Analytical Techniques for The Assessment of Physico-Chemical Properties of Ghee.

Introduction:
Ghee is complex lipids of glycerides, free fatty acids, phospholipids, sterols, sterol esters, fat soluble vitamins, carbohydrates and carotenoids (cow ghee). Ghee also contains traces of iron and calcium. It contains moisture. The major constituent of ghee is glycerides which constitutes 98% of total material in ghee and rest 2% consist of sterols most commonly cholesterol occur to the extent of about 0.5%. Ghee is most commonly prepared in India as home industry. Ghee is usually prepared by four methods: Desi Method, Creamy Butter method, Direct Cream Method and Pre-Satification Method. The desi method is most commonly used of four methods for the preparation. The quality of ghee prepared by any of this method depends upon the quality of milk, cream, dahi(Curd), butter, method of preparation, temperature, conditions of storage and type of animal feed. These factors in turn will help in determining physicochemical properties of ghee. The quality of ghee is determined by three parameters: Peroxide value, Flavour and Acidity. The quality of ghee on storage has been measured by acid and peroxide value. The temperature of clarification is most important factors that control the intensity of flavour of ghee. Ghee prepared at 120˚C or above has intense flavour which is usually referred as cooked or burnt ghee while prepared around 110˚C has somewhat mild flavour. Flavour retains longer if ghee contains 1% NaCl. The flavour of ghee is affected by the acidity of cream or butter. Cream butter having acidity of 0.15-0.25% as in ripened cream butter produces a ghee with most acceptable flavour. Rate of deterioration of ghee is less prepared from unripened butter as compared to ghee prepared from ripened butter.

Rancidity in Ghee:
Rancidity is a process which is accompanied by the formation of the unpleasant odour, taste and as a result of action of moisture, oxygen of air and enzymes. The rancidity in ghee are of two types: Hydrolytic Rancidity, Oxidative Rancidity. The hydrolytic rancidity takes place when the triglycerides are broken down to free fatty acids and simple glycerides. The action is accomplished by enzyme called Lipase. The oxidative rancidity also known as ketonic rancidity occurs in three steps where fatty acids are converted in to ketones. The enzymes responsible are Lipoxidase Enzymes, Dehydrogenase Enzymes and Decarboxylase Enzymes.

ADULTERATION IN GHEE:
The ghee is adulterated on large scale in India. The most common types of adulterants used are Vegetable oils and Animal body fats. The detection of animal body fat in ghee is difficult as the mixture has more or less the same physical and chemical characteristics. The adulterants like Starch(Potato), Sesamolin, Dyes, Synthetic colours, Vegetable fat, lard and wax. Sometimes the rancid ghee is also mixed with the fresh ghee and sold in the market.

Any type of adulteration in ghee samples bring about the changes in the physical and chemical properties of ghee. The ghee samples collected from the different place for assessment of its quality and determination of adulteration is sent to Forensic Science Laboratory. The ghee samples are tested to check whether it meets the specifications or not and the samples are analysed for the presence of adulteration. The properties of ghee are determined by the physical and chemical methods in the laboratory.

PHYSICAL METHODS:

a) Refractive Index:
The refractive index of ghee is measured at 40 °C temperature using the digital refractometer. The instrument is calibrated with the glass prism of known refractive index or by using distilled water. The light source such as sodium vapour lamp (589.3 nm) is used. The refractive index is the ratio of velocity of light in vacuum to the velocity of light in medium. The pure ghee has the refractive index of 1.45. If any adulteration in the samples is present, the refractive index of ghee will either decrease or increase. The refractive index also very helpful in determination of unsaturation. Refractive index increases with increase in unsaturation.

b) Specific Gravity:
The ghee is generally present in solid or semi-solid state. The sample is first melt and is filtered. The sample should be free from moisture. The specific gravity is measured at 30˚C. The instrument used for this purpose is pycnometer. The specific gravity of ghee is measured with respect to distilled water. The change in specific gravity of ghee indicates the adulteration.

Specific Gravity = \( \frac{W_{g} - W_{c}}{W_{S} - W_{c}} \)

\( W_{g} = \) Weight in grams of specific gravity of bottle with ghee at 30°C
\( W_{S} = \) Weight in grams of specific gravity of bottle at 30°C
\( W_{C} = \) Weight in grams of specific gravity of bottle with distilled water at 30°C

c) Melting Point:
The melting point of ghee is determined by the open tube capillary slip method. The ghee is mixture of triglycerides so they do not exhibit either sharp or definite melting point. The principle of the test is to determine the temperature at which the ghee becomes soft to slip as determined in capillary tube. The method is very useful if the ghee is suspected to have adulterated with animal fat, beef or lard.

d) Moisture Content:
The moisture content of ghee is determined by air-oven
method. It is the loss of mass of ghee on heating at 105°C temperature under operating conditions specified. The method is also useful for the determination of volatile matter in the sample if any. The moisture and volatile material is determined as percent by weight.

\[ \text{Moisture or volatile content} = \frac{W_A}{W} \times 100 \]

W = Weight in gram of material taken for test.

W_A = Loss in weight in grams of material on drying

**CHEMICAL METHODS:**

**a) Iodine Value:**

The iodine value is used to determine the degree of unsaturation of constituent fatty acids thus relative measure of unsaturated bond present in sample. It is the measure of number of grams of iodine consumed by 100 g of sample. The method is ghee is adulterated with oils or animal fat.

**b) Peroxide Value:**

The peroxide value is used to determine the oxidative rancidity in ghee. It is the milli equivalents of of peroxide oxygen per kilogram of samples. The peroxide value helps in determining the age of ghee sample. The old ghee is more rancid in nature. Higher is peroxide value, more rancid the ghee is.

**c) Acid Value:**

Free fatty acids present in ghee are readily soluble in rectified spirits. The acid value is the number of milligrams of KOH required to neutralize free fatty acids in 1 g of ghee sample. The value is determined by titrating the with standard potassium hydroxide using phenolphthalein indicator, the mixture may be warmed to about 70°C and is swirled vigorously. It is measure of fatty acids which have been liberated by hydrolysis of triglycerides due to action of moisture, temperature or enzymes.

**d) Richert-Meissl Value and Polenske Value:**

Ghee is distinguished by other fats by the presence of glycerol esters of low molecular weight fatty acids especially butyric acids but also caproic acid , capric acid, caprylic acid and myristic acid. The Richert value reflects the amount of butyrnic and caproic acids in the sample. These acids are wholly or partially soluble in steam The Polenske value reflects the caprylic acid, capric acid and lauric acid and myristic acid. The analysis of ghee for determination of RM Value and Polenske Value is to detect the presence of margarine in samples.

**f) Saponification Value:**

The Saponification value is the number of milligrams of potassium hydroxide required to saponify 1 g of ghee. The Saponification value is the index of mean molecular weight of fatty acids of glycerides comprising of fat. Lower is the Saponification value, large the molecular weight of fatty acids and triglycerides and vice-versa.

**g) Unsaponifiable matter:**

It is defined as the substances soluble in a fat which after Saponification are insoluble in water but soluble in solvents. It includes lipids of natural origin such as sterols, higher aliphatic alcohols, vitamins and hydrocarbons.

**INSTURALMENTAL TECHNIQUES:**

**a) Capillary Gas-Liquid Chromatography:**

Gas liquid chromatography is used for the determination of n-3 and n-6 unsaturated fatty acids in ghee samples. The detector used for this purpose is flame ionization detector. The column is fused silica or glass of 25-60 m in length , 0.25-0.35mm internal diameter. The stationary phase should be of moderate polarity. The carrier gas used is hydrogen.

**b) Fourier Transform Spectroscopy:**

The FT-IR is use ful for the determination of constituent of ghee samples. The FT-IR is very useful in the determination of the methyl esters, trans fat and vegetable fat and in case of adulteration of ghee samples. Also the other components of ghee such as fatty acids, sterols can also be determined by studying the spectra.

**c) EDXRF:**

The Technique can be used for the determination of Nickel in ghee samples. Nickel in ghee indicates the presence of hydrogenated fat(vegetable fat). This indicates adulteration. The hydrogenation of fat is carried out in presence of Nickel, so the traces of Nickel can be determined using EDXRF.

**DISCUSSION:**

The analysis of ghee by the analytical techniques is important for the determination of physicochemical properties and adulteration. The physical tests are employed for assessment of physical properties. Any change in physical properties of ghee gives idea about the presence of foreign substance in ghee sample. There are chemical techniques for the determination of quality of ghee but the advancement in the instrumental techniques are still in need. The confirmatory test are good and confirmatory to relate the degree of saturation and unsaturation of fatty acids in samples and thus the quality of ghee can be assessed. FT-IR is promising technique for the detection of trans fatty acids in ghee samples which could be further confirmed with the standards. EDXRF is good non destructive technique for the determination of metallic traces in the ghee samples. The effect of oxidation on samples which results in rancidity is another good discipline for the future study.

**Summary:**

The analytical techniques that are employed for the physical and chemical properties of ghee and to test the adulteration in ghee samples are discussed briefly in this paper. The aim of writing this paper is to provide brief information regarding the techniques for analysis of ghee.

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