The evolution of analytical methods of fats and oils: the role of "La Rivista Italiana delle Sostanze Grasse"

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FOREWORD: AN HISTORICAL APPROACH

The assessment of purity of foods had been an hot topic through the whole human history, the higher was the value of a food, the higher were the possibilities that is underwent to tentative of frauds
In the beginning, to check for olive oil purity and quality, analysts were compelled to use some tests of the so-called "Chemistry of indexes" because they were within the Italian legislation.
Some examples are reported in Table I; in some cases, they were chromatic (not colorimetric) reactions e.g. the Kreiss reaction for rancidity, the Halphen reaction to assess the presence of Malvaceae oils (that are characterized by the presence of fatty acids with cyclopropane and cyclopropene ring), or the reaction of Villavecchia Fabris and later Isodoro Pavolini, used to look for the presence of sesame oil that in Italy at that eve was mandatory added to seed oils. A number of false positive results occurred

Table I - The "chemistry of indexes" parameters and related meaning

Index	Nowadays
Refractive index	Purity Unsaturation degree still used
Halphen reaction sterculic acid (Malvaceae oils)	Purity No more used, GC of FAMEs and sterols
Villavecchia-Fabris and Isodoro Pavolini reactions (In Italy, for Sesame oil)	Purity No more used
Saponification Index	Purity Amount of fatty acids, no more used
Esters index	Purity Amount of TAGs, no more used
Iodine number	Purity Unsaturation degree still used
Thermosulphuric index	Purity Unsaturation degree no more used
Acidity index	Quality, still used
Peroxide value	Quality, still used

Many of the methods reported in the previous table were adopted by Italian Standards for Control of Fats and Related Substances (NGD), (Figure 1 reproduces the front cover of the second edition of the collection of methods) published in 1942, then updated in 1953 and following years; information about these methods were published on the newspaper "Oli Minerali, Grassi e Saponi, Colori e Vernici" (Mineral oils, Fats and Soaps, Paints and Varnishes), then named "Rivista Italiana delle Sostanze Grasse".

Figure 1 - Front cover of the "Norme grassi e derivati" (NGD) method collection

This scientific journal is edited by "Stazione Sperimentale per le Industrie degli Oli e dei Grassi" that was founded by Stefano Fachini in 1919, even if it already existed since 1906, as "Laboratory school for industry of oils and fats".

Stefano Fachini was a pioneer of a nowadays current methodology: in 1913 he founded the "Committee for alignment of analytical methods" and in 1924 the "Technical Governmental Committee for Mineral Oils, Fats and Soaps, Paints and Varnishes and Detergents", in 1930 he founded the "International Committee for the Study of Fats and Oils" that in 1951 became the "Division of Fatty Substances" of IUPAC.

The needed works finalised to develop and standardise analytical methods were carried out by the "Technical Governmental Committee for Mineral oils, Fats and Soaps, Paints and Varnishes and Detergents", appointed by the Italian Ministry of Industries (at that eve).

Every proposed analytical method underwent to a collective experimentation and once it was standardized, it was published on the "Rivista Italiana delle Sostanze Grasse" to undergo to a public evaluation; after a certain number of days, amendments (if any) were evaluated and eventually applied to reach the ultimate release of the method that became part of the above cited collection of official methods.

It's quite clear the key role of RISG in dissemination of information and facilitation of scientific debate.

Some examples of method development, updating and improvement:

1. FATTY ACID COMPOSITION

In 1965, the method for fatty acid composition by gas chromatographic analysis was published on RISG and adopted as Italian official one as Method NGD Ba II-15 (1965).[1]
A packed column 2 m length with polar stationary phase Polyethylene Glycol Succinate (PEGS) was adopted; separation was satisfactory, even if time of analysis were rather long, if compared to those nowadays request (about 46 minutes to obtain the elution of lignoceric acid). The text of the method clearly described many aspects of the analysis, also enclosing the instruction to calculate the separation index between some critical pairs of peaks, e.g, C16:0/C16:1, C18:0/C18:1, C18:3/C20:0.

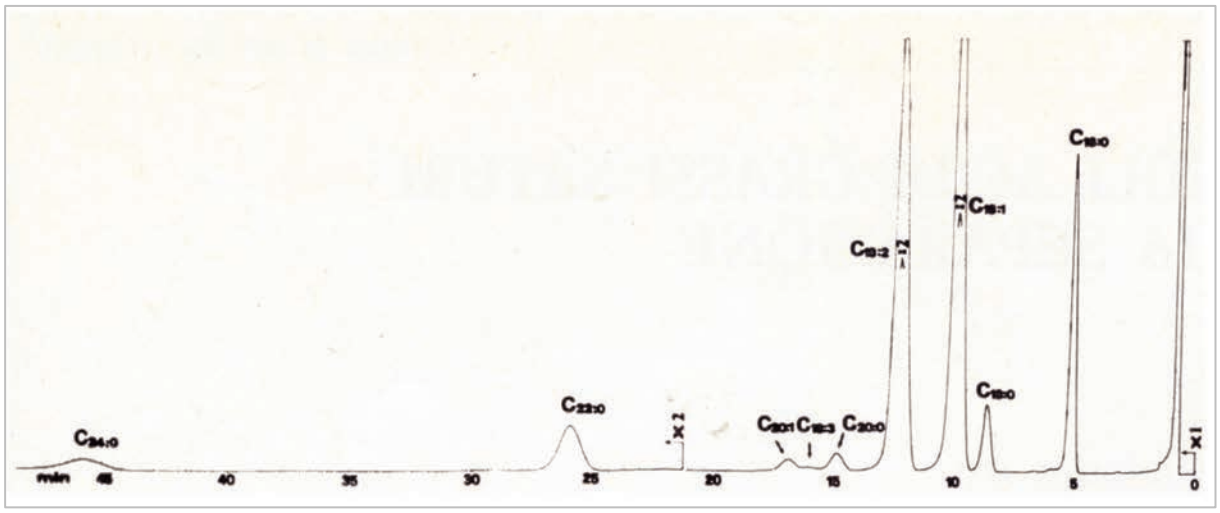

Figure 2 - Gas chromatographic trace of fatty acids methyl esters of a peanut oil, obtained by means of a packed column 2 m length, stainless steel, stationary phase polyethylene glycol succinate (PEGS) according to the NGD Ba II-15 (1965) method

Even if capillary gas chromatography was already published on RISG ad adopted within NGD for the detection of elaidinic acid (Method NGD Ba IV, 39, 1968) [2] it became a widespread used technique only more than 30 years later, when two paper were published on RISG, dealing with the determination of fatty acids trans- isomers (Morchio et al, 1989 [3], Mariani et Al., 1991 [4]); these methods, too, were then adopted within NGD and then within EEC Reg. 3682/91 [5].
The formation of fatty acids isomers early highlighted the presence of position isomers of unsaturation and was published in 1959 by Montefredine and Laporta on “Oli Minerali, Grassi e Saponi” [6], then to bypass the detection of the admixture of olive refined oil with extra virgin ones, an illegal technique was developed by using maleic anhydride, however, unexpected trans- isomers also resulted by this reaction and for this reason their detection became very important.

2. STEROLS COMPOSITION

In order to improve the oxidative stability of edible oils, researches looked at the reduction of unsaturation degree, with this aim, too, genetic improvement since 1967 obtained safflower and sunflower oils with high amount of oleic acid [7], quite similar to olive oil, at that eve, this weakened the determination of fatty acids composition as a tool to assess the admixtures of olive oils with seed oils and lead the attention to the analysis of the sterols fraction.
In 1971, the method for the determination of the sterols composition by gas chromatography with packed column was published and then, as usual, adopted within the NGD collection (NGB Ba-III-13)

[8]; the separation was rather poor, if compared to the one later obtained by several researchers, both in olive oils (Morchio and De Andreis, 1983 and 1984 [9,10]) and in other different oils (Lercker et al, 1981 [11], Giro and Marzo, 1987 [12]); these results, too, were published on RISG; a collaborative study lead to the standardization of the method on behalf of the Commissione Tecnica Governativa Italiana the method was published, too, on RISG in 1989 [13,14].
Figure 3 reports a comparison of the GLC trace obtained with a packed column and with a capillary one.

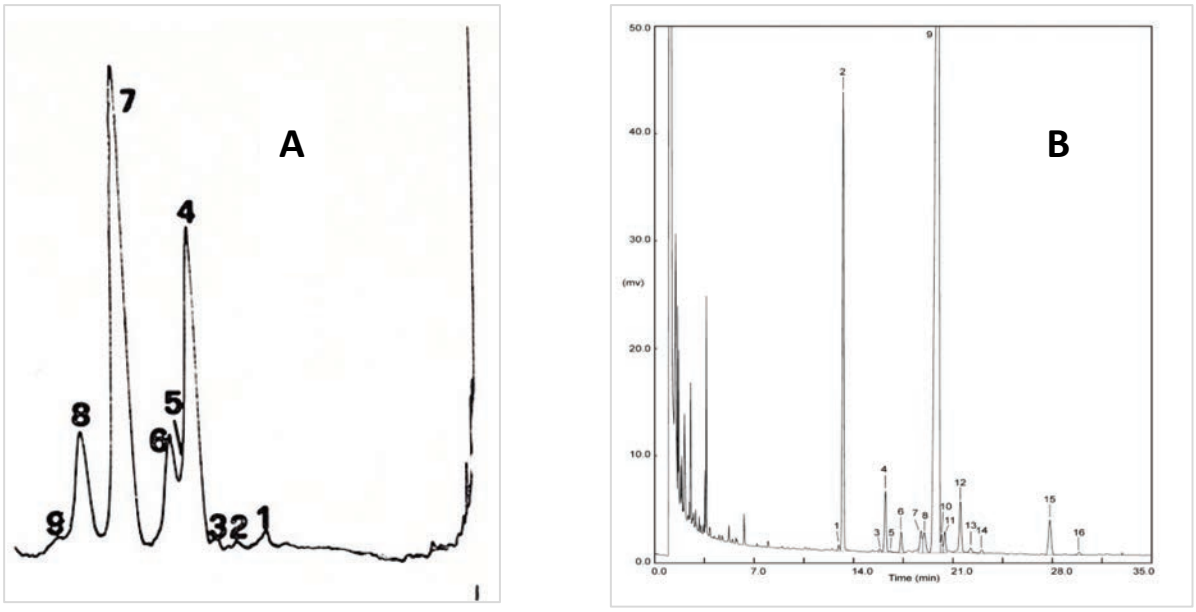

Figure 3 - Gas chromatographic analysis of sterol fraction of olive oil:
A with 3 m glass packed column (stationary phase SE30), according to the NGD Method NGD Ba III-13 1971 Method;
B with 30 m capillary column according to the NGD Method NGD C71 -1986

3. THE ASSESSMENT OF THE PRESENCE OF OTHER EXTRANEOUS OIL

Knowledges of seeds oils composition was enough deep to avoid most of the illegal admixtures, at least for the years we are speaking about, so that the only other possible oil suitable to be mixed to virgin olive oils seemed could be olive pomace oil.
Early studies highlighted the presence of two diterpenic alcohols named erythrodiol and uvaol which presence was assessed within the analysis of sterols [15], however, several years after, this parameter, too, results not so reliable; starting from the observation that the lipid fraction of drupes skin also contain waxes that after saponification undergo to hydrolysis releasing aliphatic alcohols, the quantitative determination of these compound was proposed by Camera [16] in 1981, this study was modified and published on RISG later by Tiscornia et al [17] and adopted as NGD Method in 1981[18] and EEC in 1991 [19].
Then the presence of free aliphatic alcohols was however detected in selected virgin olive oils (South Apulia, Greece) [20], so that the determination of not hydrolysed waxes was proposed as NGD Method [21] and approved as EEC official method [22].
To get success in carrying out the gas chromatographic analysis of high molecular weight and high boiling point molecules the use of the cold on column injection port is mandatory, this is true for waxes, but also for glycerol esters with fatty acids: the first paper dealing with the direct GC analysis of the reaction mixture obtained by the lipolysis with pancreatic lipase (used to assess the presence of esterified oils) was published by Motta et al in 1983 [23] and later by Lercker et al [24].
This method, too, was then validated by the Italian technical Committee [25] and approved as IOC method [26] and as EEC official method [27].

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