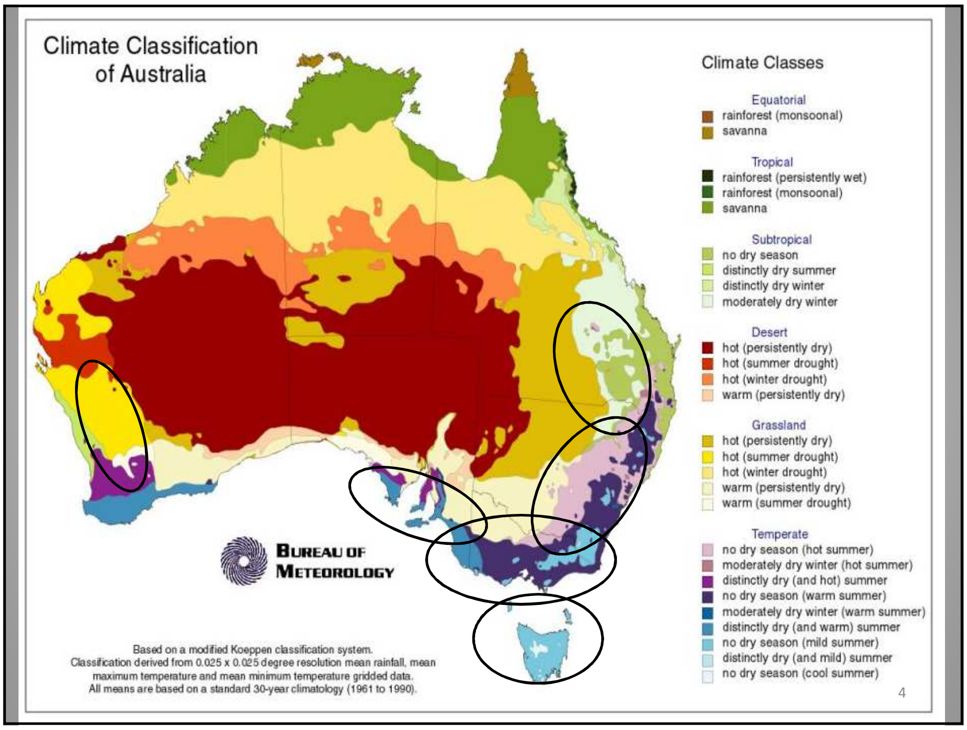
Dr Rodney Mailer
Australian Oils Research
Australia

Australian Olive Association Ltd

Traditional to Modern



Research & Development
• IOC Accreditation – 2001
• Sensory Laboratory Accreditation – 2005
• Codex Alimentarius 2001 – 2013
3



Climate Classification
of Australia
Climate Classes
Equatorial
rainforest (monsoonal)
savanna
Tropical
rainforest (persistently wet)
rainforest (monsoonal)
savanna
Subtropical
no dry season
distinctly dry summer
distinctly dry winter
moderately dry winter
Desert
hot (persistently dry)
hot (summer drought)
hot (winter drought)
warm (persistently dry)
Grassland
hot (persistently dry)
hot (summer drought)
hot (winter drought)
warm (persistently dry)
warm (summer drought)
Temperate
no dry season (hot summer)
moderately dry winter (hot summer)
distinctly dry (and hot) summer
no dry season (warm summer)
moderately dry winter (warm summer)
distinctly dry (and warm) summer
no dry season (mild summer)
distinctly dry (and mild) summer
no dry season (cool summer)
Bureau of Meteorology
Based on a modified Koeppen classification system.
Classification derived from 0.025 x 0.025 degree resolution mean rainfall, mean maximum temperature and mean minimum temperature gridded data.
All means are based on a standard 30-year climatology (1961 to 1990).
4

FATTY ACID COMPOSITION OF AUSTRALIAN OILS

	C 16:0	C16:1	C 17:1	C 18:1	C 18:2	C18:3	C 20:0	C 20:1
IOC Limits	7.5-20.0	0.3-3.5	0.0-0.3	55.0-83.0	3.5-21.0	0.0-1.0	0.0-0.6	0.0-0.4
Average	12.7	1.1	0.1	73.7	9.1	0.7	0.3	0.3
Maximum	20.3	3.6	0.5	84.2	23.8	1.7	0.7	0.6
Mimimum	6.7	0.3	0.0	53.9	2.2	0.3	0.2	0.0
No. of Samples	1935	1935	1935	1935	1935	1935	1935	1935
% Outside IOC Standard	0.4%	0.2%	0.3%	0.5%	1.5%	0.5%	0.1%	2.8%

5

STEROL COMPOSITION OF AUSTRALIAN OILS

	Cholesterol	Brassicasterol	Campesterol	Stigmasterol	D-7-Stigmastenol	β -Sitosterol	Total sterols
IOC LIMITS	0.0-0.5	0.0-0.1	0.0-4.0	< Camp.	0.0-0.5	93.0-100.0	> 1000
AVERAGE	0.2	0.0	3.7	0.7	0.2	94.2	1679.9
MAXIMUM	0.8	0.2	5.0	2.3	1.2	96.7	2862.0
MINIMUM	0.0	0.0	1.9	0.0	0.0	92.0	789.2
NUMBER OF SAMPLES	888	888	888	888	888	888	888
% OUTSIDE IOC STANDARD	0.6%	0.0%	32.8%	0.0%	0.6%	2.9%	1.7%

6

IOC Tests	Results	IOC # limits
Free Fatty Acids	**0.27**	**≤ 0.8**
Peroxide Value	**17**	**≤ 20**
UV Absorbance		
DK	**0.070**	**≤ 0.01**
K_{232nm}	**3.188**	**≤ 2.50**
K_{270nm}	**0.892**	**≤ 0.22**
Stigmastadiene Content	**6.160**	**≤ 0.10**
Unsaponifiable matter	**15.4**	**≤ 15**
Wax Content	**2002**	**≤ 250**
Trans Fatty Acids		
C18:1T	**0.071**	**≤ 0.05**
C18:2T and C18:3T	**0.099**	**≤ 0.05**

Adulterated Supermarket oils - Fatty Acid Composition

IOC LIMITS	C 16:0	C16:1	C 17:1	C 18:1	C 18:2	C18:3	C 20:0	C 20:1
	7.5-20.0	0.3-3.5	0.0-0.3	55.0-83.0	3.5-21.0	0.0-1.0	0.0-0.6	0.0-0.4
EVOO	**14.0**	**1.7**	**0.1**	**59.4**	**19.3**	**2.0**	**0.4**	**0.4**
Pure Olive Oil	**6.9**	**0.5**	**0.1**	**68.2**	**15.4**	**4.9**	**0.5**	**0.8**

2 Supermarket oils - Sterol Composition

	Cholesterol	Brassicasterol	24-Methylene-cholesterol	Campesterol	Campestanol	Stigmasterol	D-7-Campesterol	D-5,23-Stigmastadienol	Clerosterol	b-Sitosterol	Sitostanol	D-5- Avenasterol	D-5,24- Stigmastadienol	D-7- Stigmastenol	D-7- Avenasterol	Apparent β –Sitosterol	Diols	Total Sterols (mg/kg)
EVOO	0.1	3.1	0.3	12.9	0.1	1.1	0.2	0.1	0.6	72.3	0.6	6.9	0.8	0.4	0.5	81.3	1.3	2744
Pure Olive Oil	0.2	6.0	0.4	22.8	0.1	0.8	0.2	0.2	0.7	64.3	0.7	2.3	1.0	0.3	0.2	69.2	6.1	5142

IOC Limits	Cholesterol	Brassicasterol	Campesterol	Stigmasterol	D-7-Stigmastenol	β –Sitosterol	Total sterols
	0.0-0.5	0.0-0.1	0.0-4.0	< Camp.	0.0-0.5	93.0-100.0	> 1000

9

FAILED

14 in 28

10

UC Davis Studies

- 73% imported oils failed *sensory* test
- Several failed IOC UV tests
- Strong relationship between *sensory* and DAGs (65%) and PPP (49%).
- No relationship between *sensory* and FFA, FAP or PV.

- IOC & Codex limits do not account for natural variation
- IOC methods are limited in determining authenticity
- IOC limits fail to detect old oil
- Many countries are modifying the IOC regulations.

12

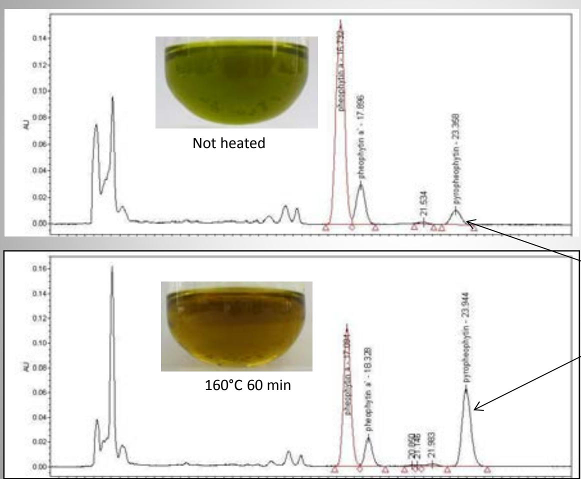
Diacylglycerol – DAGS

&

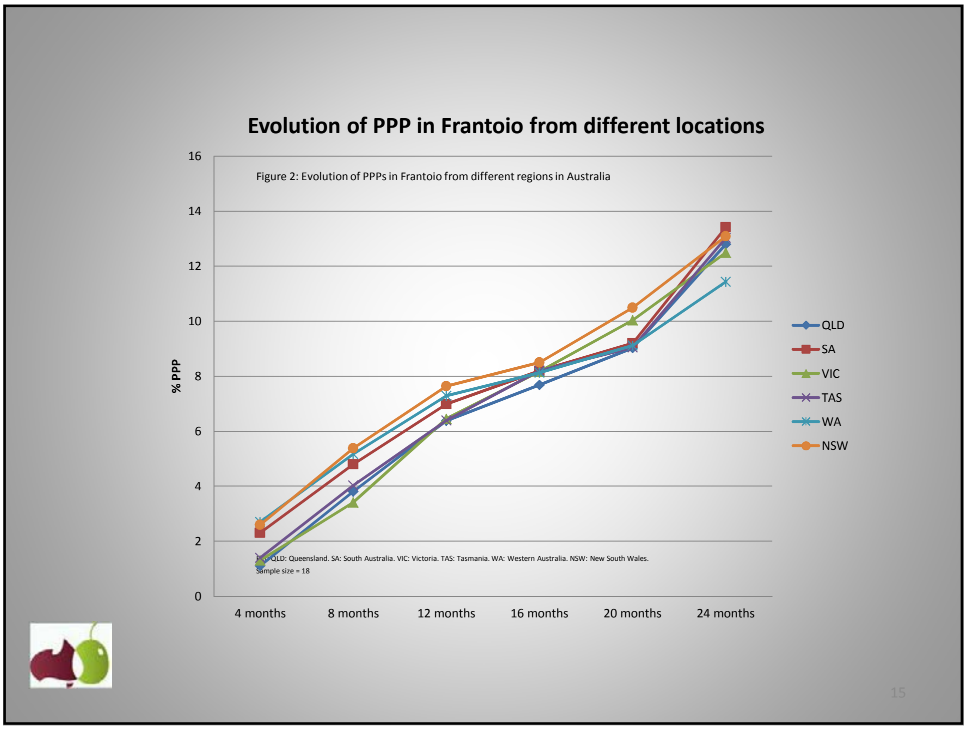
Pyropheophytin – PPP

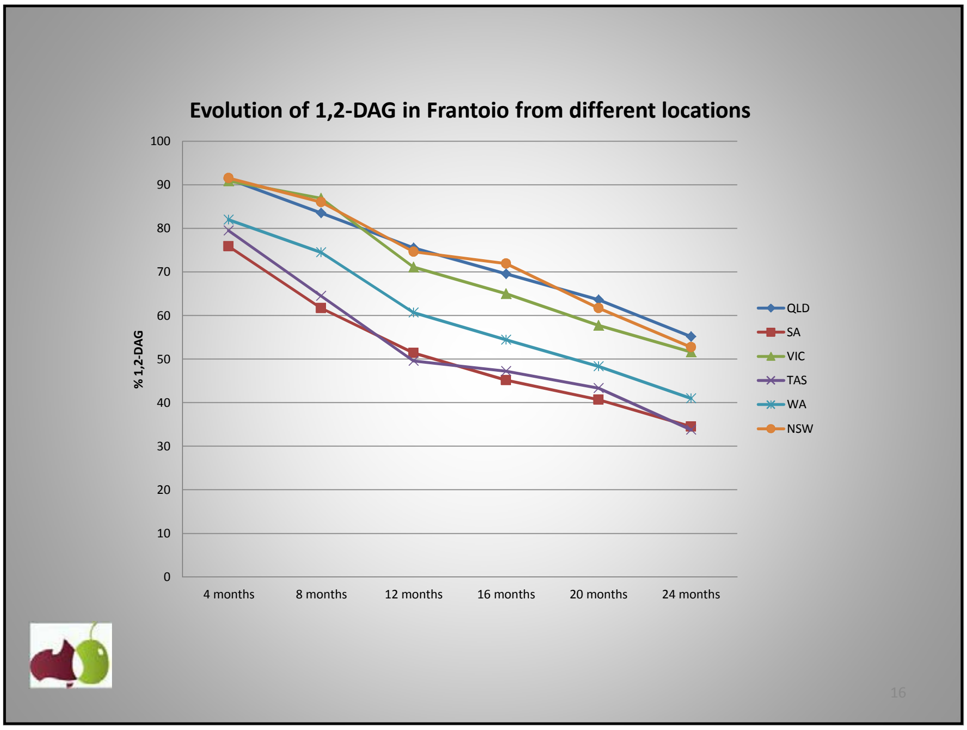
New Australian Standard

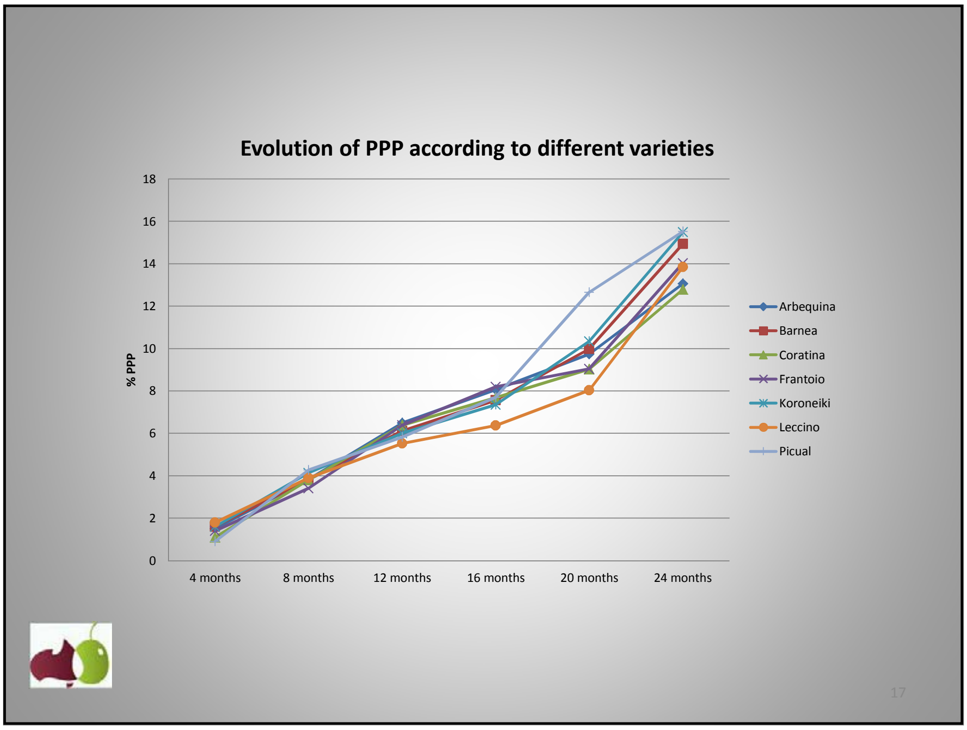
Pyropheophytin a

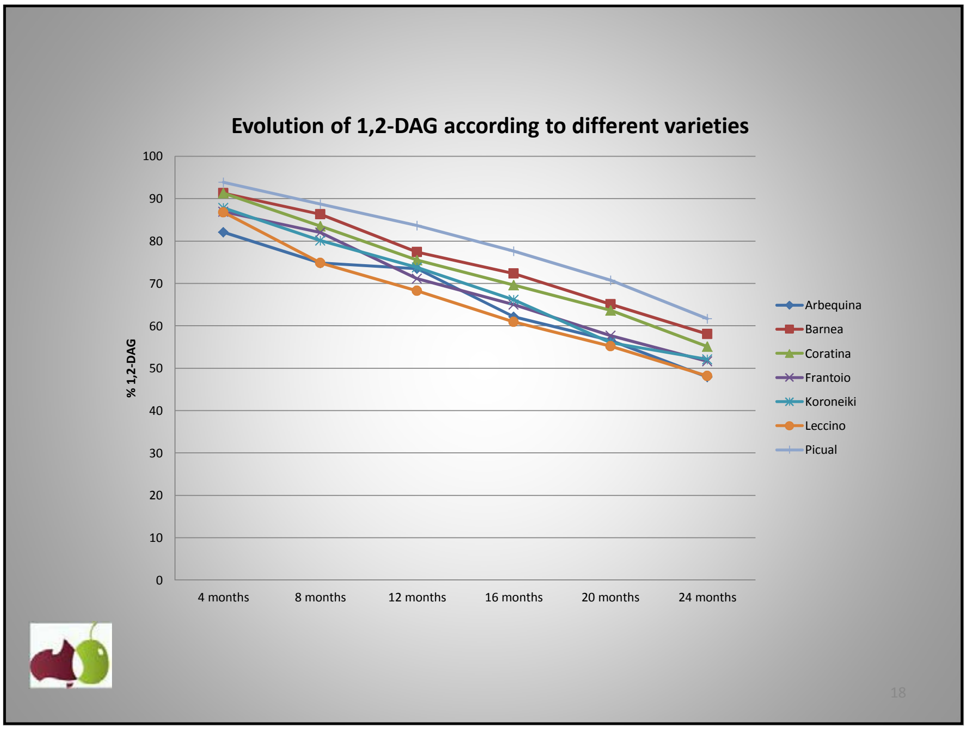


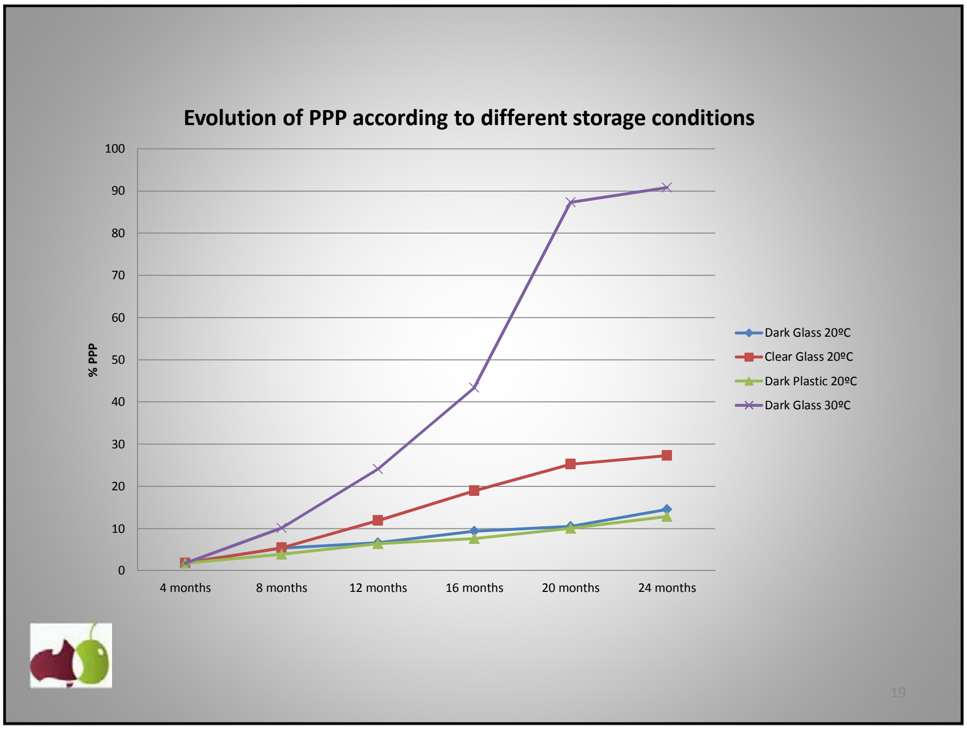
Pyropheophytin a

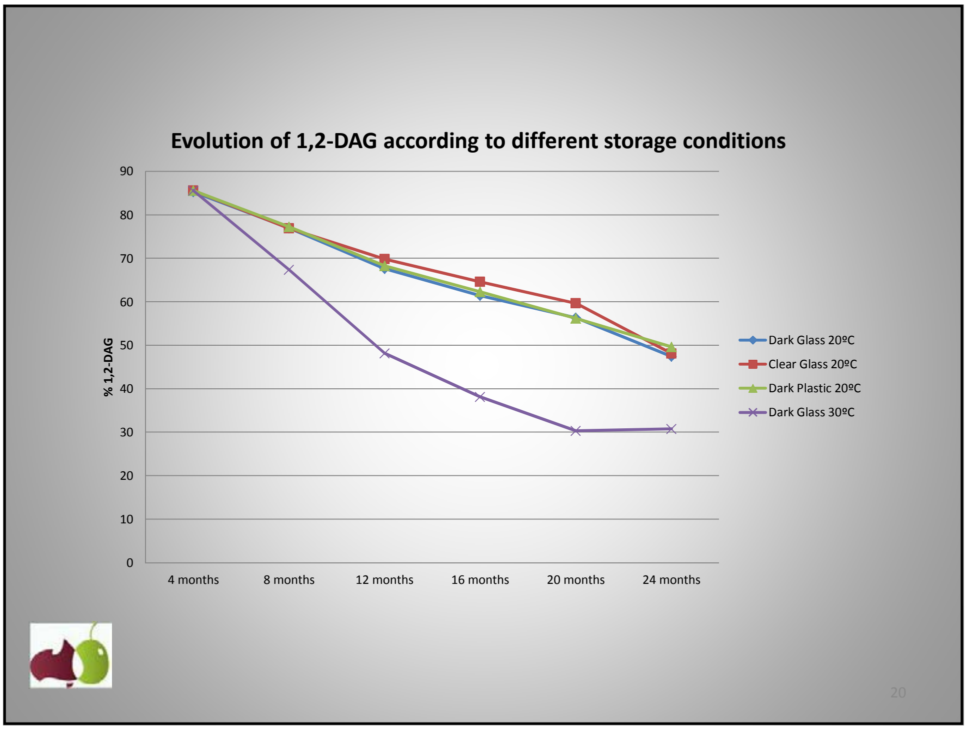

Evolution of PPP in Frantoio from different locations
Figure 2: Evolution of PPPs in Frantoio from different regions in Australia
% PPP
0
2
4
6
8
10
12
14
16
4 months
8 months
12 months
16 months
20 months
24 months
QLD
SA
VIC
TAS
WA
NSW
QLD: Queensland. SA: South Australia. VIC: Victoria. TAS: Tasmania. WA: Western Australia. NSW: New South Wales.
Sample size = 18
15


Evolution of 1,2-DAG in Frantoio from different locations
% 1,2-DAG
0
10
20
30
40
50
60
70
80
90
100
4 months
8 months
12 months
16 months
20 months
24 months
QLD
SA
VIC
TAS
WA
NSW
16


Evolution of PPP according to different varieties
% PPP
18
16
14
12
10
8
6
4
2
0
4 months
8 months
12 months
16 months
20 months
24 months
Arbequina
Barnea
Coratina
Frantoio
Koroneiki
Leccino
Picual
17


Evolution of 1,2-DAG according to different varieties
% 1,2-DAG
100
90
80
70
60
50
40
30
20
10
0
4 months
8 months
12 months
16 months
20 months
24 months
Arbequina
Barnea
Coratina
Frantoio
Koroneiki
Leccino
Picual
18


Evolution of PPP according to different storage conditions
% PPP
100
90
80
70
60
50
40
30
20
10
0
4 months
8 months
12 months
16 months
20 months
24 months
Dark Glass 20ºC
Clear Glass 20ºC
Dark Plastic 20ºC
Dark Glass 30ºC
19


Evolution of 1,2-DAG according to different storage conditions
% 1,2-DAG
90
80
70
60
50
40
30
20
10
0
4 months
8 months
12 months
16 months
20 months
24 months
Dark Glass 20ºC
Clear Glass 20ºC
Dark Plastic 20ºC
Dark Glass 30ºC
20

Fatty Acid Composition of Australian oils

	C 16:0	C16:1	C 17:1	C 18:1	C 18:2	C18:3	C 20:0	C 20:1
IOC LIMITS	7.5-20.0	0.3-3.5	0.0-0.3	55.0-83.0	3.5-21.0	0.0-1.0	0.0-0.6	0.0-0.4
AUSTRALIAN STANDARD LIMITS	**7.0-20.0**	0.3-3.5	**0.0-0.4**	**53.0-85.0**	**2.5-22.0**	**0.0-1.5**	0.0-0.6	**0.0-0.5**

Sterol Composition of Australian oils

	Cholesterol	Brassicasterol	Campesterol	Stigmasterol	D-7-Stigmastenol	β -Sitosterol	Total sterols
IOC LIMITS	0.0-0.5	0.0-0.1	0.0-4.0	< Camp.	0.0-0.5	93.0-100.0	➢1000
AUSTRALIAN STANDARD LIMITS	0.0-0.5	0.0-0.1	**0.0-4.8**	**0.0-1.9**	0.0-0.5	**92.5-100.0**	➢1000

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SUMMARY

- **Requests for changes to IOC limits have been rejected**
- **Australia, and researchers in the US, have been criticised by the IOC for exposing fraud**
- **Codex Alimentarius have been encouraged to resist change by the EU and IOC**

CONCLUSION

- International standards need to recognise natural variation
- There needs to be more evaluation of DAGs and PPPs
- EU needs to help change Codex Alimentarius

- There needs to be a more inclusive international approach to combating fraud.

11 - A critical evaluation of some methods used to assess purity of olive oils

by L. Conte

A CRITICAL EVALUATION OF SOME METHODS USED TO ASSESS PURITY OF OLIVE OILS

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The assessment of purity of olive oils, as performed by official methods (both described into law, e.g. UE Regulations and into trade standard – e.g. IOC trade standard) is carried out by means of a number of analytical evaluation that had been modified as time passed, in order to get more reliable results, as depending also on the progress of analytical instruments.
In the meantime, however, also technology applied to produce faked oils improved, so that in some cases, the official and well validated parameters and related methods seem to be no more effective.
In this presentation, some data concerning the reliability of some official and not official analytical methods are presented, when they are applied to peculiar faked oils.
In the first part of the speech, alkyl esters, pigments and diacylglycerols are evaluated in authentic extra virgin olive oils as well as in deodorised olive oils and in mixtures of these two. Pigments derived by chlorophyll degradation and diacylglycerols isomerisation had been proposed by Serani et al and then by Gertz et al, while alkyl esters were proposed later by Perez Camino et al.
Results highlighted that alkylesters were the more reliable approach, as the other two can be influenced by the characteristics of oils used to produce the mixtures.
Official parameters as stigmastadienes and ΔECN42 are usually retained as the most suitable parameters to assess the presence of extraneous oils, however, selected mixtures can not be detected by these parameters, while triacylglycerols when analysed by gas chromatography can have some more chance of success. Some data will be presented concerning this approach and some hypothesis of limit for selected triacylglycerols will be presented

References

Serani A., Piacenti D. (2001), Analisi dei pigmenti clorofilliani in oli vergini di oliva" Sistema analitico per l'identificazione di oli deodorati in oli vergini di oliva, *Rivista Italiana delle Sostanze Grasse, 78, 459-463*

Gertz C., Fiebig H.J., (2006). Determination of thermal degradation products of chlorophyll a in virgin olive oil. *European Journal of Lipid Science and Technology, 108, 1062-1065*

Serani A., Piacenti D., (2001) Analisi HPLC/VIS delle feofitine e GC/FID dei digliceridi in oli vergini di oliva. Sistema analitico per l'dentificazione di oli deodorati in oli vergini di oliva, *Rivista Italiana delle Sostanze Grasse, 78,* 567- 570

Serani A., Piacenti D., Staiano G. (2001) Cinetica di isomerizzazione dei digiceridi in oli vergini di oliva Sistema analitico per l'identificazione di oli deodorati in oli vergini di oliva, *Rivista Italiana delle Sostanze Grasse, 78,* 525-528

Gertz C., Fiebig H.J., (2006) Determination of 1,2- and 1,3-diacylglycerols in virgin olive oil . *European Journal of Lipid Science and Technology, 108, 1066-1069*

Perez-Camino M.C, Cert A., Romero-Segura A., Cert-Trujillo R., Moreda W. Alkyl Esters of Fatty Acids a Useful Tool to Detect Soft Deodorized Olive Oils (2008) *Journal of Agricultural and Food Chemistry, 56, 6740–6744*

A CRITICAL EVALUATION OF SOME METHODS USED TO ASSESS PURITY OF OLIVE OILS

Lanfranco Conte[1], Carlo Mariani[2]

1 Dept of Food Science – University of Udine Via Sondrio, 2/A 33100 Udine – IT
2 Innovhub – Stazione Sperimentale per le Industrie degli Oli e dei Grassi-Via G. Colombo. 79, 20133 Milano

- **Are the official / validated parameters / methods nowadays applied to assess quality and purity of olive oils still so effective?**
- **Do some frauds exists suitable to skip the control net at present used?**
- **Is it possible to change some limit / parameter without a real risk that more and more faked oils reach the market?**

2

Speech agenda

- Fatty acid composition and purity
- ΔECN42 and triacylglycerols GLC analysis
- Diacylglycerols, PPP and alkyl esters

3

Fatty acid composition

- The ancient approach of separative techniques for oils and fats analysis
- Olive oil composition modification depending on the cultivation area
- Need for new limits vs need for purity guarantee

4

TABLE I: MIX HIGH OLEIC SUNFLOWER OIL (HOSO)/PALM OIL (LOWER CONCENTRATION) AND OLIVE OIL (SOURCE OF DATA: MARIANI- SSOG AND FABERI IT. FRAUD DETECTION INSPECTORATE)

Sample	C12:0	C14:0	C16:0	C16:1w9	C16:1w7	C17:0	C17:1	C18:0	C18:1t	C18:1c	C18:2tt	C18:2tc	C18:2cc	C20:0	C18:3tcc	C18:3ccc	C20:1	C22:0	C24:0
HOSO	0,09	0,28	11,02	0,04	0,16	0,04	0,04	3,26	0,13	75,45	0,12	0,11	7,60	0,33	0,02	0,11	0,26	0,66	0,26
Olive			12,47	0,08	0,73	0,04	0,07	2,09	0,01	76,27			6,82	0,35		0,65	0,27	0,11	0,04
O + H 2%	0,00	0,01	12,45	0,08	0,72	0,04	0,07	2,11	0,01	76,26	0,00	0,00	6,84	0,34	0,00	0,63	0,27	0,12	0,05
O + H 4%	0,00	0,01	12,42	0,08	0,71	0,04	0,07	2,14	0,02	76,24	0,00	0,00	6,85	0,34	0,00	0,62	0,27	0,13	0,05
O + H 6%	0,01	0,02	12,39	0,08	0,70	0,04	0,07	2,16	0,02	76,22	0,01	0,01	6,87	0,34	0,00	0,61	0,27	0,14	0,06
O + H 8%	0,01	0,02	12,36	0,07	0,69	0,04	0,07	2,18	0,02	76,21	0,01	0,01	6,88	0,34	0,00	0,60	0,27	0,15	0,06
O + H 10%	0,01	0,03	12,33	0,07	0,67	0,04	0,07	2,21	0,02	76,19	0,01	0,01	6,90	0,34	0,00	0,59	0,27	0,16	0,07

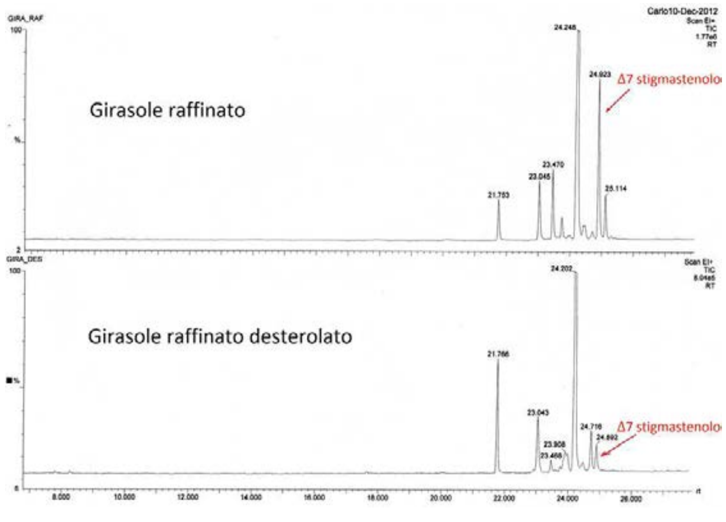
TABLE II: MIX SOYBEAN OIL AND OLIVE OIL (SOURCE OF DATA: CODEX STAN 210-1999, PAGE 6 OF 13)

Sample	C12:0	C14:0	C16:0	C16:1w9	C16:1w7	C17:0	C17:1	C18:0	C18:1t	C18:1c	C18:2tt	C18:2tc	C18:2cc	C20:0	C18:3tcc	C18:3ccc	C20:1	C22:0	C24:0
SOYBEAN	0,10	0,20	13,50	0,00	0,20	0,10	0,10	5,40	0,05	59,00	0,05	0,05	59,00	0,60	0,02	11,00	0,50	0,70	0,50
OLIVE			12,47	0,08	0,73	0,04	0,07	2,09	0,01	76,27			6,82	0,35		0,65	0,27	0,11	0,04
O + S 2%	0,00	0,00	12,49	0,08	0,72	0,04	0,07	2,16	0,01	75,93	0,00	0,00	7,86	0,35	0,00	0,85	0,28	0,12	0,05
O + S 4%	0,00	0,01	12,52	0,07	0,71	0,04	0,07	2,22	0,01	75,58	0,00	0,00	8,91	0,36	0,00	1,06	0,28	0,13	0,06
O + S 6%	0,01	0,01	12,54	0,07	0,70	0,04	0,07	2,29	0,01	75,24	0,00	0,00	9,95	0,36	0,00	1,27	0,29	0,14	0,07
O + S 8%	0,01	0,02	12,56	0,07	0,69	0,04	0,07	2,35	0,01	74,89	0,00	0,00	10,99	0,37	0,00	1,47	0,29	0,15	0,08
O + S 10%	0,01	0,02	12,58	0,07	0,68	0,04	0,07	2,42	0,01	74,54	0,01	0,01	12,04	0,37	0,00	1,68	0,29	0,17	0,09

TABLE III: MIX PALM OIL AND OLIVE OIL (SOURCE OF DATA: MARIANI- SSOG AND FABERI IT. FRAUD DETECTION INSPECTORATE)

Sample	C12:0	C14:0	C16:0	C16:1w9	C16:1w7	C17:0	C17:1	C18:0	C18:1t	C18:1c	C18:2tt	C18:2tc	C18:2cc	C20:0	C18:3tcc	C18:3ccc	C20:1	C22:0	C24:0
Palm+HOSO	0,07	0,22	15,64	0,04	0,19	0,05	0,05	2,80	0,06	70,48	0,10	0,09	8,99	0,24	0,03	0,14	0,19	0,44	0,18
Olive		0,00	12,47	0,08	0,73	0,04	0,07	2,09	0,01	76,27	0,00	0,00	6,82	0,35		0,65	0,27	0,11	0,04
O + PG 2%	0,00	0,00	12,54	0,08	0,72	0,04	0,07	2,10	0,01	76,16	0,00	0,00	6,86	0,34	0,00	0,64	0,27	0,11	0,05
O + PG 4%	0,00	0,01	12,60	0,08	0,71	0,04	0,07	2,12	0,01	76,04	0,00	0,00	6,91	0,34	0,00	0,63	0,27	0,12	0,05
O + PG 6%	0,00	0,01	12,66	0,08	0,70	0,04	0,07	2,13	0,01	75,92	0,01	0,01	6,95	0,34	0,00	0,62	0,27	0,13	0,05
O + PG 8%	0,01	0,02	12,73	0,07	0,69	0,04	0,07	2,15	0,01	75,81	0,01	0,01	6,99	0,34	0,00	0,60	0,26	0,13	0,05
O + PG 10%	0,01	0,02	12,79	0,07	0,68	0,04	0,07	2,16	0,02	75,69	0,01	0,01	7,04	0,34	0,00	0,59	0,26	0,14	0,06

What about sterols?



Probably ,a 20% of such an oil in OO could not be detectded

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Sample	ΔECN 42	THEORETICAL VALUE
OLIVE	0,05	
SUNFLOWER	12,15	
PALM + SUNFLOWER	0,74	
OLIVE + SUNFLOWER 2%	0,04	0,31
OLIVE + SUNFLOWER 4%	0,01	0,31
OLIVE + SUNFLOWER 6%	0,02	0,30
OLIVE + SUNFLOWER 8%	0,06	0,30
OLIVE + SUNFLOWER 10%	0,00	0,29
OLIVE + PALM + SUNFLOWER 2%	0,11	0,17
OLIVE + PALM + SUNFLOWER 4%	0,10	0,17
OLIVE + PALM + SUNFLOWER 6%	0,15	0,18
OLIVE + PALM + SUNFLOWER 8%	0,12	0,18
		Quantità
OLIVE	0,01	100,000
SUNFLOWER	0,28	10,000
PALM	0,37	25,000

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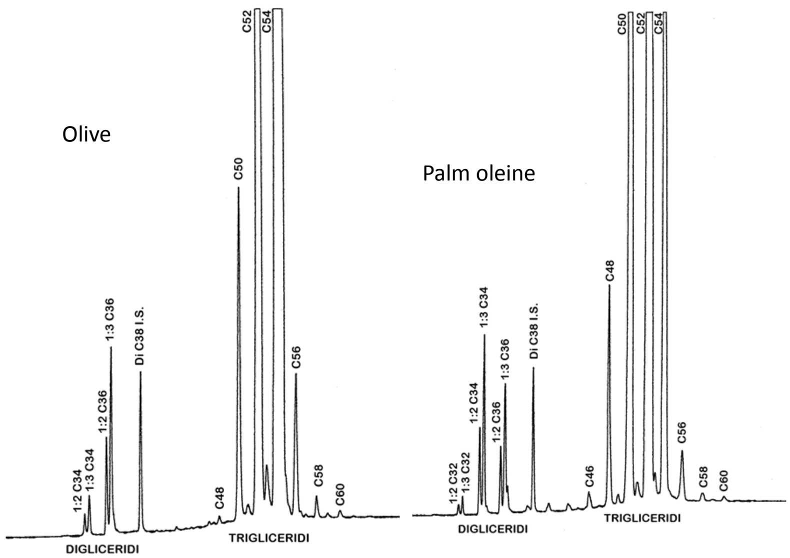
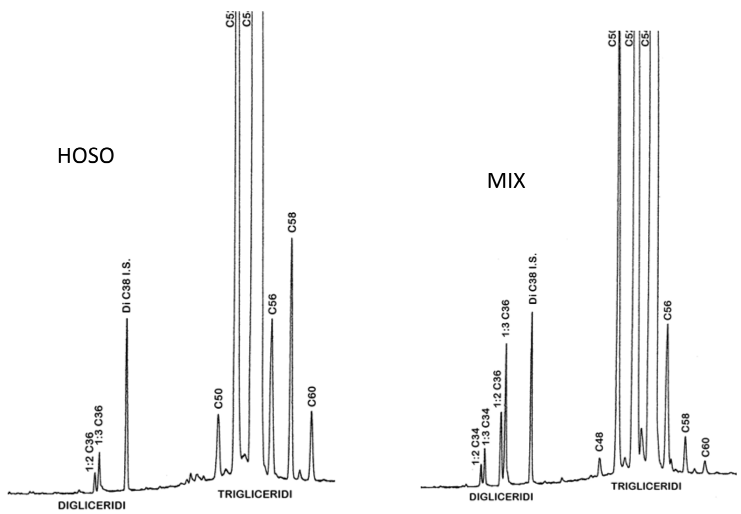
	Extra Vergine	Palm + Sunflower	Mix 9% Palm + Sunflower
Trigliceride C 48	0,06	0,38	0,15
Trigliceride C 50	3,12	9,60	4,72
Trigliceride C 52	27,11	29,88	27,44
Trigliceride C 54	67,10	57,01	65,10
Trigliceride C 56	2,19	1,62	2,02
Trigliceride C 58	0,31	1,12	0,42
Trigliceride C 60	0,10	0,39	0,14

Maximun value (%) of C58 IN EVOO and OO is about 0,32% (assessed on the basis of results of 120 samples)

8


Olive
Palm oleine
1:2 C34
1:3 C34
1:2 C36
1:3 C36
Di C38 I.S.
C48
C50
C52
C54
C56
C58
C60
DIGLICERIDI
TRIGLICERIDI
1:2 C32
1:3 C32
1:2 C34
1:3 C34
1:2 C36
1:3 C36
Di C38 I.S.
C46
C48
C50
C52
C54
C56
C58
C60
DIGLICERIDI
TRIGLICERIDI

9


HOSO
MIX
1:2 C36
1:3 C36
Di C38 I.S.
C50
C56
C58
C60
DIGLICERIDI
TRIGLICERIDI
1:2 C34
1:3 C34
1:2 C36
1:3 C36
Di C38 I.S.
C48
C56
C58
C60
DIGLICERIDI
TRIGLICERIDI

10

Thermal degradation

Peak Number #	IN A	IN B
C 48	0,23	0,00
C 50	2,71	3,08
C 52	25,75	25,72
C 54	71,42	69,18
C 56	0,00	1,68
C 58	0,00	0,26
C 60	0,00	0,08
C 48	0,41	0,74
C 50	11,63	11,60
C 52	29,96	28,64
C 54	57,41	56,86
C 56	0,59	1,27
C 58	0,00	0,64
C 60	0,00	0,25
C 48	0,09	0,06
C 50	3,99	4,20
C 52	32,86	31,89
C 54	62,43	62,14
C 56	0,63	1,43
C 58	0,00	0,18
C 60	0,00	0,10

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Conclusions

- **In some cases, when selected mixtures are used to prepare faked oils, GLC analysis of TAGs seems to be a very performanting approach, however, care must be payd to the injection mode, to avoid thermic degradation, the use of a cold O.C. in jection port is mandatory.**

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Deodorized oils

Deodorization is a step of vegetable oils refining

Virgin oils cannot undergo to this technolgycal treatment

However, some lampante oils (olive oils with chemical or sensory characteristics not fitting the standard for extra virgin olive oils) are submitted to a "soft" deodorisation in order to remove undesiderable odour.
These oils present chemical characteristics fitting chemical standards without any flavour.
Such an oil is then mixed to extra virgin olive oil to produce faked oils

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"Classical" chemical parameters and related analytical methods are not suitable to detect the presence of deodorised olive oils when mixed to extra virgin oils.

Some analytical approaches were proposed:

1. Serani & Piacenti, 2001: "Cold index"

- [Theoretycal amount of Pyropheophytin A] = [Total Pheophyitin A] * 0,075 + 0,199
Cold Index = (Δ Effective -Theorethycal) * ([Pheophyitin A] / [Total Pheophytins]
- 1,2 DAG/1,3 DAG

2. Gertz 2005

$$\text{- \% Pyropheophytin A (Peak area)} = \frac{\text{PPPA (Peak area)} * 100}{\text{PPPA + PPA + PPA'}}$$

PPPA= Pyropheophytin A PPA = Pheophytin A PPA' = Pheophytin A'

1,2DAG/ΣDAG

3. Perez.Camino et al, 2006 alkyl esters

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Aim of this work:

Compare results of Serani's, Gertz's and Perez Camino's methods by applying them to a number of deodorysed oils and to a number of authentic extravirgin olive oils and their mixtures

Check for influence of time on the considered parameters

Check for reliability of considered methods

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Analytical results oils one year old oils

Sample	% FFA	%PPPA	1,2 / 1,3 DAG	%(1,2 / DAGtot.)	ME	EE	ΣAlkyl Esters	PV
1	0,47	22,25	0,89	47	10,38	9,46	19,84	6,4
4	0,45	11,71	0,62	38	23,93	33,19	57,12	7,7
8	0,36	61,8	0,5	33	99,20	198,40	297,60	14,5
9	0,42	16,51	0,63	39	13,90	31,34	44,83	11,8
10	0,25	12,44	1,27	56	6,38	4,94	11,33	10,4
11	0,24	12,16	0,97	49	18,00	41,11	59,11	10,7
14	0,36	17,13	0,71	30	11,75	15,52	27,27	7,9
15	0,64	16,9	0,52	34	78,92	286,67	365,59	9,7
16	0,7	53,22	0,28	22	31,68	67,33	99,01	11,5
17	0,65	49,58	0,22	16	69,63	99,61	169,24	8

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Analytical results fresh oils

Sample	% FFA	% PPA	1,2 / 1,3 DAG	%(1,2 / DGtot.)	ME	EE	Σ Alkyl esters	P.V.
2	0,24	0,39	7,3	88	2,8	1,33	4,13	5,5
3	0,48	0,44	7,81	89	2,62	1,08	3,7	6,4
5	0,46	1,78	7,08	88	23,36	32,86	56,21	10,8
6	1,8	1,36	2,58	72	62,49	107,54	170,03	8,6
7	2,5	0,81	2,53	72	75,49	201,13	276,3	8,7
18	0,36	1,82	5,09	84	9,15	7,26	16,41	6,7
19	0,32	94,03	1,62	62	54,51	182,31	236,82	5,5
32*	0,19	0,38	8,22	89	4,1	4,3	8,39	8,6
12	0,13	87,89	0,79	43	15,49	39,93	55,42	8,3
13	0,24	36,45	0,43	27	79,99	100,92	180.91	7,8

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A number of results seem be able to assess if a sample is or not a deodorysed oil

But what happens in case of mixing such an oil

With a fresh extracted one?

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Extra Virgin Olive Oil n°	% Extra virgin	Deodorised oil n°	% Deodorised oil
2	90	16	10
2	80	16	20
2	90	19	10
2	80	19	20
2	90	8	10
2	20	8	20
3	90	8	10
3	20	16	20
3	90	19	10
3	80	19	20
3	90	8	10
3	80	8	20

19

Sample n°	Fresh/1n year Old	mg PPPA	mg PP A	mg PP A'	PPPA Theoric	Δ PPPA experimental - theoric	Cold Index
1	O	7,41	20,45	5,03	2,11	5,30	1,54
2	O	0,37	22,01	4,84	2,21	-1,84	-0,03
3	O	0,44	32,86	7,64	3,24	-2,79	-0,03
4	O	2,36	13,41	2,92	1,42	0,94	0,14
5	F	0,72	22,86	2,74	2,12	-1,40	-0,04
6	F	0,39	7,91	1,88	0,93	-0,54	-0,02
7	F	0,33	6,32	1,51	0,79	-0,46	-0,02
8	O	13,54	5,83	2,91	0,85	12,69	19,66
9	O	9,22	37,49	8,31	3,63	5,59	1,13
10	F	3,72	20,38	4,48	2,06	1,66	0,25
11	O	6,73	38,74	8,49	3,74	2,99	0,43
12	F	10,49	0,95	0,99	0,34	10,15	54,85
13	F	11,08	14,96	4,43	1,65	9,43	5,39
14	O	3,51	13,21	3,09	1,42	2,09	0,45
15	O	3,73	14,18	3,40	1,52	2,21	0,47
16	O	15,80	11,47	2,72	1,26	14,54	16,19
17	O	18,35	15,13	3,79	1,62	16,73	16,22
18	F	0,72	23,25	1,80	2,08	-1,36	-0,04
19	F	63,79	1,44	3,13	0,54	63,25	882,57
32	F	0,77	100,28	28,03	9,82	-9,06	-0,05

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Sample n°		mg PPPA	mg PP A	mg PP A'	PPPA Theoric	Δ PPP experimental –theoric	Cold Index
20	Mix	0,63	22,93	5,62	2,34	-1,71	-0,04
21	Mix	0,82	21,78	5,07	2,21	-1,39	-0,04
22	Mix	8,29	26,80	5,98	2,66	5,63	1,42
23	Mix	17,17	17,46	4,40	1,84	15,33	12,05
24	Mix	2,68	29,14	7,20	2,92	-0,25	-0,02
25	Mix	3,73	23,18	5,09	2,32	1,41	0,19
26	Mix	0,70	35,13	7,29	3,38	-2,69	-0,04
27	Mix	0,64	21,81	4,46	2,17	-1,53	-0,04
28	Mix	5,16	25,01	5,30	2,47	2,69	0,46
29	Mix	8,26	23,12	5,34	2,33	5,93	1,72
30	Mix	1,68	29,68	6,40	2,91	-1,22	-0,06
31	Mix	4,43	43,29	10,50	4,23	0,19	0,02

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T = Theoretic

S = Experimental

	Acidità	%Pirof	!,2-1,3Ratio	%1,2 Dig	ME	EE	AE TOT
20 T	0,3	5,67	6,60	81,40	5,69	7,93	12,72
20 S	0,3	1,27	4,77	82,00	5,90	7,70	13,57
21 T	0,3	10,96	5,90	74,80	8,58	14,53	22,31
21 S	0,3	2,07	3,15	75,00	8,40	13,20	21,59
22 T	0,2	9,75	6,73	85,40	7,97	19,43	26,50
22 S	0,2	19,90	4,83	82,00	8,90	19,20	28,12
23 T	0,3	19,12	6,16	82,80	13,14	37,53	49,87
23 S	0,2	44,00*	3,27	73,00	17,00	39,10	56,08
24 T	0,3	6,53	6,62	82,50	11,63	41,85	52,58
24 S	0,3	6,30	3,95	78,00	10,50	34,50	45,06
25 T	0,3	12,67	5,94	77,00	20,47	82,37*	102,02*
25 S	0,2	11,10	2,74	73,00	14,80	56,50*	71,32
26 T	0,5	5,72	7,06	82,30	5,53	7,71	13,23
26 S	0,3	1,00	3,52	78,00	5,30	7,70	13,04
27 T	0,5	11,00	6,30	75,60	8,43	14,33	22,76
27 S	0,2	1,40	2,99	74,00	8,40	14,10	22,52
28 T	0,5	9,80	7,19	86,30	7,81	19,20	27,01
28 S	0,2	14,00	4,18	80,00	8,50	19,60	28,15
29 T	0,4	19,16	6,57	83,60	13,00	37,33	50,32
29 S	0,2	22,20	2,39	70,00	13,70	36,00	49,71
30 T	0,5	6,58	7,08	83,40	11,47	41,63	53,09
30 S	0,2	3,80	2,45	71,00	11,10	43,40	54,49
31 T	0,5	12,71	6,35	77,80	20,32	82,17*	102,48*
31 S	0,2	7,20	1,46	59,00	18,60	75,70*	94,24*

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Conclusion

1. Determination of DAG isomerisation: strong influence of time of storage: in the case of mixtures with fresh extracted olive oils, the high amount of 1,2DAG of the latter could mask the presence of deodorised oils
2. PPPA also seems not always useful to highligh the presence of deodorised oils, as they too are strongly influenced by time and conditions (light) of storage
3. Alkyl esters seems could be useful, as they do not depend on time of storage, but on quality of olive fruits, furthermore, they seems more related to theoric values in the case of mixtures.

   The lower will be the limit adopted for this parameter, the higher will be its efficiency

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Alkyl esters

- Nowadays the official parameter is the sum of Fatty acids ethyl esters (FAEE) and fatty acids methyl esters (FAME).
- Results of a recent experimentation by SISSG confirmed that to use FAEE only could be a better approach

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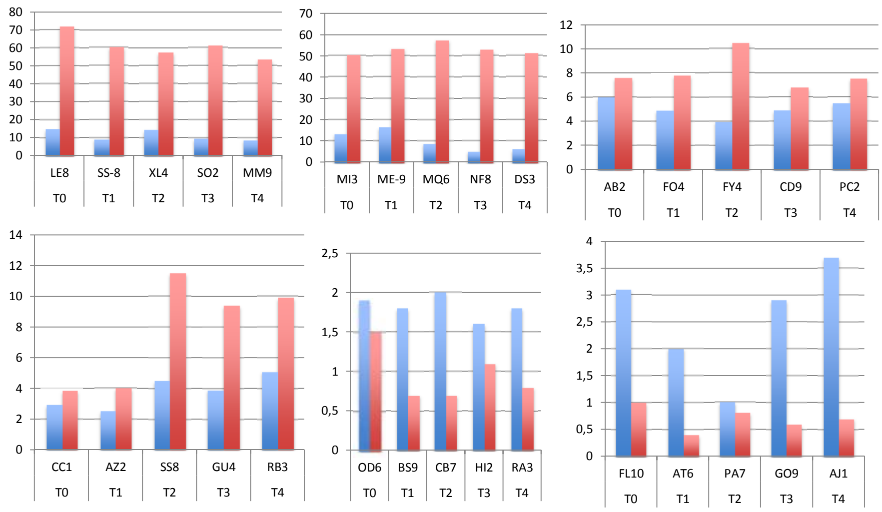
METHYL ALCOHOL ■ **ETHYL ALCOHOL** ■

SAMPLES 1 -6



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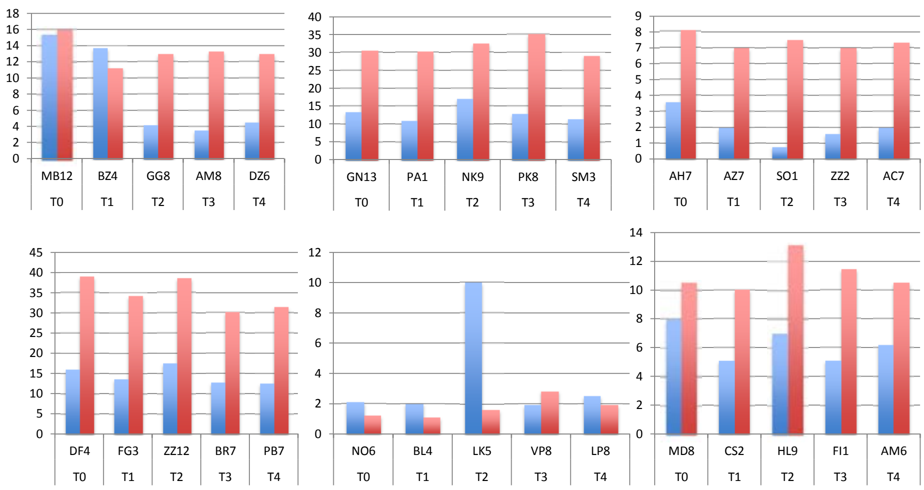
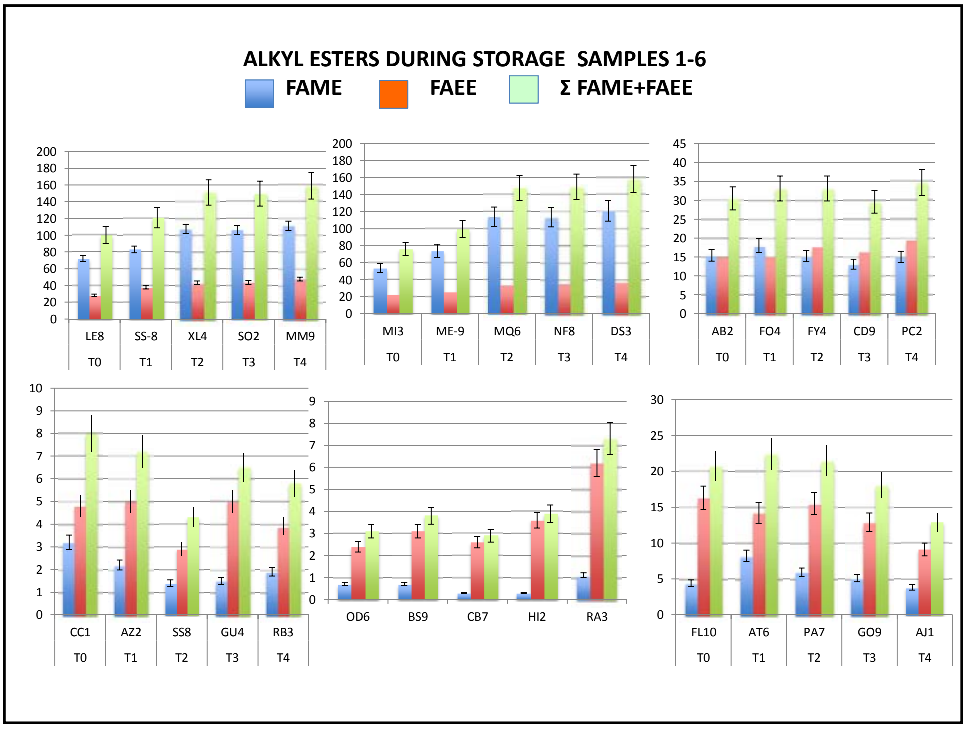
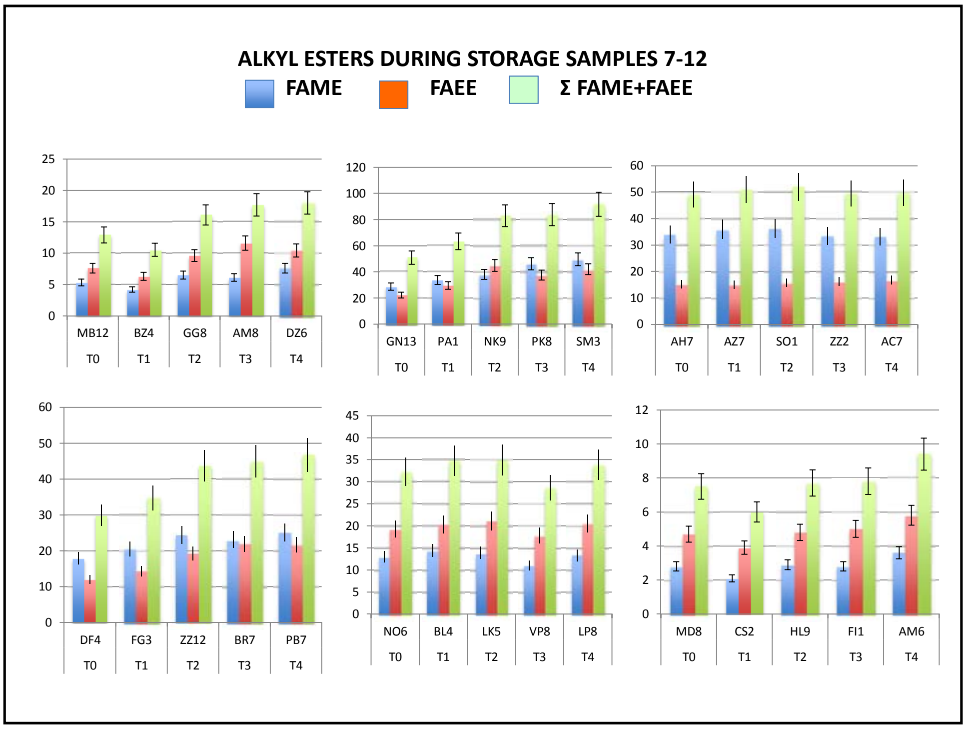
METHYL ALCOHOL ■ **ETHYL ALCOHOL** ■

SAMPLES 7-12



26


ALKYL ESTERS DURING STORAGE SAMPLES 1-6
FAME
FAEE
Σ FAME+FAEE
LE8 SS-8 XL4 SO2 MM9
T0 T1 T2 T3 T4
MI3 ME-9 MQ6 NF8 DS3
T0 T1 T2 T3 T4
AB2 FO4 FY4 CD9 PC2
T0 T1 T2 T3 T4
CC1 AZ2 SS8 GU4 RB3
T0 T1 T2 T3 T4
OD6 BS9 CB7 HI2 RA3
FL10 AT6 PA7 GO9 AJ1
T0 T1 T2 T3 T4


ALKYL ESTERS DURING STORAGE SAMPLES 7-12
FAME
FAEE
Σ FAME+FAEE
MB12 BZ4 GG8 AM8 DZ6
T0 T1 T2 T3 T4
GN13 PA1 NK9 PK8 SM3
T0 T1 T2 T3 T4
AH7 AZ7 SO1 ZZ2 AC7
T0 T1 T2 T3 T4
DF4 FG3 ZZ12 BR7 PB7
T0 T1 T2 T3 T4
NO6 BL4 LK5 VP8 LP8
T0 T1 T2 T3 T4
MD8 CS2 HL9 FI1 AM6
T0 T1 T2 T3 T4

Thanks for your kind attention!

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