



## Adulterations in Some Edible Oils and Fats and Their Detection Methods

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### Abstract

Vegetable oils and fats have a big contribution in our diet as cooking or frying oil, salad oil or in food products formulation. They are important from nutritional and economical point of views. Their authenticity is a serious issue since old time. Some edible oils and fats such as olive oil, cocoa butter and milk fat are so expensive which makes tempting to adulterate them with other lower price vegetable oils and fats to achieve more profit. The need for authentication is a necessity of the food industry. Today, adulterations are more sophisticated. Therefore, it is necessary to use advanced and suitable methods to detect adulteration. Adulteration can cause several problems in edible oils application and industry. To detect edible oils and fats adulteration, it is possible to use both major and minor components as detection tool. Since each oil and fat may have an especial component at a known level, their presence and amounts should be considered as a detection tool. This paper is a brief review on adulteration of edible oils and fats and their detection methods. Several methods have been used to check the purity of edible oils and fats. There is a necessity for food related organization to develop and utilize reliable methods to detect such adulterations, which can make consumers and markets more certain on authenticity and purity of edible oils and fats.

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### Introduction

Vegetable oils and fats (VOFs) have a big contribution in our diet as cooking or frying oil, salad oil or in food products formulation. Also, VOFs are so important by regard to economic point of view. Some VOFs have high price which is tempting for defrauders to adulterate them with less expensive oils and fats to get more profit. Issue of vegetable oils adulteration is not a new problem and even in one given region or country (Jee, 2002). Usually, health problems may not be an issue in adulteration of VOFs, if edible expensive oil such as olive oil is admixed with less expensive edible one; however, it has been reported that adulteration of vegetable oils caused serious health problems in some cases like Spanish toxic oil syn-

drome or Spanish olive oil syndrome due to selling non-edible rapeseed oil as an edible rapeseed oil and even as olive oil. Another example is adulteration of mustard oil with poisonous argemone oil (Clemente and Cahoon, 2009; Posada et al., 1996).

Today, adulterations are more sophisticated. Therefore, it is necessary to use advanced and suitable methods to detect adulteration. Generally, using physical properties like refractive index, viscosity, melting point, saponification and iodine value are not anymore practical to detect adulteration. These properties are well arranged in adulterated VOFs to mask the adulteration. Edible oils and fats consist of major and minor components. Major components in edible oils and fats are triacylglycerols (TAG) and minor components are sterols, carotenoids, tocopher-

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rols, chlorophylls and other minor compounds. Among VOFs, some have particular component which is absent in other one. For example, (E)-5-methylhept-2-en-4-one (filbertone) has been identified as the flavouring component of hazelnuts which is present in some level in hazelnut oil (Fox et al., 1998; Ntakatsane et al., 2013; Ruiz del Castillo et al., 1998). Other examples are brassicasterol, a sterol which is almost exclusively present in canola oil and sesamol, sesamin or sesamolin are present exclusively in sesame oil.

To detect edible oils and fats adulteration, it is possible to use both major and minor components as detection tool. Since each oil and fat may have an especial component at a known level, their presence and amounts should be considered as a detection tool. For example brassicasterol is present in rapeseed/canola oil; therefore, it is possible to use brassicasterol to detect other VOFs adulteration with canola oil. Another example like phytosterols (i.e. campesterol, stigmasterol and sitosterol) are present in small amount in butter, if there was high level of phytosterols in butter, it can be concluded that it has been admixed with VOFs (Clemente and Cahoon, 2009; Liu et al., 2002).

This paper is a brief review on adulteration of edible oils and fats and their detection methods. Several methods have been used to check the purity of edible oils and fats. There is a necessity for food related organization to develop and utilize reliable methods to detect such adulterations, which can make consumers and markets more certain on authenticity and purity of edible oils and fats.

### Types of adulteration

There are two major adulterations in edible oils and fats namely 1) admixing cold press oil with refined one and 2) replacement of more expensive oils and fats with cheaper one (Jee, 2002).

There are certain edible oils and fats which are expensive and have special place in our diet, food preparation and formulation. These are different types of olive oils, cocoa butter and milk fat. Demands for cold press vegetable oils are increasing, since it is produced only by pressing and further simple filtration without refining. Therefore, cold press oil has no contact with chemicals and solvents as it happens for refined oils. Some minor compounds are also well preserved in cold press oil compared with refined oils. Cold press oil is more expensive than refined oil; therefore, there is a temptation to admix them by refined one (Jee, 2002).

The replacement of more expensive oil by cheaper one is so profitable for producers and there are inspries to do it (Siger et al., 2008).

### Detection of cold press oil adulteration

All crude oils obtained by solvent extraction contain variable amounts of non-TAG components such as fatty acids, mono-and diacylglycerols, phosphatides, and etc. The amount of the non-TAG varies with the oil source, extraction process, season and geographical source. Removal of non-TAG constituents from the oil with the least possible damage to the TAG and minimal loss of desirable constituents is the objective of the refining process. Low quality vegetable oils are also refined to produce higher quality edible oil (Amiot et al., 1986; Romani et al., 1999).

Refining processes generally comprise various steps including degumming, neutralization, bleaching and deodorisation. Refining can affect minor components present in the unsaponifiable fraction of vegetable oils. During refining processes, particularly during deodorisation and bleaching, trans fatty acids and steradienes are also formed (Ferrari et al., 1996) which are generally absent in cold press VOFs.

Virgin olive oil adulteration with refined vegetable oils can be detected using trans fatty acid or steradienes as markers (Grob and Bronz, 1994; Lanzón et al., 1989). Detection of stigmastadienes in virgin olive oil at levels in excess of 0.15 mg/kg is regarded under EC regulations as evidence of the presence of refined oils. It should be noted that detection of steradienes and trans fatty acids isomer in other cold press oils are also a sign of adulteration with refined VOFs. Stigmastadiene level in unrefined cocoa butter are well below 0.1 mg/kg whereas in refined butters it may present up to several hundred mg/kg (Crews, 2002). Determination of trans fatty acid in cocoa butter can be a useful tool to detect hydrogenated VOFs in cocoa butter since some cis fatty acid isomers are converted to trans fatty acid isomers during hydrogenation (Jee, 2002).

### Adulteration of admixing oil and fats

As mentioned before, the replacement of expensive oil by lower price one is usual respecting economic point of view. Some oils are more prone to be adulterated due to their higher price and limited accessibility. To make it easy for readers, three of the most common adulteration would be investigated in the next sections.

Virgin olive oil is obtained by the fruit of the olive tree solely by mechanical or other physical means under certain thermal conditions that do not alter the oil, and the oil will not undergo any treatment other than washing, decantation, centrifugation and filtration.

Because of the high price of virgin olive oil, there is a great temptation to adulterate it with oils with similar

fatty acid and sterol profiles (Aparicio, 2000; Baeten et al., 2005). Olive oil adulteration with most vegetable oils can be detected by conventional methods.

Fatty acid composition is useful to detect adulteration of olive oil with the following vegetable oils including soybean, walnut, canola, rapeseed, peanut and mustard, even at level of adulteration below 5% (Christopoulou et al., 2004).

$\Delta$ ECN42 (calculated from the difference between the theoretical and experimental equivalent carbon number 42 in TAG) can be used to detect olive oil adulteration with the following vegetable oils including sunflower, soybean, cotton, corn, safflower, canola and rapeseed at levels as low as 1% (Christopoulou et al., 2004).

Olive oil adulteration with sunflower, soybean, cotton, corn, walnut, sesame, safflower and canola oils can also be detected based on the differences in triacylglycerol and fatty acid composition between the olive oil and these vegetable oils (Christopoulou et al., 2004).

Hazelnut oil has been used to adulterate olive oil due to its similar composition of TAG, fatty acids and major sterols (Cercaci et al., 2003; Christopoulou et al., 2004). It is difficult to detect olive oil adulteration with hazelnut oil lower than 20% concentration using conventional methods (Bøwadt and Aparicio, 2003; Christopoulou et al., 2004). Table 1 shows the composition of hazelnut and olive oil. Different methods have been proposed to detect adulteration of olive oil with hazelnut oil (Azadmard-Damirchi, 2010; Bøwadt and Aparicio, 2003). The sterol profile can be used as a mean of differentiating between vegetable oils or detecting their authenticity (Azadmard-Damirchi and Dutta, 2006a; Azadmard-Damirchi and Dutta, 2006b; Azadmard-Damirchi and Dutta, 2007; Itoh et al., 1973). Phytosterols (plant sterols) comprise a major proportion of the unsaponifiables in vegetable oils. Phytosterols are classified into three separate groups based on the methyl groups at the C<sub>4</sub> position in the A ring including 4-desmethylsterols (without methyl group), 4-monomethylsterols (one methyl group) and 4, 4'-dimethylsterols (triterpene alcohols, two methyl groups) (Akihisa et al., 1991).

Composition and amount of 4, 4'-dimethylsterols are more varied in vegetable oils compared to 4-desmethylsterols, so it can be used as a greater mean to detect vegetable oil adulteration (Azadmard-Damirchi et al., 2005; Itoh et al., 1973). 4-desmethylsterols have been used to detect olive oil adulteration with vegetable oils at levels as low as 5% (Bohačenko and Kopicova, 2001).  $\Delta^7$ -stigmasterol and campesterol have been used to detect olive oil adulteration with sunflower and soybean oil (Bohačenko and Kopicova, 2001). Brassicasterol has also been used to detect olive oil adulteration with rapeseed oil. Different types of olive oil (virgin, refined and solvent-

extracted) could be classified using some 4-desmethylsterols (stigmasterol, clerosterol,  $\Delta^5$ -avenasterol,  $\Delta^7$ -stigmasterol and  $\Delta^7$ -avenasterol) as differentiating factors. 4, 4'-dimethylsterols have been used to detect virgin olive oil adulteration with pomace olive oil at levels as low as 5%. Lupeol and  $\alpha$ -amyrin have also been used to detect olive oil adulteration with almond and hazelnut oils at levels as low as 5%, analyzed by GC (Jiménez de Blas and de Valle-González, 1996).

Lupeol and an unknown (lupane skeleton) compound were exclusively present in hazelnut oil. 4,4'-dimethylsterols could be used as markers to detect virgin olive oil adulteration with hazelnut oil at levels lower than 4% (Azadmard-Damirchi et al., 2005).

Phytosterols are present in free and esterified forms, i.e. as fatty acid esters, steryl glycosides or acylated steryl glycosides (Azadmard-Damirchi, 2007; Moreau et al., 2002). In free form, the hydroxyl group at the C<sub>3</sub> in the A ring is underivatized, whereas in esterified form, the hydroxyl group is covalently bound to other constituents (Moreau, 2005). Some esterified 4-desmethylsterols (campesterol,  $\Delta^7$ -stigmasterol and  $\Delta^7$ -avenasterol) have been used to detect olive oil adulteration with hazelnut oil (Mariani et al., 2006).

Two unknown polar components present in hazelnut oil have been used for tracing olive oil adulteration with hazelnut oil (Zabaras and Gordon, 2004). However, there was large variability in these polar components amount. Therefore, this method could not be used for the quantitative determination of the level of adulteration.

TAG could be used to classify hazelnut and olive oil and admixtures of hazelnut oil in olive oil at level as low as 10% (Parcerisa et al., 2000). However, it has been reported that TAG composition could not be used to detect olive oil adulteration with hazelnut oil at levels lower than or equal to 5% (Christopoulou et al., 2004). Olive oil adulteration with hazelnut oil could be detected using fourier transform infrared (FT-IR) spectroscopy at levels of 25% and higher (Ozen and Mauer, 2002).

Peña et al. (2005) suggested direct coupling of headspace with mass spectrometry to detect adulteration of olive oil with hazelnut oil. The system was applied to analysis of the volatile fraction, which can be used for detection of crude hazelnut oil in olive oil. It was concluded that the proposed method was rapid and reliable but disadvantages included the need for multivariate statistical techniques for data treatment.

The minimum adulteration levels detected by analysis of volatile fraction by coupling of headspace with mass spectrometry were 7 and 15% of crude hazelnut oil in adulterated refined and virgin olive oil, respectively (Peña et al., 2005). However, this method could not be used to detect adulteration with refined hazelnut oil, because volatile component are removed during refining.

García-González et al. (2004) used <sup>1</sup>H and <sup>13</sup>C nuclear magnetic resonance (NMR) techniques to detect olive oil adulteration with hazelnut oil. The detection of olive oil adulteration by NMR is based on the qualitative and quantitative chemical information obtained from resonance data. <sup>1</sup>H NMR spectra provide information on major compounds such as fatty acids and also on minor compounds such as aldehydes, terpenes and sterols. <sup>13</sup>C NMR is a technique that is capable of characterising vegetable oils according to the acyl positional distribution in the glycerol moiety. An artificial neural network based

on <sup>1</sup>H- and <sup>13</sup>C-NMR data could be used to detect olive oil adulteration with hazelnut oil at a level of 8%, with some limitations (García-González et al., 2004).

(E)-5-methylhept-2-en-4-one (filbertone) has been identified as the flavour impact component of hazelnuts. There are many studies on using this compound as a marker to detect olive oil adulteration with hazelnut oil. Filbertone could be used as a chiral marker to detect olive oil adulteration with hazelnut oil at levels higher than 10% by direct reversed-phase (RP)-LC-GC under conditions proposed by Ruiz del Castillo et al. (1998).

**Table 1:** Major and some minor lipid components present in hazelnut and olive oils\*

| Compound                      | Virgin olive oil       | Hazelnut oil |
|-------------------------------|------------------------|--------------|
| TAG by carbon number (CN) (%) |                        |              |
| CN50                          | Tr <sup>**</sup> -10   | 0.7-0.9      |
| CN52                          | 17-53                  | 16-20        |
| CN54                          | 30-91                  | 70-84        |
| CN56                          | Tr-1                   | 0.2-0.6      |
| Fatty acids (%)               |                        |              |
| Palmitic acid (C16:0)         | 7.5-20                 | 5-7          |
| Stearic acid (C18:0)          | 0.5-5                  | 1-3          |
| Oleic acid (C18:1)            | 55-83                  | 70-82        |
| Linoleic acid (C18:2)         | 5-21                   | 8-17         |
| Linolenic acid (C18:3)        | 0.0-0.9                | 0.1          |
| Tocopherols (ppm)             |                        |              |
| α-Tocopherol                  | 33-219                 | 329-448      |
| β-Tocopherol                  | 0.6-4.0                | 2-6          |
| γ-Tocopherol                  | 0.1-11.9               | 5-47         |
| δ-Tocopherol                  | ND <sup>***</sup> -0.7 | 0.3-4.5      |
| Phytosterol classes (ppm)     |                        |              |
| 4-desmethylsterols            |                        |              |
| Sitosterol                    | ≥750                   | 1050-1700    |
| Campesterol                   | ≤40                    | 50-95        |
| Stigmasterol                  | <campesterol           | 10-18        |
| Δ5-Avenasterol                | 40-140                 | 20-80        |
| 4-monomethylsterols           |                        |              |
| Obtusifolol                   | ND-59                  | Tr3-18       |
| Gramisterol                   | ND-48                  | Tr-17        |
| citrostadienol                | 17-576                 | 17-122       |
| 4,4'-dimethylsterols          |                        |              |
| β-Amyrin                      | 8-108                  | 12-192       |
| Butyrospermol                 | 6-104                  | Tr-27        |
| Cycloartenol                  | 36-856                 | Tr-96        |
| 24-Methylenecycloartanol      | 203-2190               | Tr-72        |
| Wax esters (ppm)              |                        |              |
| C36                           | 37-74                  | 42-186       |
| C38                           | 19-55                  | 21-97        |
| C40                           | 3-53                   | 18-80        |
| C42                           | Tr-76                  | Tr           |
| C44                           | 13-133                 | 1-16         |
| C46                           | 7-96                   | 3-17         |
| Aliphatic alcohols (ppm)      |                        |              |
| C23                           | ND-11                  | ND-20        |
| C24                           | 11-204                 | 4-34         |
| C25                           | 4-36                   | 6-34         |
| C26                           | 9-256                  | 5-59         |
| C27                           | 2-18                   | ND-12        |

\* Data reported from IOOC (2003); Benítez-Sánchez et al. (2003)

\*\*Trace

\*\*\* Not detected

**Table 2:** Major fatty acids in bovine milk fat

| Fatty acid      | Range (% w/w) |
|-----------------|---------------|
| 4:0             | 3.1-4.4       |
| 6:0             | 1.8-2.7       |
| 8:0             | 1.0-1.7       |
| 10:0            | 2.2-3.8       |
| 12:0            | 2.6-4.2       |
| 14:0            | 9.1-11.9      |
| 14:1            | 0.5-1.1       |
| 15:0            | 0.9-1.4       |
| 16:0            | 23.6-31.4     |
| 16:1            | 1.4-14.6      |
| 18:0            | 14.9-22.0     |
| 18:1 cis        | 3.2-4.2       |
| 18:1 trans      | 1.2-1.7       |
| 18:2            | 0.8-1.5       |
| 18:2 conjugated | 0.9-1.2       |
| 18:3            | 4.8-7.5       |

### Detection of cocoa butter adulteration

Cocoa butter is derived from the *Theobroma cacao* tree, which grows in several tropical areas, including Indonesia, the Ivory coast, Malaysia, New Guinea and Brazil. Cocoa butter is used mainly in the manufacture of chocolate confectionery, but it has also applications in cosmetics and pharmaceuticals. Because of the high price of cocoa butter, there is a place of adulteration for fraudsters. The non-cocoa fats also used in confectionery are known as cocoa butter alternatives, of which the most important are cocoa butter equivalents, cocoa butter replacers and cocoa butter substitutes (Buchgraber et al., 2007; Ulberth and Buchgrabe, 2003).

Cocoa butter has identical fatty acid composition. Despite of the other edible oils and fats which has several fatty acids with different ratio (Zhang et al., 2014), cocoa butter has three major fatty acids, palmitic acid (16:0) 25-30%, stearic acid (18:0) 24-37% and oleic acid (18:1) 29-38% and in minor amount linolenic acid (18:3) 0-4%. Changes and variation from this identical composition can be a sign of adulteration. TAG is different in VOFs and therefore, it can be as a useful tool to detect adulteration. TAG analysis can be used to detect adulteration of cocoa butter with other VOFs and even it can be used to determine its origin. Phytosterol composition can also be used to detect cocoa butter adulteration. Cocoa butter has a high proportion of stigmasterol compared with other confectionery fats. Significant difference were in the ratio of stigmasterol to campesterol when comparing cocoa butter (mean ratio for three samples=3.18) with ten CBAs and fats used in cocoa butter formulation (range=0.38 to 1.47) (Buchgraber et al., 2007; Crews, 2002).

### Detection of milk fat adulteration

Dairy products have great importance in our diet. Milk fat is present in dairy products such as milk, cream, butter, whole milk powder and cheese. Milk fat is more

expensive than VOFs; therefore, it is tempting to admix it with VOFs (Lipp, 1995).

Milk fat has special fatty acid composition (Table 2) which can be vary by changes in factors such as breed of cow, diet and stage of lactation. Fatty acid composition can be used to detect VOFs with different fatty acid composition in dairy products. However, differences in fatty acid of VOFs and milk fat should be very distinct to be applicable to use as a detection tool (Fox et al., 1988; Ntakatsane et al., 2013; Ulberth, 1994).

Soybean and canola oils have high amount (7-10%) of linolenic acid (18:3) but milk fat has very low amount (0.9-1.2). Therefore, detection of higher amount of linolenic acid in dairy products can be a sign of adulteration with soybean or canola oils (Clemente and Cahoon, 2009). Cottonseed, sunflower and corn oils have high amount (40-70%) of linoleic acid (18:2), but milk fat has low amount of this fatty acid (1-2%). Therefore, linoleic acid can also be used to detect some VOFs in dairy products (Liu et al., 2002). In some cases, it is difficult to use fatty acid composition to detect VOFs such as palm oil in dairy products, because there is similarity in their fatty acid composition and also variation in fatty acid composition of palm oil and milk fat can make the detection of adulteration difficult (Edem, 2002). Sterols are very useful tool to detect almost all types of VOFs in dairy products. Main sterol in milk fat is cholesterol, and phytosterols are present in trace amount (1-3% of total sterols) in milk fat. Phytosterols are present in all VOFs at high amount (1000-12000 ppm), therefore detection of phytosterols in higher level in dairy products can be an indication of adulteration with VOFs. Therefore, in cases fatty acids are not suitable marker, such as detection of palm oil in dairy products, it is possible to use phytosterols as a detection tool (Fox et al., 1998; Ntakatsane et al., 2013; Oguntibeju et al., 2009; Ulberth, 1994).

### Conflicts of interest

None declared.

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