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Review Article

Adulteration in edible oil (mustard oil) and ghee; detection and their effects on human health

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Abstract: Edible oils and ghee are vital parts of our daily culinary practices. In recent years, owing to heightened demand in the domestic and global markets, consistent reports regarding the adulteration of edible oils and ghee with substandard ingredients have been reported. Adulteration in edible oils is widespread, with distinctive contaminants, including cottonseed, mineral, and lower-cost oils like palm olein. In the case of ghee, it is repeatedly combined with animal fats, synthetic materials, or vanaspati. The consumption of contaminated oils and trans-fats within the human diet has resulted in adverse health effects, including cardiovascular diseases, digestive disorders, and even cancer. The review aims to summarize various adulterants found in edible oil (mustard oil) and ghee, their detection techniques, and harmful effects. This review provides an overview of the contemplation linked to the adulteration of edible oils and ghee, the compliance with relevant regulations, and the technological approaches available for detection. The detection technologies for identifying adulteration in edible oils are chromatography and spectroscopy biochemical methods and the use of high-precision analytical instruments. The presence of adulterants in edible oil and ghee undermines our societal integrity and our ethical standards. Awareness among consumers is essential for effectively combating these adulterations.

Keywords: Food, edible oil, ghee, adulteration, health

Introduction

Food is a vital energy source for living things that support growth and survival on the planet [1, 2], but it has been accountable for adulteration since classical times [3]. Food adulteration encompasses a range of activities, including combining, switching, adding harmful ingredients, mislabeling food to hide its quality, and storing expired or decomposing food. Adulteration intentionally contaminates food with inferior, inexpensive, non-edible, or hazardous materials [3]. Essential nutrients, including proteins, lipids, carbohydrates, minerals, and vitamins, are found in food, often derived from animals or plants. However, due to the expanding population and rising food consumption, there is a lack of high-quality food to satisfy the demand. Food adulteration is a long-standing issue that impacts individuals across all social classes. Either the addition of harmful compounds or the removal of essential elements may pose health risks [4]. So, ensuring consumers' safety and checking food quality has become necessary worldwide. Food adulteration acts are natural or unintentional and intentional. Producers find replacing more expensive oils with less expensive ones highly profitable, which motivates them to do so. Insufficient facilities to control food quality leads to unintentional adulteration. Ecological adulteration occurs when certain substances, radicals, or organic compounds harmful to health are not added purposefully and accidentally get up in food. People add adulterants to food products to slake their greed and maximize profits by selling low-value goods at higher prices. As a result, food adulteration has been a profitable business in recent days. Food adulteration affects almost all of the reported foods, including grains, oils, dairy products, alcoholic drinks, spices, honey, and some other foods. In addition, the market's vegetables and fruits are not as pure because dangerous chemicals and pesticides are sprayed on them [3, 4].

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Table 1. Different types of fats and oils and their adulterants

Fats & oils	Adulterants
Mustard oil/Edible oil	Argemone oil (Seed)
	Papaya seed, Butter Yellow
	Mineral oil, castor oil, Karanja oil, olive oil, cottonseed oil
Ghee	Vanaspathy, or margined in Ghee, Palm oil, Cottonseed oil, coal tar dye, etc.
	Boil potato, Starch
	Goat body fat, coal tar dye, Anatta
	Paraffin oil
Butter (Milk fat)	Sterols, tocopherol, rapeseed, palm, soybean, vegetable, etc.
	Anatta, mashed potato, Banana, Sweet potato
Cold press oil	Refined oil
Virgin olive oil	Vegetable oil, Soybean oil, Mustard oil, etc.

A collection of fatty liquids physically eliminated from various agricultural products, some animal tissues or microorganisms are called "edible oils". Mustard oils are commonly grown as a vegetable; their seeds are also utilized in many other ways, including as a seasoning for food and as an element in cosmetics and pharmaceuticals. In 2015-16, edible oil production in India totaled 25.3 million tonnes, with 26.13 million hectares under cultivation. In 2013-14, 32.75 million tons of edible oil were recovered from 28.05 million hectares of land. Edible oils and fats or Ghee are a significant supplement in our diet. Edible oils are essential for cooking, frying, or in food product formulations. So, their originality is a serious matter. Clarified butter or Ghee is a necessary component in Indian kitchens and is widely used. It is also seen as representing a family's prosperity and fortune. Ayurveda considers Ghee a crucial element in herbal remedies since it promotes growth and well-being. Adulteration of Ghee is a typical occurrence due to its great demand in Indian markets [5].

The present review summarizes the different adulterants in edible oil (Mustard oil) and Ghee, their detection methods, and how adulterants impose health effects.

Common adulterants

An analysis of the primary sources of oils indicated that most edible oils come from seeds, although fruits are sources of olive, coconut, and palm oils. Various types of oils, including Argemone oil, Mineral oil, Castor oil, Karanja oil, Olive oil, Cottonseed oil, and paraffin oil, are often mixed into edible oils and Ghee, shown in **Table 1**. Some adulterated products may contain harmful substances like cyanide and prohibited colors [6]. The authenticity of mustard oil and soybean oil is frequently compromised through the intentional addition of extraneous substances, including cyanide, mineral oil, karanja oil, Argemone oil, and linseed oil, thereby posing a significant risk to consumer health and safety. Avocado oil, olive oil, canola oil, walnut oil, peanut oil, perilla oil, and other oils are frequently used with Sesame oil [7]. Argemone oil is extracted from the Argemone *Mexicana* plant's seeds and added to mustard oil. Its spherical, netted, blackish-brown seeds are remarkably similar to mustard seeds [8].

For this reason, it sometimes happens that mustard seeds are accidentally or purposely tempered with Argemone Mexicana seeds [8, 9]. Cow and buffalo ghee is often mixed with other oils and animal body fat. Palm oil is typically used in cow and buffalo Ghee to increase the volume of cooked potatoes and other non-edible oils like soybean oil, which can change its quality and make it less pure. Synthetic flavors and colors are mixed with fats to appear as Ghee. Additionally, it uses animal fat products like tallow, which is quite concerning for India's cultural and religious heritage, vegetarianism, and maternal attitude towards cows. Adulteration is sometimes not detected as they are added in tiny quantities and cause chronic effects [10].

Detection techniques

There are different sophisticated methods of adulteration analysis in edible oil and Ghee. Even though we have many advanced analytical techniques, some conventional tests are still essential and helpful in identifying adulteration in food products. In many analytical methods, costly instruments are required, which are unavailable to everyone. Many biochemical tests are available to detect adulterants in edible oil and Ghee, which are cost-effective [7].

Several instrumental methods are available for detecting butter and olive oil adulteration, including differential scanning chromatography, thin-layer chromatography, and near-infrared spectroscopy (NIR) [11]. Thin-layer chromatography of glycerides was described by Chakraborty et al. as a method for identifying adulteration in fats and oils. Spectroscopic PAT techniques, Mid-infrared spectroscopy (MIR), FT-IR spectroscopy, Raman spectroscopy (RS), Nuclear Magnetic resonance (NMR) spectroscopy, Hyper Spectral Imaging (HSI) spectroscopy, oil ultraviolet spectroscopy are used to detect adulteration in edible oil.

Biochemical test for detection of adulteration in edible oil and ghee [12]

Mustard oil: 1. Detection of Argemone oil [9, 13]: (1) Sulfuric acid test: Argemone oil primarily consists of the alkaloid sanguinarine, which is responsible for the color change observed in the test. First, take three drops of sample oil in a test tube and add one drop of liquid phenol and 3 ml of conc-sulfuric acid (98%) sequentially. After vigorously shaking the test tube, the appearance of a red color within 10-20 seconds indicates the presence of Argemone oil. (2) Nitric acid test: Take 5 ml of mustard oil sample and then add 5 ml of concentrated Nitric acid and mix correctly. The appearance of orange-yellow signifies the adulteration of Argemone oil. (3) Cupric acid test: Take 5 ml of contaminated mustard oil sample in a test tube, followed by adding 1 ml of glacial acetic acid and 2 mg of cupric acetate solution. The green color indicates the presence of Argemone oil in the test sample. (4) Ferric chloride test: 5 ml of mustard oil sample is mixed with 2 ml of concentrated hydrochloric acid in a test tube. Agitate it for 1 minute and then heat gently in a boiling water bath for 2 minutes. After cooling, the layers separate, and add 1 ml of ferric chloride reagent. The appearance of a reddish-brown, needle-shaped crystalline precipitate indicates the presence of Argemone oil in the test sample.

2. Detection of Castor oil: To detect the existence of castor oil, take 3 ml of oil sample in a test tube and add 2 ml of petroleum ether. Shake it vigorously to ensure thorough blending of the substances. Keep the test tube immersed in salt ice, and note the adulteration. The rapid appearance of turbidity confirms the presence of castor oil.

3. Detection of Prohibited color: Take 1 ml sample in four test tubes and add 2 ml, 3 ml, and 4 ml of distilled water and HCl acid. Shake the mixture. If a prohibited color were used as an adulterant, it would have been present in the acid or water layer.

4. Detection of Karanja oil: In a 5 ml test sample, add 10 ml ethyl alcohol and five drops of ferric chloride solution. Shake the mixture vigorously for 2 minutes and keep it at room temperature for 2 hours. The appearance of light blue to dark color in the alcoholic layer indicates the presence of Karanja oil due to the phenolic group in Karanja oil.

5. Detection of Mineral oil: Add 25 ml of alcoholic caustic potash in 1 ml sample. Boil in a water bath till the mixture becomes clear. Add 25 ml distilled water along the side of the tube, with gentle shaking. The presence of turbidity indicates the presence of mineral oil.

6. Detection of Cotton seed oil: Add 2.5 ml alcohol and 2.5 ml solution of Sulphur in Carbon disulfide (1%) in a 2 ml oil sample. Heat the mixture and observe the pink-red color for the presence of cottonseed oil.

7. Detection of Rancidity: Add 3 ml of oil sample and 3 ml of HCl acid in a test tube. Seal the test tube and mix the contents. Subsequently, add 3 ml of a solution containing 0.1% phloroglucinol in diethyl ether to the mixture. Shake the mixture vigorously and set aside for 30 minutes. The presence of rancidity is indicated by the appearance of a pink or red coloration in the acid layer [10, 14].

8. Detection of Rice Bran Oil (Azo Dye Test): Several nations that produce rice, including China, India, and Japan, use rice bran oil as edible. The color and density of physically processed rice bran oil are similar to those of mustard oil. Because rice bran oil is less expensive than Mustard oil, it is commonly added as an adulterant. The literature describes a quick and

easy colorimetric technique for identifying rice bran oil in vegetable oil. Oryzanol is one of the significant components of rice bran oil. Oryzanol was once thought to consist of just one ingredient, but further research revealed that it also included plant sterols, ferulic acid (4-hydroxy-3-methoxy cinnamic acid), esters of triterpenoid alcohol, and crude and physically processed rice bran oil.

For detecting rice bran oil, take 1 ml of rice bran oil alone or rice bran oil containing another oil in a test tube, mix it with 2-4 ml of 10% (w/v) sodium hydroxide solution, then shake the mixture for 5-10 minutes to create an emulsified solution (a). In a different dry test tube, take one or two drops of aniline and dissolve it in diluted hydrochloric acid. Cool it between 0 and 5 degrees Celsius. Add 2-3 ml 5% (w/v) sodium nitrite solution and shake it to generate benzene diazonium chloride solution (b). After combining solutions (a) and (b), shake briefly. The presence of rice bran oil as an adulterant in another oil is indicated by the development of an orange-red color in 10-20 seconds. This novel method is very cost-effective and can identify adulteration of rice bran oil down to 2.5 percent. This approach cannot identify the adulteration of rice bran oil in corn oil because ferulic acid ester is also present [15].

Ghee: A famous Indian dairy product, Ghee is made by heating milk or butter to a clear consistency. Diverse thermal analysis (DTA) can identify adulteration of goat body fat in Ghee [16]. Some other biochemical techniques for the detection of adulteration in Ghee are summarized as:

1. Starch or Mashed Potato: Take half a teaspoon of Ghee and add two to three drops of iodine tincture. The appearance of a blue color signifies the presence of starch adulteration in the ghee sample.

2. Vanaspathy in Ghee: Mix a teaspoon of liquefied Ghee in a test tube with an equal quantity of concentrated sulphuric acid and a small portion of sugar. Vigorously shake the mixture for 1 minute and subsequently allow it to rest undisturbed for 5 minutes. A transformation in color to a vibrant crimson signifies the presence of vanaspati or margarine within the sample [17].

3. Palm oil: Take 1 ml of fat/Ghee. Add 2 ml of DPPH solution, and the test tube lasts 30 seconds. The color of the pure Ghee remained violet, while the color of the Ghee contaminated with palm oil transitioned from violet to yellow. The yellow hue signifies the presence of palm oil adulteration. In another method, add 2 ml of Ghee and 1 ml of Ferric Chloride (0.008 M) and 0.3 ml (0.03 M) of Potassium ferricyanide, respectively. The blue color indicates the presence of palm oil contamination in the ghee sample [18].

4. Coal tar dye: Take one teaspoon of melted Ghee, add 5 ml of diluted sulfuric acid, and mix well. The pink color indicates the presence of coal tar dye [19].

5. Cotton Seed oil: Take 5 g of melted Ghee and add 0.1 ml of a 0.1% Methylene blue solution. Mix vigorously before solidification. A decrease in the intensity of the dye coloration signifies the existence of cottonseed oil in the ghee sample [20].

Spectrometry-based methods for detection of edible oil adulteration

1. Ultraviolet spectrophotometry: Different kinds of edible oil absorb light in the UV zone at various wavelengths. Various ultraviolet spectroscopy detection tasks can prompt the associated quantitative and qualitative analysis [21].

2. Near-infrared spectroscopy: Apart from UV spectroscopy, near-infrared spectroscopy will also be utilized for diverse testing tasks during the testing procedure. The general rule is to choose representative samples and then use the spectrum and sample concentration data to do the subsequent quantitative and qualitative detection, which is crucial for identifying the adulteration effect of edible oil [22].

3. Fluorescence spectroscopy: Fluorescence spectroscopy is another technique used in adulterant detection. The adulteration of edible oils can be easily detected during the detection procedure since different edible oil types have different fluorescence components [23].

4. FT-IR (Fourier transform infrared) spectroscopy: FTIR-spectroscopy is a potent and dependable analytical instrument for the quantitative and qualitative investigation of edible oil and fat adulteration. It requires less sample preparation and is a quick, non-invasive proce-

dure [22]. FT-IR spectroscopy makes it possible to analyze a wide range of sample types in both the scientific and industrial domains very quickly, significantly, and without any problems. The basis of FT-IR spectroscopy is the theory that, upon exposure to infrared radiation, functional groups within the sample may absorb the radiation and begin to vibrate in a specific way, such as bending or stretching vibrations. These vibrations are then directly correlated with the biochemical species found within the sample. Since the generated spectrum represents a characteristic of the unusual sample being studied, it can now be called a chemical "fingerprint". The most crucial determining factor in the analysis of FTIR spectra is selecting an appropriate statistical tool [24].

5. Raman spectroscopy: The Raman effect helps us to understand how molecules move vibrationally and how one molecule interacts with its surroundings. While NIR spectroscopy relies on light absorption, RS data is produced by a light diffusion method, much like FTIR. Raman spectroscopy provides additional insight into a reaction by depending on intra- and intermolecular motions. The FTIR and Raman techniques provide a set of characteristics of specific molecule vibrations known as a "molecular fingerprint", which is crucial for identifying a material. The non-destructive nature of RS, its low cost and efficiency, its resistance to water infiltration, the need for tiny sample quantities, and its reduced sample preparation are among its benefits. However, RS also offers additional information on vibrations inherent to the molecular backbone structure and lower spectrum frequencies. A Raman spectrum is distinguished by a band of individual spectra, in contrast to the infrared absorption band, which has regularly broad peaks. It also provides strong responses to nonpolar bonds, such as CH, CCl, and CC, making it a reliable tool for evaluating adulteration in edible oil [25].

Nuclear magnetic resonance (NMR) method

In food science, nuclear magnetic resonance (NMR) spectroscopy is becoming increasingly popular for assessing and evaluating various foods, including vegetables, beverages, meats, lipids, and dairy products. It is one of the most reliable and versatile analytical techniques for studying solid and liquid products [21]. The advantages of the NMR technology include excellent selectivity, easy automation, immediate quantification of many products, non-invasive and concurrent identification, and increased repeatability and speed.

A vast amount of information could be gathered via NMR, particularly regarding the molecular makeup of the organic substances. It is based on measuring radiofrequency radiation absorbed by atoms' nuclei in a strong magnetic field. The nuclei may be incorporated more heavily based on the local environment in the atom-to-atom area, causing a negligible change in the magnetic field. NMR spectroscopy uses a variety of isotopes, such as ^{15}N, ^{31}P, and ^{17}O, although the most common ones are ^{1}H and ^{13}C. The purity assessment and chemical structure classification were initially done using the NMR spectroscopic approach. The use of NMR as a mixture analysis screening tool has recently increased. This evolution can be attributed to advancements in routine sampling techniques, the effectiveness of NMR spectrometers, and the development of innovative software for analyzing spectrum data. The study of edible oil has also made significant use of NMR spectroscopic methods. The combination of these tools with chemometric tools has improved authenticity and categorization. Without modifying the sample, the NMR approach can provide comprehensive information about the molecular makeup of fatty acids, acids/esters, squalene, sterols, wax esters, alcohols, and phenols in edible oils [26].

Chromatography method

Chromatography is another approach that's frequently used to find adulterated edible oils. Currently, liquid and gas chromatography are the primary methods utilized for detection work. Edible oil's precise fatty acid composition can be ascertained before gas chromatography is used for various detection tasks. Based on this, the adulteration of edible oil and the precise adulteration ratio may be evaluated through comparison analysis, combining it with pertinent national requirements [27].

Harmful effect on human health

Adulteration makes edible oil and Ghee tainted and unsafe for human consumption. Edible oil adulteration has reportedly led to severe health problems. Foods containing hazardous chemicals pose a health risk to consumers and reduce the meal's nutritional value. Adulterants are dangerous and harm the organs permanently, according to the Indian Council of Medical Research. According to Azadmard-Damirchi and Torbati, non-edible rapeseed oil was marketed as edible and olive oil, resulting in over 600 deaths from the Spanish toxic oil syndrome or Spanish olive oil illness [11]. If an adulterant that contains peanut oil or another type of allergy is employed, additional health issues could potentially result since some consumers may be allergic to the peanut proteins in the impurity. Due to the presence of poisonous compounds called dihydrosanguinarine and sanguinarine, adulteration in edible *Argemone mexicana* seed oil has been linked to epidemic dropsy in several cases [28].

Additionally, Argemone oil mixed with food oils can lead to glaucoma and blindness in addition to widespread sleepiness. Also, the gallbladder can develop cancer due to the usage of butter yellow, Argemone oil, and edible oil adulterants. Loose edible oils are more contaminated. Edible oils contaminated with castor oil result in stomach issues. Conversely, edible oils tainted with mineral oils are cancerous and harmful to the liver [29].

One of the most severe cases of pandemic dropsy occurred in 1998 in the Indian capital of New Delhi and was caused by the consumption of edible oil tainted with *Argemone mexicana* (Mexican Poppy) oil. The outbreak impacted both the political and health communities. Argemone poisoning was first reported in Calcutta in 1877; since then, there have been several cases in various countries, including the Fiji Islands, Mauritius, Madagascar, South Africa, and Burma (Myanmar). Retinal hemorrhages, hypoproteinemia, mild to severe renal azotemia, and anemia are some of its symptoms. Due to the presence of polycyclic aromatic hydrocarbons (PAH), a potent carcinogen, it is very harmful to health [8].

Another adulteration in edible oil is cottonseed oil. It contains a significant amount of polyunsaturated fatty acids or PUFAs, which can lead to lifestyle diseases like obesity, diabetes as well as cancer. There are few reports of adulteration of soybean and mustard oil with burnt mobil oil, which contains polychlorinated biphenyl (PCB). PCBs are dangerous for people. They can cause cyanosis, weight loss, dehydration, nausea, eye and respiratory tract irritation, and changes in the liver, kidneys, pancreas, and neuromuscular system activity [30].

Conclusion

Edible fats and oils, such as mustard, olive, sunflower, Ghee, butter, and cocoa butter, are often adulterated with lower-quality seed oils and inexpensive vegetable oils. Food adulteration can cause several health problems, such as kidney problems, certain diseases resulting from inadequate nutrition, and failure of the heart, kidney, and liver [31]. In light of this challenge, the development of rapid detection kits that employ acid-based or alkali-based color change techniques has emerged, enabling the quick identification of adulteration in food products. Consumers must be educated about these adulteration methods to safeguard the integrity of edible oils and fats. Law enforcement agencies must adopt a more proactive approach to enforcing applicable laws. Many different material analysis applications can benefit from the versatility of ultrasonic nondestructive testing. Oils have been characterized using a range of methods, including X-ray diffraction, Refraction measurements (RI), UV-spectroscopy, nuclear magnetic resonance (NMR), neutron scattering, and differential scanning calorimetry (DSC). Other nondestructive techniques include using biosensors in oil assessment, X-ray imaging, electronic sniffing, and NIR spectroscopy.

Food adulteration is a severe issue that needs to be addressed, and awareness is necessary among the people. Although several methods and techniques are available for detecting adulterants in oil and ghee, there are still many limitations and challenges. Proper training and expertise are required to detect adulterants adequately in food products. Further, many techniques, instruments, and chemicals are costly, making these methods not feasible. The sensitivity of each test or method is also important before using them for adulteration analysis. Food adulteration affects not just our morality but also our societal values. People are experiencing a variety of health problems because they cannot tell the difference between pure and impure meals with their naked eyes as a result of consuming contaminated food. Therefore, the general public should know basic screening tests. If people are aware enough and alert to the adulteration in our food products, it will help us to cope with many health issues. Different food safety organizations should implement effective strategies to ensure the authenticity and purity of edible oils and fats. To save society against the unhealthy routines that the sellers have adopted, the Prevention of Food Adulteration Act (PFA), 1954, was authorized by the Parliament of India and has been now changed by the Food Safety and Standards Act, 2006, governed by Food Safety & Standards Authority of India (FSSAI). We should be aware of adulteration's harmful effects on food safety and health. In addition to legal action, consumer education is also necessary. Governments must impose severe sanctions on those who participate in this detrimental behavior. Increasing public knowledge regarding adulteration in food products can stop dishonest people from preying on people's ignorance.

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Disclosure of conflict of interest

None.

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