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# Qualitative Analysis of Indian Ghee and Mustard Oil Samples

***[1]Damini*, [2]Deepa Pundir****

[1]Department of biotechnology, KVA DAV College for Women, Karnal, Railway Road, Karnal (132001), Haryana, India
[2]Department of biotechnology, KVA DAV College for Women, Karnal, Railway Road, Karnal (132001), Haryana, India

**ABSTRACT**

The purpose of the current research was to analyze the qualitative characteristics of pure Indian Ghee and the Mustard oil available in local areas of North India. Ghee is the clarified butter obtained from buffaloes, cows, sheep, and goats. It is prepared by melting the butter, boiling of the moisture, and decanting the clarified fat. Whereas the mustard oil is the pressed oil used for cooking purposes in some cultures. Elevation in the intake of dairy products mainly in Haryana region make these eatables a critical target for adulterations with financial gains for the selfish producers. An experimental study conducted, 5 samples of the mustard oil and the 4 samples of Indian ghee were taken for the research were collected under aseptic conditions from different houses. In present work, adulteration detection tests were carried out on 9 unknown fat samples. The samples were coded and qualitatively tested for adulteration. These all tests were carried out at room temperature. Various biochemical tests were performed to check the quality of fat samples and presence of adulteration in those samples.

Keywords: Qualitative, adulteration, saponification, decanting, clarified fat.

## 1. Introduction

Lipids are a heterogenous and organic type of molecules that are important for energy storage and cell structure. They include fats, oil, waxes, sterols and pigments such as chlorophylls, carotenoids, etc. (Fahy et al., 2005). Lipids are one of the important biochemical components of plant cell (Hayun Uk Kim, 2020). Fats and oils are storage form of energy to be utilized during respiration, growth and repair. These are abundantly present as storage food in fatty or oily seeds and fruits. Some sources of healthy fats include avocados, nuts, and olive oil. The term lipids were coined by a German biochemist Bloor in 1943**.** The term 'lipid' comes from the Greek word 'Lipos', which means "fat". Lipids are made up of carbon, hydrogen, and oxygen atoms (Ahmed et al., 2023). Lipids require more oxygen for their complete oxidation but yield more energy than carbohydrates. Lipids are insoluble in water and soluble in polar organic solvents. Simple lipids are called Fats (Bhat et al., 2019). So, all fats are lipids but all lipids are not fats.

Fatty acids are carboxylic acids with hydrocarbon side chain (Tvrzicka et al., 2011) (Bhat et al., 2019). They are the building blocks of the fats. And the simplest form of lipids. Fats are mainly found in endosperm of seeds. Fruits coconut, fatty meats, nuts, oil seeds and seeds of conifers are especially rich in fats. A little amount of fats is also present in the stem, leaves and roots. Fats are subgroup of lipids are called triglycerides. Lipids also encompass molecules such as fatty acids and their derivatives (W.M. Nimal Ratnayake and Claudio Galli, 2009) as well as other sterol-containing metabolites such as cholesterol.

Primarily, Healthy fats are unsaturated. Fats are originated from plants and they are generally in liquid form at room temperature. This is not exclusive, but a general rule (i.e. avocados are solid at room temperature, but contain healthy fats).

Second, unhealthy fats, or saturated fats, are originated from animals and they are solid at room temperature The most important exception to this rule is fish, with generally have healthy fats.

Third, a trans-fat is a normal fat molecule that has been present in processed food. Partially hydrogenating vegetable oils makes them more stable and less lightly to spoil, which is very good for food manufacturers and very bad for you. No amount of trans fat is healthy. Trans fat contributes to major health problems, from heart disease to cancer (Dhaka et al., 2011).

Omega-3 Fatty acids are a type of polyunsaturated fat (Philip C. Calder, 2009). While all types of unsaturated fats are good for you, omega-3 fats are providing to be especially beneficial. Research has shown that they can: protect against memory loss and dementia and reduce the risk of heart disease, stroke, and cancer, among other (Swanson et al., 2012). The rural population in North and East India consumes more rapeseed-mustard oil (*Brassica Juncea*) and grains, which are considered a poor man's food. In urban areas, Indian ghee and vegetable ghee are substituted for mustard oils and whole grains. Rapeseed-mustard oil contains high amount of erucic acid, varied from 14% to 33% in the lipids. High erucic acid content in foodstuff has been associated with myocardial lipidosis and heart lesions in laboratory rat's experiments (El-Shenawy et al., 2014); therefore, not safe for human consumption (Pasalic et al., 2012). Mustard oil also has a high ratio of oleic to linolenic fatty acid and linoleic (ω-6) to linolenic (ω-3) fatty acid. The limit for human consumption of trans fatty acid as a percentage of energy should be less than 1% in fats and oils according to WHO/PAHO.

"Ghee," the Indian name for clarified butterfat, is usually prepared from cow's milk, buffalo milk or mixed milk. According to The International Diary Federation (1977), ghee is a product exclusively made from milk, cream, or butter from various animal species. During the manufacturing process of ghee, there is almost total removal of moisture (almost anhydrous fat) from the milk and the milk becomes solids-not-fat (ghee), which is only produced in India. Analysis by different groups showed that ghee contains 45-65% saturated fat and 32% Monounsaturated Fatty Acids (MUFA). It is also a good source of lipid nutrients, fat-soluble vitamins, and essential fatty acids. Because of its high saturated fat content, consumption of ghee is expected to be associated with high coronary heart disease (CHD). But, study on healthy young Indian by Shankar et al., indicated that there is no serious adverse effect of ghee on lipoprotein profile. Consuming ghee at the level of 10% of total energy intake in a vegetarian diet generally has no effect on the serum lipid profile of young, healthy, physically active individuals. Similarly, a study showed that the prevalence of CHD in men were low, who consumed more ghee in their diet (Gupta R and Prakash H, 1997).

In this study, different samples were taken from various regions of the Haryana, and qualitative tests were performed to determine their quality status whether the adulterants were present in it or not. Current study was intended to detect the quality of fat samples in context of adulterations by depiction of specific colors signifies the presence and absence of specific components as chemicals.

## 2. Methodology

**Study sample**

Total nine unknown fat samples (ghee and mustard oil) were collected under aseptic conditions from different local regions. The samples were coded and qualitatively tested for adulteration. These all tests were performed at room temperature. Myriads of biochemical tests were performed to check the quality and detection of adulteration in those samples. These tests are described as below:

**Study design**

Random sampling was used to choose the fat sample, and commonly used fat varieties were selected for the analysis. Fat samples stored in safe and hygienic condition with proper coding. Particularly, ghee samples needed a little thawing before performing the tests. The protocols of Standard Operating Procedures (SOP) were followed with accuracy to analyze the adulteration in fat samples.

### *2.1 Test of free fatty acids*

Took10ml of 0.1N NaOH in flask and added two drops of phenolphthalein. Add the test solution perform titration, till the pink color in flask get disappeared and note the final reading.

### *2.2 Test of acid value*

To estimate the Acid value of fat samples, took 1g of fat sample and 10ml of ethanol-ether mixture in flask. Added and mixed few drops of phenolphthalein solution. Prepared 0.1N KOH solution and titrate it until the pink colors appears for 20-30 sec. Note the volume of KOH used and repeat the titration with other samples. Also, a blank should be coded with fat sample for the accuracy.

### *2.3 Test of saponification value*

To determine the saponification value measured 1g of fat sample in flask and dissolved it in 5ml of solvent {ethanol (95%)-ether (1:1)}. Added 25 ml of alcoholic KOH and kept in boiling water bath for 30 min. Cool & added few drops of phenolphthalein solution. Titrate it till the pink color disappeared. Repeat it with other samples and run a blank without adding fat sample.

### *2.4 Test of pH*

In litmus test, test the fat sample (ghee and mustard oil) by using pH strips.

### *2.5 Test of solubility*

To analyze the solubility of samples, took 1g fat sample in a 3 dry test tubes and patterned its solubility in water, alcohol/benzene, chloroform/ether.

### *2.6 Test of unsaturated fats*

To determine the unsaturated fats, took 3 drops of fat sample with 1ml of ethyl alcohol and solution of bromine drop by drop & mixed after each addition until a pale-yellow color is seen.

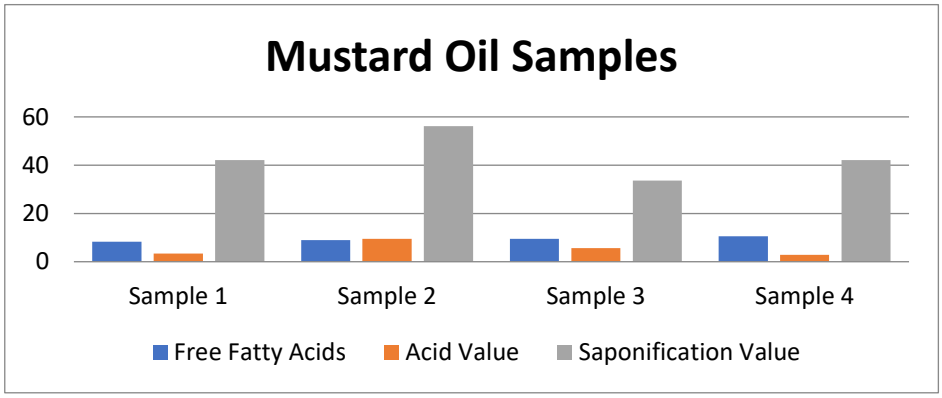
## 3. Results

As Shown in the Table 1, initial experiments showed the mixed results whereas all samples were acidic in nature and unsaturated fats were also present in all. Somehow, solubility and saponification results were less positive.

**Table 1 - Identification of Mustard Oil Samples**.

| **Experiments** | **Sample 1** | **Sample 2** | **Sample 3** | **Sample 4** |
|---|---|---|---|---|
| Free Fatty Acids | 8.3 | 9 | 9.5 | 10.5 |
| Acid Value | 3.36 | 9.53 | 5.61 | 2.80 |
| Saponification Value | 42.07 | 56.1 | 33.6 | 42.07 |
| Litmus Test | Acidic | Acidic | Acidic | Acidic |
| Solubility<br>a) Water<br>b) Alcohol<br>c) Chloroform | <br>No<br>No<br>Yes | <br>No<br>No<br>Yes | <br>No<br>No<br>Yes | <br>No<br>No<br>Yes |
| Unsaturated Fats | Present | Present | Present | Present |

**Graph 1. Mustard Oil Samples.**



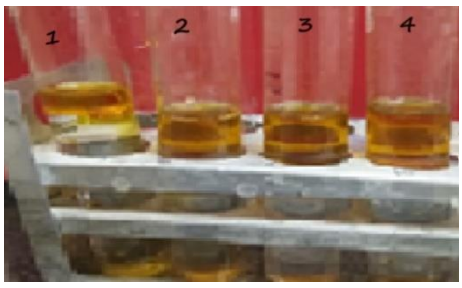




**Figure a: Solubility(Water).**

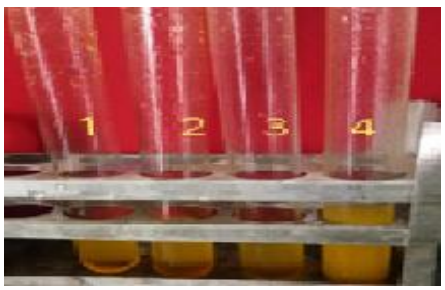



**Figure b: Solubility (Alcohol).**

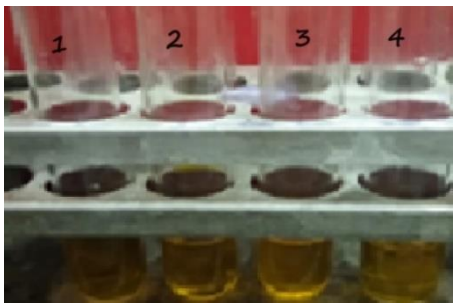



**Figure c: Solubility (Cholorofoam).**

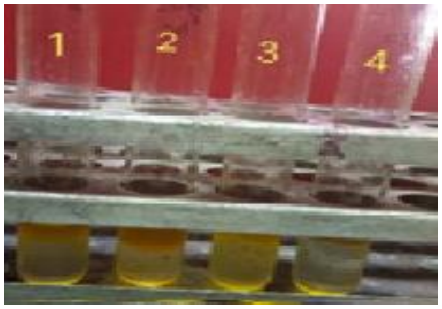


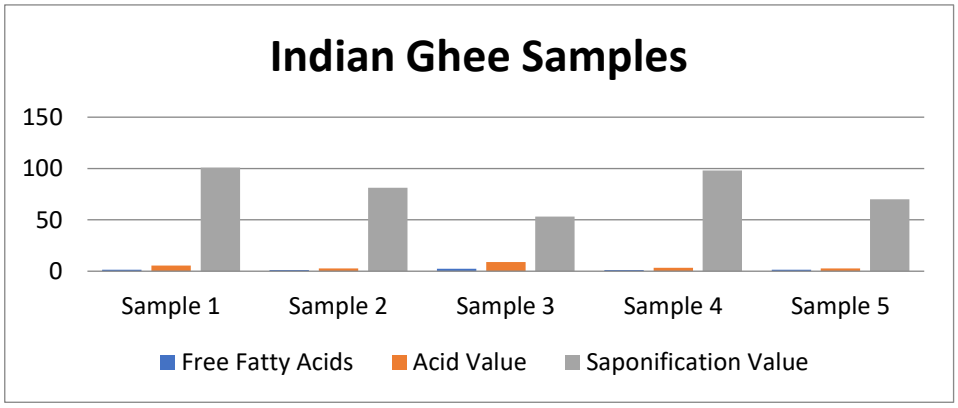

**Figure d: Presence of unsaturated fats.**

In table 2, Not only free fatty acids, acid value, saponification value were comparatively differed from oil samples. But also, two sample were neutral and other were acidic in nature. Although, unsaturated fat results were common in both samples. Lastly, solubility case was partially in favor.

**Table 2 - Identification of Indian Ghee Samples.**

| Experiments | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|---|---|---|---|---|---|
| Free Fatty Acids | 1.5 | 1 | 2.5 | 1 | 1.5 |
| Acid Value | 5.61 | 2.80 | 8.97 | 3.36 | 2.80 |
| Saponification Value | 100.9 | 81.3 | 53.2 | 98.1 | 70.1 |
| Litmus Test | Neutral | Acidic | Acidic | Acidic | Neutral |
| Solubility | | | | | |
| a) Water | No | No | No | No | No |
| b) Alcohol | No | No | No | No | No |
| c) Chloroform | Yes | Yes | Yes | Yes | Yes |
| Unsaturated Fats | Present | Present | Present | Present | Present |

**Graph 2. Indian Ghee Samples**



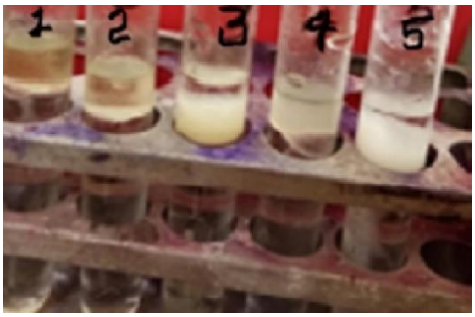




**Figure e: Solubility(Water).**

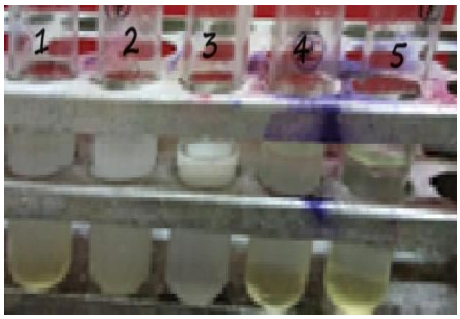



**Figure f: Solubility (Alcohol).**

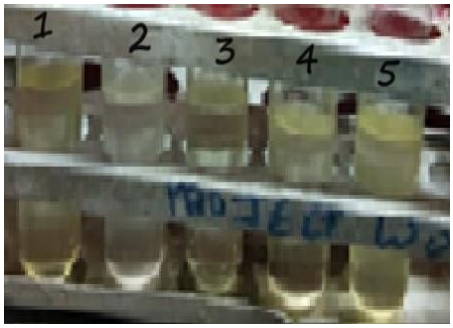



**Figure g: Solubility (Cholorofoam).**

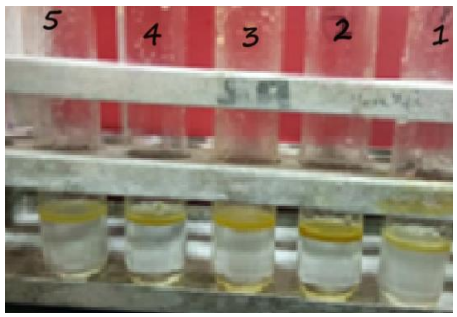



**Figure h: Presence of unsaturated fats.**

## 4. Discussion

Fats are an essential part of our diet. They provide energy, help absorb vitamins, and support cell growth. Due to the crucial part of diet, fat products are targeted for adulteration. These adulterants have adverse effect on human health (Momtaz et al., 2023) and causing serious disease related to heart, obesity, cholesterol etc. Adulterants in fat samples can be detected by commonly used biochemical tests. These tests can include measuring acid value, free fatty acids, saponification value, solubility in water, alcohol and cholesterol, litmus test, unsaturated fats were done. These values indicate the occurrence of impurities or contaminants. Although these experiments are only valid for small amount. There are several potential adulterants that can

be found in fat samples, such as synthetic dyes, vegetable oils, animal fats and even harmful substances like chemical or contaminants such as hydrogen peroxide, synthetic antioxidants, and food coloring etc. In current study, qualitative analysis was done to detect the adulterants in different fat samples. Undoubtedly, high amount of free fatty acids is undesirable for health. It is important to consume fats with balanced levels of fatty acids, preferably below 0.1%. Second, consumable healthy fat should have low acid value, typically around 1-3mg KOH/g, high acid value fat can disturb your life style. Third, fats having saponification value below 60 contains higher proportion of saturated fatty acids and they are considered less healthy whereas above 60 can be considered as beneficial for health as they contain higher proportion of unsaturated fats. Indian Ghee samples were having high saponification value and can be considered good for consumption. Generally, healthy range is between 180 and 200. Certain acidic fats such as omega-3 fatty acids, can be beneficial for health whereas neutral fats also known as triglycerides area an essential component of or diet and play a crucial role in energy, storage, insulation, and hormone production. However, it is important to consume them in moderation as excessive intake can contribute to weight gain and health complications. Both soluble and non-soluble fats have different contribution for overall health like, soluble fat can help lower cholesterol levels whereas insoluble fat is important for digestion and maintain bowel regularity.

## 5. Conclusion

Additives are added in the fat sample to extend shelf life, prevent spoilage, prevent oxidation, improve stability, texture, quality, flavor, and overall functionality of fats, ensuring a better experience for consumers. Fats are most adulterated with recycled oils, preservatives like BHA (butylated hydroxytoluene), BHT (butylated hydroxytoluene). And anti-oxidants such as tocopherols, ascorbic acids, and rosemary extract. And emulsifiers like mono and diglycerides, lecithin and polysorbates. In this study we can screen the presence of some of these adulterants in given fat sample of mustard oil and ghee. These all adulterants of fats compromised the quality and make it hazardous for human health.

## 6. Recommendations

This study was only about the qualitative analysis of mustard oil and ghee samples. For more accurate results, further results can be carried out for quantitative analysis of fat adulterants.

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