GENERAL RULES IN FOOD TOXICOLOGICAL ANALYSIS

1. Rules of Collection of Samples in Food Toxicology Analysis

Collection of samples in food toxicology analyzes is very important. A sample should represent the properties of the food. The amount of sample taken from the food should be able to analyze at least three times.

Considering that the result of the analysis may be objected, a sufficient amount of sample for an analysis should be separated and stored. Original packaged food samples (cheese, milk, soft drinks, spirits, oil etc.) are taken from the same brand and the same manufacturer. Various samples are taken from the manufacturer and mixed samples are prepared.

2. Packaging:

Liquid food samples are filled in well-cleaned bottles which are closed with unused stoppers. Foods including fats (such as butter), honey, sausages are placed in bottles and their bottle cap are closed tightly; it is wrapped with parchment paper when necessary.

After the packages of the samples are wrapped and tied with string, the sample should be sealed by the authorized person. The name and address of the seller; the type, quantity and price of the sample; the date and place of the sample; the name of the person or authority taken the sample should be given.

3. Preparing Samples for Analysis

Samples are send to the laboratory to be analyzed as soon as possible. In the laboratory, the packaging of the food is opened carefully. Information about the seal, label and packaging is recorded.

The sample is thoroughly mixed before analysis. The food sample must be crushed, chopped or macerated for the analysis. Liquid or pulverized substances are easily mixed by transferring or shaking from one container to another. Frozen foods are cut or crushed and taken from various parts.

Each analysis first begins with organoleptic examination. Shape, definition, net and gross weight of the sample are recorded. Also the appearance and characteristics of the food; smell, taste, pH are determined. Then the chemical analysis is started.

Food Sample	Sample Quantity	Sampling Type
Oils (solid) (butter, margarine etc.)	125 g	In wide-mouthed jars, tightly closed (should be sent immediately to analysis)
Fish and canned fish	3 or more fishes	Unopened canned (should be sent immediately to analysis)
Meat, canned meat	125 g original pack	Paper should be wrapped well. Sent only in glass container.
Soft drinks (mineral water, fruit juice etc.)	1 original pack (250-500 ml)	Clean, dry bottles
Honey, artificial honey	Min 125 g original pack	Wide-mouthed glass jars
Cheese	250 g or 1 piece	Parchment paper or original packaging
Milk	1 ¼ liter or original packing	Clean dry bottles Should be sent immediately
Cereals	125 g	Paper bag

Water	1 liter	Mouth closed, dry and clean bottles
Sausage, sausage, sausage	125 g or original packing (min 4 pieces)	Wide-mouthed glass containers or original packages
Sugar and sugary ingredients	125-250 g	In a jar or suitable packaging
Wine and other spirits	1 liter	Clean and dry bottles
Beer	2 original pack (250 ml)	Clean and dry bottles
Vinegar	1 liter	Clean and dry bottles

EXPERIMENT NO: 8

ANALYSIS OF DECAY PRODUCTS AND NON-LEGAL ADDITIVES OF OILS

The edible oils are classified as vegetable and animal oils according to the Regulation on Turkish Food Codex. There are different index for oils; free fatty acids index, refraction index, saponification index, iodine index. Foreign oils, mineral oils and dyes are searched in samples. Bitterness and also the amount of moisture and salt are determined.

1. Determination of Refractive Index of Oils

The refractive index of a pure substance is constant under certain temperature and pressure conditions. The refractive index is used to determine the purity of the oils. Although the oils are not completely pure, refractive indexes vary within a narrow boundary.

Tools for determining refractive index are called refractometers. The refractive indexes of the liquid oils are measured at 25ºC and the others at 40ºC. A thermostat is connected to the refractometer to adjust the temperature.

In Abbe and Zeiss refractometers, refractive indexes are measured between 1.325 and 1.492. In order to measure the refractive indexes of fats with a simpler instrument called butirorefractometer, the values between 1.422 and 1.489 are evaluated between 0 and 100 and this is called a butiro index. When evaluating the oils, it is necessary to convert the refractive index to the butiro index from the charts. Olive oil is considered to be mixed with other oils if the refraction index of the olive oil examined at 25ºC in abbe refractometer shows a deviation from the butiro index value (59.4 - 63.6).

2. Determination of Bitterness in Oils with Kreis Test

During the degradation of fats, epihidrin aldehyde occurs with other aldehydes. The Kreis experiment is based on the color reaction of this aldehyde with phenols such as resorcin and fluoroglucin.

The epihydrin aldehide is bound in acetal form with glycerin in oils. The oil is first treated with HCl to release it.

a. Melted 5 g of oil is shaken with 5 mL of HCl for 1 minute. 5mL of 0.6% resorcin solution is added and shaken again. After 5 minutes, the color of the separated acid layer is checked. This layer changes to a dark violet red color in bitter oil or bleached oils in light.

b. When the same experiment is made with 0.1% fluoroglucine solution, the colour of the oil changed to red. According to the regulations, the Kreis test in olive oil should not exceed the pink border.

3. Investigation of Mineral Oil

1mL of oil, 1 ml of saturated KOH solution and 25 mL of alcohol is placed into a balloon. Then the mixture is saponified in a vertical cooler. As a result of the saponification, 25 ml of water is added to understand the presence of mineral oils. If the mixture becomes cloudy, it means that there is mineral oil.

4. Determination of Saponification Value:

Saponification value number represents the number of milligrams of potassium hydroxide required to saponify 1g of fat under the conditions specified.

The distortion and adulteration of the oils is understood with the saponification value. There is a value between 221 and 230 for butter and 187-198 for oils.

Experiment: 5 g of oil is heated in a vertical cooler with 50 mL ½ N alcoholic potassium hydroxide solution for one hour. The excess amount of alkaline is titrated back with ½ N HCl using the phenolphtalein.

$$\text{Saponification value} = \frac{(a-b)\times 28.05}{\text{Weight (g) of the sample}}$$

a: Acid spent to neutralize 50 mL of alcoholic potassium hydroxide
b: Acid spent to neutralize potassium hydroxide without saponification

5. Determination of Other Oils in Olive Oil:

Experiment: 5 mL of oil is mixed with 5 mL of nitric acid and allowed to stand for 15 minutes. There is no discoloration in pure olive oil, but different colors are formed in other oils. It is forbidden to mix various vegetable oils with each other according to the Regulation on Turkish Food Codex, olive oil should not have any color change.

6. Determination of Free Fatty Acids in Olive Oil:

Natural fats include a small amount of free fatty acids and their amount increases with time. The acid value is the number that expresses, in milligrams the quantity of potassium hydroxide required to neutralize the free acids present in 1 g of the substance.

Experiment: About 5 g of oil, which is weighed in an erlenmayer, is dissolved in 25 ml of the ether-alcohol mixture, which has already been neutralized. A few drops of phenolphthalein solution are added and titrated with 1/10 N KOH. The amount of KOH spent is recorded until the pink color is obtained.

$$\text{Acid Value (Acid index)} = \frac{5{,}6 \times a \times f}{T}$$

$$\%\ \text{Free Fat Acids} = \frac{M \times a \times f}{100 \times T}$$

a: Amount of 0.1 N base spent (mL)
f: Base Factor
T: The weighed amount of oil (gram)
M: Molecular weight of fatty acid

According to the Regulation on Turkish Food Codex; free fatty acids does not exceed; 0.8% in the filtered olive oil; 1.5% in extra-extra olive oil, 2.5% in extra olive oil; 3.5% in 1. edible olive oil; 4.5% in 2. edible olive oil.

7. Determination of Acidity in Butter:

The amount of alkali in milligrams, which should be used to neutralize the free fatty acids in 1 gram oil, gives the acid index of that oil.

Experiment: 5 g of oil is dissolved in 25 mL of the ether-alcohol mixture, which has been previously neutralized. A few drops of phenolphtalein solution are added and titrated with 1/10 N NaOH. If the amount of mL spent is multiplied by 2, the acidity of the oil is found. To show the acidity of the butter as % milk acid, the acidity is multiplied by 0.09 (1mL N NaOH = 0.09 g milk acid).

The degree of acidity in non-stale (fresh) butter is between 1 to 5. It is much more in stale butter which is kept in poor conditions. The acidity for butter is considered to be 3, and for others it is accepted to be between 3-10 according to the Food Regulation. If the degree of acidity is 10-15 in butter, it is adulterated. If it is higher than 15, these butter are considered to be harmful to health.

8. Determination of Margarine in Butter:

Experiment: 5 g of butter is melted in a beaker. After removal from the water with anhydrous Na_2SO_4 added to the filter paper, it is filtered into a tube. Take 2 mL of the mixture and add 2 mL amyl alcohol. The mixture is heated until it becomes clear. Then the tube is taken away from the heat and mixed until it becomes cloudy again.

The formation of the turbidity between 48-53°C indicates that the butter is pure. In the margarine, turbidity occurs between 70-74°C. In margarine mixed oils, depending on the mixing ratio, turbidity occurs mostly between 60-63°C, above 53°C.