Detection of Adulterants and Mastitis in Milk Samples and Major Milk Fatty Acid Composition Estimation using Gas Chromatography

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Abstract

Milk is the only substance which gives life to infants. The first food for survival comes from milk so basically we call it life. Every infant starts journey from milk being fed by mother milk, cow milk, buffalo milk or packaged, it is a birth right of every human to have hygiene food. The present case study carried out keeping in view the recent increasing trends of adulterations which poses inimical effects on human health. Some locally available milk brands in the vicinity of Secunderabad were procured along with fresh raw milk from goat, cow, buffalo and breast milk. The objective of the study was to check whether the procured samples conform to the legal standards prescribed by the Food Safety and Standards Authority of India (FSSAI) using milk adulteration kit manufactured by Himedia, Mumbai, India.

Detection of mastitis causing bacteria in milk by gas chromatography using the Sherlock MIS was other objective. The characteristic feature of milk is its fatty acid composition, which mainly consists of short chain fatty acids, saturated and unsaturated fatty acids. Comparative study of fatty acids in different milk samples was done using FAME analysis. 2–D plot which uses a principle components analysis of FAME profiles was used to group entries in a two dimensional space. Prominent milk Fatty acids in present study were elaborated in accordance to their specific functions.

The test results clearly suggest that most of the milk samples were adulterated even though no positive sample was found for mastitis. This portrays that most of the milk samples were prepared with added adulterants during their production and processing or added intentionally according to one’s own choice to generate money.

Key words: Mastitis, Fame, Fatty Acids, Gas Chromatography, Himedia
Introduction

Milk has been part of the human diet for millennia and is valued as a natural and traditional food. Milk and dairy foods are considered to be one of the main food groups important in a healthy balanced diet. Milk consumption has been called a marker for an overall healthy diet because of its association with increased nutrient intake. Cows’ milk provides a wide range of essential nutrients to the diet. Whilst milk as a source of calcium is often recognized, it is perhaps less commonly known that milk and milk products are also an important source of good quality protein, the B vitamins, B2 (riboflavin) and B12, and the minerals iodine, potassium and phosphorus (Foods Standards Agency, 2002). The demand for milk in developing countries is expected to increase by 25 percent by 2025 (FAO, 2008a).

Small-scale livestock holders supply the vast majority of this milk, and dairy animals provide household food security and a means of fast returns for them. In countries such as the US where milk is fortified, dairy foods can make an important contribution to vitamin D intake, but this is not the case in India due to milk adulterants. It is sad to note that most Indians are resigned to drinking milk diluted with water which not only reduces the nutritious value of the beverage but also poses risk to health. Milk, if present in its natural form has high food value. It supplies nutrients like good quality proteins, fat, carbohydrates, vitamins and minerals in significant amount than any other single food (Neumann et al., 2002). Considering the huge gap between demand and supply, some companies and dairy farms are accused of involving in illegal activities taking public health for granted.

They are adulterating milk using harmful detergents, formalin, glucose and starch powder, sodium carbonate, sodium bicarbonate, sodium hydroxide and milk powder containing animal fats. Another factor may be of traditional milk distribution system in the country which involves middlemen. In such condition, to compensate this gap between demand and supply, the people adulterate the milk to maximize their profit (Lateef et al., 2009). Milk dealers may either dilute the milk or extract valuable components and there after add cheap substances to maintain its compositional parameters. These substances include starch, urea, preservatives like formalin, hydrogen peroxide, boric acid and various antibiotics (Tippu et al., 2007). Urea is added for whitening of milk and only few grams of urea are enough to bring milk in its original state (Walker et al., 2004). Hydrogen peroxide is used as preservative usually in summer season when environmental temperature is very high. This unethical activity is usually adapted to prevent the financial losses due to the spoilage of milk during its transportation and sale (Naz, 2000). At the same time, in order to preserve, to enhance taste and to add tinge, it is learned that some hazardous chemicals are being mixed in the milk, often described as nature’s wholesome food.

Adulteration is rampant in India and its main intention to earn huge profits and the main driver of this phenomenon is the absence of vigilance by authorities. For most dairy producers, mastitis is the costliest animal disease that must be managed. When the udders of cows become infected, a host of expenses and losses occur: milk must be discarded and production potential is reduced, animals sometimes must be culled which results in added replacement costs, potential superior genetics are lost, treatment expenses reduce profitability, reproductive performance can be reduced, extra labor must be expended, and an immeasurable level of stress on the management personnel occurs.

The significance in the fat acids composition from milk was reported by a number of authors (Gollomb et al., 2004; Jensen, 2002). The objective of our study was to establish the content of the main groups of fatty acids using FAME by Gas chromatography in the milk produced from cow, buffalo, goat, breast milk and some packaged milk brands and interpretation of their compositional parameters.

Material and Methods:

Study was conducted to observe the extent of adulteration on market milk sold at Secunderabad city. A total of fifteen market milk samples were collected randomly from each area and examined at the laboratory using a standard milk adulteration kit manufactured by HIMEDIA laboratories, Mumbai, India. Samples were collected in clean, dry and sterilized glass bottles. The milk samples were tested for the following adulterants – formalin, urea, starch, neutralizers, detergents, sodium chloride, skim milk powder, sucrose, glucose/dextrose, hydrogen peroxide, pond water etc.

Analysis:

Fat and fatty acids are extracted from milk by hydrolytic method using standard MIDI method. Fat is extracted into ether, and then methylated to fatty acid methyl esters (FAMEs). FAMEs are quantitatively measured by gas chromatography. The system analyzes fatty acids of 9 to 20 carbons in length and automatically identifies and quantitates them. Determined mastitis infection using a novel The Sherlock Microbial Identification System (MIS), developed and marketed by MIDI, Inc., Newark, DE, USA which analyzes and identifies microorganisms isolated in pure culture on artificial media.

Data analysis:

The similarity between obtained fatty acid profiling was done using 2-D plot. Cluster analysis uses mathematical techniques to display similarities among objects in a set. Using MIDI’s cluster analysis tools, histogram and 2-D plot, samples with common fatty acid compositions are identified and grouped2-D plot is a cluster analysis tool that can be used to track similarity between milk samples. In its normal operating mode, 2-D plot uses a principle components analysis of FAME profiles to group entries in a two-dimensional space.

Results and Discussion:

Results presented in Figure 1 show that the extent of adulteration in the market milk samples collected from Secunderabad city which agrees with the findings of Singuluri et al. (2014), who observed elevated adulteration practice in different localities in Hyderabad, India.

![Figure 1: Adulterants observed in market milk samples of Secunderabad city.](image-url)
On the basis of data obtained in the present study, conclusion may be drawn that milk quality is not completely as per standards and adulteration in milk is still in practice and has not been checked completely. Consumption of lower quality milk may lead to serious human health problems. To eradicate this malpractice by local dairy owners which is deep rooted in the cities more than rural areas, steps should be taken from the door steps of local consumers. The consumers must be more active against milk adulteration going on in whole country. In the present study none of the milk samples found to be within the pure standard.

Mastitis in dairy cows, which is most often result of a bacterial infection (contagious or environmental), causes an increased somatic cell levels in milk.

Unhealthy cow’s milk has the potential to yield milk that is lower in quality. However, in the present study no positive sample was found for mastitis tested using Microbial identification Sherlock system (MIS).

Fatty acid analysis:
Gas chromatography was used for the analysis of fatty acid profiles. The Sherlock® MIS software includes many powerful tools, including: PLFA Analysis (PLFA Tools) Library Generation (LGS), Principal Component Analysis (PCA), and Electronic Records & Signatures (ERS) can estimate major fatty acids from milk samples. Packaged milk labeled as sample 1 to 8 for reference.

It was found that about a quarter of the fat in milk is monounsaturated. The remainder was mostly saturated fat, although some polyunsaturated fats and other minor fatty acids were also present. Palmitic, stearic and myristic acids were the major saturated fats found in milk. Dairy fat was also comparatively rich in the short and medium chain saturated fats. Oleic acid (18:1, ω−9), which is a main component of olive oil, macadamia oil and other monounsaturated fats found in major packaged milks.

Fatty acids are the main components of food fats, oils and fat deposits in animals and man. Besides the work they do inside the body, monounsaturated fats like oleic acid are less susceptible to spoilage than some other fats, which make them useful in food preservation Sales et al. (2013). Oleic acid regulates the activity of adrenoreceptor signaling pathways which direct the adrenergic receptors (α – and β –adrenoceptors) that help regulate blood pressure (Teres et al., 2008). Eating a diet high in oleic acid for 4 weeks reduced blood pressure while increasing good HDL cholesterol in women (Ruiz-Gutierrez et al., 1996). Oleic acid normalizes or increases fat oxidation (burning). Lim et al. (2013) found that oleic acid increases the expression of genes involved in fat burning. This means that your body is more efficient at using fat as fuel.

In addition oleic acid accelerated rates of complete fat oxidation in muscle cells. People consuming the highest amounts of oleic acid were 89% less likely to have ulcerative colitis than those consuming the least amount of oleic acid (de Silva et al., 2014). Oleic acid reverses the symptoms caused by type 2 diabetes in mice (Vassilious et al., 2009). Consuming oleic acid inhibited the inflammatory cytokine TNF–α which is produced by fat cells in obese people and contributes to metabolic syndrome (Yudkin 2007, Vassilious et al., 2009). A diet high in oleic acid may reduce the inflammation seen in obesity and non-insulin dependent obesity (Vassilious et al., 2009). When you consume oleic acid, it replaces other omega fatty acids in cell membranes. Since oleic acid is less susceptible to oxidation damage than omega-6 and omega-3 fatty acids, replacing these fatty acids with oleic acid protects your cell membranes from free radicals and other oxidative stressors (Haug et al., 2007).

20:4 w6,9,12,15c

Dairy products are loaded with fats that are easily stored under your skin as “body fat.” Cow’s milk contains a unique kind of fat with double bonds.
2-D plot is a cluster analysis tool that can be used to track similarity between milk samples. The x-axis represents principle component 1, and y-axis represents principle component 2. The 2-D plot is most useful for finding relationships among large numbers of samples or for visualizing the relationships of distantly related samples.

In the first step the 2-D plot was generated in command center’s analysis view. The data is exported using the export excel sheet. Cluster analysis uses mathematical techniques to display similarities among objects in a set. Using MIDI’s cluster analysis tools, histogram and 2-D plot, samples with common fatty acid compositions are identified and grouped as follows.

### Table 1 System generated PC1 & PC2 Data

| Sl.no | Volume | Seq Name | Index | ID #  | Sample ID  | (-1) PC1  | (-2) PC2  |
|-------|--------|----------|-------|-------|------------|-----------|-----------|
| 1     | DATA   | E152046.87A | 12    | 1338  | Sample 1   | -2.047    | 0.580     |
| 2     | DATA   | E152046.87A | 13    | 1339  | Sample 2   | -11.024   | 25.936    |
| 3     | DATA   | E152046.87A | 15    | 1340  | Sample 3   | -1.109    | 26.976    |
| 4     | DATA   | E154204.38A | 26    | 1549  | Buffalo    | -1.901    | -6.481    |
| 5     | DATA   | E154204.38A | 27    | 1550  | Cow        | -2.036    | -6.695    |
| 6     | DATA   | E154204.38A | 28    | 1551  | Goat       | -6.852    | 7.555     |
| 7     | DATA   | E154204.38A | 29    | 1552  | Mother milk| 7.223     | 19.475    |
| 8     | DATA   | E154204.38A | 34    | 1554  | Sample 4   | 3.600     | 28.066    |
| 9     | DATA   | E154204.38A | 35    | 1555  | Sample 5   | -2.051    | -6.428    |
| 10    | DATA   | E154204.38A | 36    | 1556  | Sample 6   | -2.210    | 30.621    |
| 11    | DATA   | E154204.38A | 37    | 1557  | Sample 7   | -2.436    | 26.144    |
| 12    | DATA   | E154204.38A | 38    | 1558  | Sample 8   | -2.817    | 31.954    |

* Figure 4 PC1 Vs PC2 cluster analysis tool data

This paper reports on the employment of PCA, a multivariate statistical method, to discriminate and classify various milk fats based upon their fatty acid profiles. Based on the defined groups it can be inferred that milk sample 1, 5, 6, 7 and 8 has PC1 readings similar to that of fresh cow milk. It may be concluded that these milk samples use cow milk as sole raw material and processed before packaged. Sample 3 milk showed similarly with buffalo milk constituents. Goat milk was not noticed in any of the packaged milk. Whereas mother milk noticed to have positive PC1 value as compared to other milks may be attributed to its nutritive fatty acids and complexity.

**Conclusion:**

Study concludes that the milk dealers of Secunderabad city adulterate milk to overcome the gap between demand and supply. In a country such as India where milk and milk products play an important role in different foodstuffs, this analysis carried out should bring about more awareness to the general public about the malpractices or negligence in milk production. In the present study no positive sample was found for mastitis indicating no microbial contamination. Even though, these adulterants poses health hazards on long run consumption. Milk fat is the most variable component of milk, both in concentration and composition. In dairy cattle, both the concentration and composition of milk fat are influenced by the diet. Oleic acid was found in significant amount in most samples which has many important health benefits. Evidences in the last years have showed the effects of oleic acid (OA) in human health and disease. Olive oil, rich in oleic acid, is supposed to present modulatory effects in a wide physiological function, while some studies also suggest a beneficial effect on cancer, autoimmune and inflammatory diseases, besides its ability to facilitate wound healing. Then, novel putative therapies for inflammatory and infectious diseases could be developed based on the characteristics presented by unsaturated fatty acids like OA. In comparison of olive oil, milk is cheap and provides many other essential nutrients to body. Every infant starts journey from milk being fed by mother milk, cow milk, buffalo milk or packaged, it is a birth right of every human to have hygiene food. As these are preliminary results, further investigations of fatty acid adulteration from a larger number of vegetable samples and milk from different geographical areas would show real assessment of methods and the potentials for further development and application of methods in practice.

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