UNDERGRADUATE OF NUTRITION PROGRAM
FACULTY OF PUBLIC HEALTH
UNIVERSITAS AIRLANGGA

PRACTICUM GUIDELINE

NUTRIENT ANALYSIS

2ND EDITION

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APPROVAL SHEET
PRACTICUM GUDELINE
NUTRIENT ANALYSIS

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PRACTICUM RULES

1. Practicum participants are not allowed to enter the laboratory before the practicum begins.
2. Participants must have taken a test about the event that will be worked on before the practicum
3. Participants must be present on time. Late participants must report and get permission from the laboratory assistant that day.
4. Participants must fill in / sign the attendance list before starting the practicum.
5. Participants are required to wear a practicum coat during the practicum.
6. During the practicum, participants are not allowed to eat, drink, smoke, joke, or do other things that can disrupt the course of the practicum.
7. Participants who do something that is considered to endanger laboratory equipment will be asked to leave the laboratory.
8. Fifteen minutes before the end of the practicum, the work is stopped, and the remaining time is used to tidy up the equipment and clean the practicum site.
9. The duty laboratory assistant must authorize all results / observation data, and this data is made into a report, which is then authorized again by the laboratory assistant concerned.
10. At the end of each practicum, participants must report all equipment they have borrowed to the laboratory staff.
11. Practicum participants who damage or lose equipment must replace the equipment before the next practicum day or before taking the final test/response.
12. To keep the practicum going well, all participants must obey this order.

I. DETERMINATION OF MOISTURE CONTENT

Determination of moisture content is an essential and most widely performed analysis in food processing and testing. The amount of dry matter in a material sample is opposite to the amount of water it contains, so moisture content is directly related to the economic importance of the material. The water content of an ingredient is also related to the quality and stability of the ingredient. Mold, heating, insects, and the risk of germination will easily damage grains with high moisture content. The browning rate of dried vegetables and fruits and the absorption of O_2 by egg powder increase with higher water content.

To meet composition standards and regulations, moisture content needs to be known in determining the nutritional value of food. Moisture content is also needed to calculate the composition of ingredients presented on a dry matter basis. Analysis of the moisture content of food ingredients can use several methods:

- Drying method (thermogravimetry)
- Distillation method (thermovolumetry)
- Chemical method (Fischer method)
- Physical method

Drying/Heating method (thermogravimetry) (AOAC, 1925)

Working principle: Evaporate water from the material by heating to a constant weight until all the water has evaporated. Weaknesses of this method: Volatile substances at 100˚C evaporate and are counted as water.

Cara Kerja :

1. Weigh the sample in the form of powder or material that has been mashed as much as 1-2 grams in a clean and dry weighing bottle and know its constant weight.
2. Dry in an oven at 100-105˚C for 3-5 hours, depending on the material. The higher the water content of the ingredients, the longer the drying time.
3. Cool the weighing bottle and sample in an applicator and weigh. Heat again in the oven for 1 hour, cool in an applicator, and weigh. This treatment was repeated until a constant weight (difference in successive weighings <0.2 mg) was achieved.
4. Weight reduction is the amount of water in the material.
5. Calculate the moisture content on a % wet basis.

II. DETERMINATION OF ASH CONTENT

Ash is the residue or residual inorganic substances from the combustion of organic matter. Its content and composition depend on the nature of the material being burned and the method of ashing. Mineral elements in ignition will produce ash in the form of their salts or oxides.

The form of the mineral component in the sample may differ from its form in the ash. For example, Ca-oxalate will turn into Ca-carbonate and, on further ashing, will become CaO. Some "trace minerals" or minerals bound to biologically active systems are converted to inorganic compounds.

Water-soluble ash is sometimes used to index fruit content in jelly or other fruit preserves. Insoluble ash is useful as an index of dust fouling in spices, talc in confectionery, the presence of sand in sugar, seeds, and so on. Insoluble ash is determined by boiling the ash in 10% HCl.

Ash from fruits is alkaline, which results from converting organic salts into carbonate salts. Foodstuffs high in fruit acids or salts produce ash alkalinity, an index of the fruit portion of the ingredient.

In determining total ash, the method that gives satisfactory results is ashing in porcelain crucibles at 400-700ºC (most commonly ±550ºC). For individual mineral analysis, it is necessary to asphyxiate in a platinum crucible.

When the ashing fails to obtain carbon-free ash, it is moistened, dried, and re-fired until it is white/gray-white. Sometimes it is necessary to add H O22 or nitric acid to help the ashing reaction.

Dry ignition to destroy organic matter in the determination of trace minerals is rarely applied because the minerals can be lost to evaporation. Thiers (1957) recommends dry ignition with a special tool with the help of a hot plate & infrared lamp with temperatures increasing gradually to 450ºC.

The formula for calculating ash content is as follows:

Ash content (*wb*) = [(ash weight) : (sample weight)] x 100%

Ash content (*db*) $= \frac{\text{ash weight}}{\text{water free sample weight}}\ x\ 100\%$

= Ash content (*wb*) x [100 / (100 - % water)]

How to work Dry Ignition (AOAC Method, 1925)

1. Incinerate the porcelain crucible with a lid in a muffle furnace, cool it in an oven, and then transfer it to an applicator and weigh it.
2. Weigh the sample in a porcelain crucible with a known weight (approximately 2-10 grams of the sample), and burn it on an electric stove until it becomes charcoal (no longer smoking, do it in a fume hood). Then incinerate in a muffle furnace until it becomes a whitish ash.
3. Place in a 100ºC oven to cool the temperature, continue cooling in an exicator, and weigh.
4. Repeat heating for 30 minutes, then cooling and weighing until a constant weight is obtained.
5. Calculate the ash weight and determine the ash content in % wet-basis and % dry-basis.

Notes :

- If the ash obtained is still dark (gray), wet the ash with an ammonia solution (NH3) or hydrogen peroxide (H O22) and then ash it again.
- For mineral analysis, especially trace minerals, soaking does not use the 'dry' method as above, but the 'wet' method, namely the process of deconstruction or digestion by boiling in concentrated strong mineral acids (hydrochloric acid, sulfuric acid, perchloric acid, or their mixtures). The organic compound components will completely oxidize while the mineral elements will form inorganic salts. The procedure is the same as the digestion procedure in total nitrogen analysis for the determination of protein content (See Chapter IV). These inorganic salt solutions can then be analyzed for individual mineral components by gravimetric (settling and weighing), titrimetric, or spectrophotometric (Atomic Absorption spectrophotometry or AAS) methods.

III. DETERMINATION OF CARBOHYDRATE CONTENT

Total carbohydrate is sometimes not determined by separate analysis. However, it is calculated from the results of the determination of water, ash, lipid, and protein content, assuming that substances other than these components are carbohydrates. This method of determination expresses the result as a **carbohydrate by difference**.

So :

Carbohydrate *'by difference'* % (*wb*) = 100% - %wb (water + ash + lipids + protein)

Carbohydrate *'by diference'* % (*db*) = 100% - %db (ash + lipids + protein)

With this method of determination, the results cannot describe the nutritional value of the food ingredients being analyzed. Carbohydrates consist of digestible carbohydrates consisting of sugar compounds (monosaccharides and oligosaccharides), dextrins, and starch, and non-digestible carbohydrates, which are fibers (consisting of cellulose, hemicellulose, pectin, glucomannan, galactomannan, lignin and from seawater plants: agar, carrageenan, furcelaran, hypnan, alginate).

Digestible carbohydrates will be absorbed by the small intestinal wall, carried by the blood to the muscles and liver to be burned to produce energy for our daily needs/activities. Analysis of digestible carbohydrates is often based on the reducing sugar analysis method.

Determination of reducing sugars (spectrophotometric method, Nelson-Somogyi method)

Standard curve generation:

1. **Penentuan Determination of reducing sugars (spectrophotometric method, Nelson-Somogyi method)**

   Standard curve generation:

- A standard curve was prepared from 10 mg/100 ml pure glucose solution, which was filled into test tubes in the following amounts:

Test Tube Number	1	2	3	4	5	6
Glucose solution (ml)	0	0,2	0,4	0,6	0,8	1
Aquadest (ml)	1	0,8	0,6	0,4	0,2	0
Total Volume (ml)	1	1	1	1	1	1
Glucose level (mg/ml)	0	0,02	0,04	0,06	0,08	0,1

- Add to each of the above tubes 1 ml of Nelson's reagent, and heat all tubes in a boiling water bath for 20 minutes.

- Take all tubes, cool them together in water to 25ºC. Each added 1 ml of Arseno-molybdate reagent, mix until all precipitates dissolve again, then each tube plus 7 ml of distilled water, mix until homogeneous.
- Measure the optical density or absorbance with a spectrophotometer at a spectrum of 540 nm and tabulate the readings as follows:

No	*x* (sugar content)	*y* (absorbance)	*x2*	*y2*	*Xy*
1	0 = x_1	y_1	x_1^2	y_1^2	x_1y_1
2	2 = x_2	y_2	x_2^2	y_2^2	x_2y_2
3	4 = x_3	y_3	x_3^2	y_3^2	x_3y_3
4	6 = x_4	y_4	x_4^2	y_4^2	x_4y_4
5	8 = x_5	y_5	x_5^2	y_5^2	x_5y_5
6	10 = x_6	y_6	x_6^2	y_6^2	x_6y_6
N	$\sum x$	$\sum y$	$\sum x^2$	$\sum y^2$	$\sum xy$

- Linear standard curve equation: y = a + bx

where $b = [n\sum xy - \sum x \sum y] / [n\sum x^2 - (\sum x)^2]$

$a = [\sum y - b\sum x] : n$

with regression coefficient $r = \dfrac{[n\sum xy - \sum x\sum y]}{[n\sum x^2 - (\sum x)^3]^{1/2}[n\sum y^2 - (\sum y)^2]1/2}$

- Prepare a sample solution that is chemically purified (e.g. with Pb-acetate or Al-hydroxide) and filtered or centrifuged.
- Dilute the sample solution such that it contains reducing sugar in the range of 2-8 mg/100ml.
- Pipette 1 ml of clear sample solution and transfer to a clean test tube, then add 1 ml of Nelson's reagent and proceed as with the sugar solution in the standard curve preparation above.
- The OD or absorbance readings results are entered into the Y value in the linear line equation Y = a + bX. Then the value of X = reducing sugar content in mg / 10 ml units will be obtained.

- By using the solution's dilution factor, the sample's reducing sugar content can be calculated

2. Determination of Total Sugar:

Determination of Total Sugar:

- Pipette 25 ml of the lead (Pb) free filtrate from the above-reducing sugar determination, put in an erlenmayer, and add 15 ml of distilled water and 5 ml of 30% HCl.
- Heat on a water bath at 80ºC for 10 minutes, then cool rapidly to 20ºC, and neutralize with the addition of 40% NaOH and further diluted in such a way that it contains reducing sugar between 2-8 mg/100ml.
- Pipette 1 ml of the aqueous solution and determine the reducing sugar as in the standard solution preparation procedure above.

Catatan:

Supposing the sugar hydrolyzed is sucrose (non-reductive hydrolyzed to reductive), the difference between the reducing sugar after hydrolysis and before hydrolysis is the same as the reducing sugar from sucrose hydrolysis.

Sucrose + water → glucose + fructose

BM = 342 BM =18 180 180

sucrose = [(342) / (180+180)] x reducing sugar resulting from sucrose hydrolysis

Sucrose = 0,95 x reducing sugar resulting from sucrose hydrolysis

3. Determination of Total Starch

Starch (amylum = amylose + amylopectin) can be hydrolyzed into monosaccharides (glucose) by hydrolyzing the oligosaccharides in no.2 above. However, a slightly higher acid level and ± 3-4 hours are required. The glucose solution from hydrolysis is neutralized and diluted until the reduction sugar level is between 2-8 mg/100ml. Then the sugar content is analyzed using the Nelson-Somogyi method as in no.1. Starch is a glucose polymer, so the hydrolysis equation is as follows:

(glucose) n + n water → n glucose

Starch (BM = n x 162) (n x 8) (n x 180)

Starch weight = (BM starch) / (n x BM glucose) x glucose weight (reduced sugar)

= (162 n) / (180 n) x reduced sugar weight = 0,9 x reducd sugar weight

Starch content = 0,90 x reduced sugar content

4. Qualitative Analysis of Carbohydrates

Qualitative analysis aims to determine the presence or absence of carbohydrate content in a sample. One method that can be used in the qualitative test of carbohydrates is the Benedict test. Benedict's reagent consists of sulfate copper salt, sodium citrate, and sodium carbonate. The principle of Benedict's test is the heating of carbohydrates, given that Benedict's solution will change color to form a brick-red precipitate of cuprooxide..

Steps:

1. Put 1 ml of Benedict's reagent in a 10 ml test tube.
2. Add 0.5 ml of the sample to be tested, then vortex.
3. Observe the color change that occurs.
4. Heat in a water bath for 5 minutes.
5. Observe the color change that occurs.

IV. DETERMINATION OF PROTEIN CONTENT

1. Protein Determination by Kjeldahl Method

Determination of crude protein content of a food sample can be approached through the determination of total nitrogen content. This is because in addition to protein, there are other compounds that contain nitrogen elements such as urea, nitrate, nitrite, ammonia, nucleic acids, purines, pyrimidines and amides. Only the nitrogen that makes up proteins should be counted, but this is technically very difficult to do. Nitrogen that makes up compounds other than proteins is usually so low that for this purpose it can be ignored.

The most well-known determination of protein by total nitrogen content determination is the macro Kjeldahl method which includes the following steps:

(a) **Digestion or deconstruction**. At this stage the sample material (± 2 grams) is boiled in concentrated H2SO4 until all the elements C and H are burned out which is marked by clear liquid. This digestion can be accelerated by adding Zink or Selenium metal catalysts, and K_2SO_4 or Na_2SO_4 salts to increase the boiling point of the mixture, and some porcelain granules to expand the heating contact. In this process the N element will form the ammonium-sulphate salt $(NH_4)_2SO_4$.

(b) **Distillation**. The liquid from digestion is very acidic, when added with concentrated NaOH until alkaline, the Ammonium sulfate compound will turn into ammonium hydroxide (NH_4OH, which is the form of ammonia (NH_3) solution in water). $_3$When this solution is distilled by flowing hot water vapor, the NH gas will evaporate and then condense in the cooling pipe and drip into the collection flask which was previously filled with 0.1 N HCl solution in a certain volume (the NCl concentration must be greater than the ammonia concentration being distilled).

(c) **Titration.** The distilled ammonia will react with some of the HCl in the collection flask, and then the remaining HCl is measured by titration using 0.1 N NaOH standard solution. Perform a blank analysis which means that the total amount of HCl ≈ the amount of NaOH for titration.

The macro Kjeldahl method is slow and uses relatively many reagents (chemicals) so it is expensive. Then the micro Kjeldahl method was developed which only uses the number of samples and the number of chemical reagents about 10-15% x the needs of macro Kjeldahl, also the working time is shorter. In micro Kjeldahl, the distillation collection solution is boric acid (HBO3) and the solution for titration is standard HCl.

Distillation and titration reaction equations:

Kjeldahl macro : $(NH_4)_2SO_4 + 2\ NaOH \rightarrow Na_2SO_4 + 2\ (NH_4)OH$

$(NH_4)OH + \text{excess } HCl \rightarrow (NH_4)Cl + H_2O$

titration : $HCl\ (\text{residual}) + \text{standard } NaOH \rightarrow NaCl + H_2O$

Kjeldahl micro : $(NH_4)_2SO_4 + 2\ NaOH \rightarrow Na_2SO_4 + 2\ (NH_4)OH$

$(NH_4)OH + HBO_3 \rightarrow (NH_4)BO_3 + H_2O$

titration : $(NH_4)BO_3 + HCl\ standard \rightarrow (NH_4)Cl + HBO_3$

Calculation of analysis work results:

Kjeldahl macro : mgrek N = mgrek NH_4OH = mgrek (HCl-NaOH)

mgram N = mgrek N x 14 (≈ BA Nitrogen)

Micro Kjeldahl : mgrek N = mgrek NH_4OH = mgrek HCl = (ml x Normal) HCl

mgram N = mgrek N x 14 (≈ BA Nitrogen)

From many studies, it is found that most proteins contain an average Nitrogen element of about 16% (in pure protein), so the amount of Protein weight can be calculated = (100 / 16) x Nitrogen weight.

Protein Weight = 6.25 x Nitrogen Weight

The conversion factor = 6.25 is used in general, including for ingredients where the amino acid composition of the protein is not known. However, there are ingredients that use different conversion / multiplication factors.

N to protein conversion factor of some ingredients

Material Type	Multiplication Factors
Beer, syrup, seeds, yeast, fruits, tea, wine, malt, fodder	6,25
Rice	5,95
bread, wheat, macaroni, noodles	5,70
peanuts	5,46
Soybeans	5,75
Milk	6,38
Gelatin	5,55

Kjeldahl Micro Working Procedure:

(a) Weigh 50-60 mg dry matter or 0.2 - 0.5 g wet matter and place in a Kjeldahl flask (50ml) and add 2 ml concentrated sulfuric acid. Add 0.5 - 2 g of a mixture of $Na_2\ SO_4$: HgO (20:1) as catalyst.

(b) Simmer in the smoke chamber until clear and continue boiling another 30 minutes. After cooling, wash the inner walls of the flask with a little distilled water and boil again for 30 minutes.

(c) After cooling transfer to a Kjeldahl micro distillation flask and add 5-10 ml of distilled water and 6-15 ml of NaOH-Na S O_{223} solution (40:5 g and dissolve with distilled water to 100 ml).

(d) Immediately perform steam distillation, the distillate is collected in a 100 ml erlenmayer that has contained 5 ml of 4% boric acid solution (saturated solution) and add 1 ml of mixed indicator (methyl red-methylene blue) or (methyl red-brom cresol green). Distillation is terminated when all N has been distilled, i.e. when the distillate droplets are no longer basic.

(e) Titrate the distillate with 0.02 N HCl.

(f) Calculate the total N or % protein in the ingredients using the formula:

$$\%\, N = \frac{(N\ x\ ml)\ HCl\ x\ 14{,}008}{mg\ ingredients}\ x\ 100\%$$

% protein = % N x conversion factor

2. Protein Determination by *Lowry-Folin* Method

The Lowry-Folin method is also called the *Folin-Ciocalteu test*, which can be used for protein determination. This method can measure the protein content of snippets up to 5µg. The blue color that occurs by Folin Ciocalteaeu reagent is due to the reaction between proteins with Cupri (Cu) in alkaline solution and the reduction of phosphomolybdate-phosphotungstate salts by tyrosine and tryptophan present in proteins, because the content of the two kinds of amino acids varies widely between types of proteins, the intensity of the color caused per milligram of protein is different.

To measure the amount of protein in a solution, a standard curve is needed, which describes the relationship between protein concentration and absorbance at a wavelength of 540 nm.

Material:

1. Reagent A: dissolve 100 g $Na_2\ CO_3$ in 0.5 N NaOH to a volume of 1000 ml.
2. Reagent B: dissolve 1 g $CuSO_4\ .5H_2\ O$ in distilled water to a volume of 100 ml.
3. Reagent C: dissolve 2 g K-tartrate in distilled water to a volume of 100 ml (Solutions A, B, and C can be stored).
4. Dissolve 20mg/100ml bovine serum albumin standard. Prepare the concentration series for the preparation of the standard curve as follows:

Test tube number	1	2	3	4	5	6
BSA solution (ml)	0	0,2	0,4	0,6	0,8	1
Aquadest (ml)	1	0,8	0,6	0,4	0,2	0
Total volume (ml)	1	1	1	1	1	1
Protein content (mg/ml)	0	0,04	0,08	0,12	0,16	0,2

5. Reagent D: mix 15 ml of solution A; 0.75 ml of solution B; and 0.75 ml of solution C then mix until being homogeneous.
6. Reagent E: dilute 5 ml of 2 N Folin Ciocalteaeu reagent to a volume of 50 ml and mix well.

Steps:

1. Put 1 ml of snippet in a test tube then add 1 ml of reagent D, immediately vortexed and incubated at room temperature for 15 minutes.
2. Add 3 ml of reagent E to the sampling tube and vortex immediately, then incubate at room temperature for 45 minutes and immediately measure the absorbance at 540 nm. The blue color formed remains stable for 45-80 minutes after incubation.
3. Make a standard curve of bovine serum albumin with a concentration of 0; 0.04; 0.08; 0.12; 0.16; 0.2 mg/ml so that a regression line is obtained between absorbance and protein concentration. Based on this regression line, the protein content of the snippet can be determined.

V. DETERMINATION OF LIPIDS (FATS & OILS)

By definition, lipids are all organic compounds that are soluble in non-polar organic solvents. Most (>90%) lipid compounds in food are triglyceride compounds (fats & oils) and others can be fatty acids, free, phospholipids (e.g. lecithin), waxes, steroids (sterols & sterones), carotenoids, vitamins (A, D, E, K), terpenoids, or isoprenoids (essential oils, among others).

Triglycerides and waxes are called neutral lipids which are very non-polar in nature so they are very difficult to dissolve in water but on the contrary they are very soluble in non-polar solvents / organic solvents (benzene, petroleum ether, diethyl ether, hexanes, chloroform, etc.). Therefore, to determine the fat & oil content of food ingredients can be done by extraction using nonpolar solvents, evaporating the solvent from the extract and followed by weighing the residue. Solvents commonly used are hexane, and petroleum ether.

Some lipids in food are bound (not tightly) to other food components (proteins, carbohydrates) which are polar and hydrophilic (like to bind water). Therefore, in order for the extraction to run perfectly, the material must be dry and in the form of fine flour. For materials with high fat/oil content, when ground, they tend to clump and are difficult to make into fine flour. Such materials before extracting lipids, can be added fine sand and mixed until homogeneous / evenly distributed. Well-known extraction tools for lipid determination are *Soxhlet extraction tools*, *Goldfish* extraction tools, and their developments such as *micro Soxhlet* and *Soxhtec*.

Micro Soxhlet Extraction Procedure:

1. Weigh 1-2 g of dried and powdered material (passing 40 mesh), place in a Soxhlet extraction tube.
2. Attach the extraction tube to a micro Soxhlet distillation device with sufficient petroleum ether solvent (± 10 ml), for 4 hours of distillation.
3. Petroleum ether that has contained fat / oil is transferred into a clean weighing bottle that has known weight, then the solvent is evaporated on a waterbath, and then dried in a 100ºC oven until constant weight.
4. The weight of the residue in the weighing bottle is expressed as the weight of fat & oil.

Quality of Food Fats & Oils

The quality of lipids in food tissues or those that have been extracted as fats and oils is influenced by physical, chemical, and organoleptic (sensory) properties. Triglycerides or the full name tri-acyl-glycerol is a compound composed of one glycerol molecule that forms an ester bond with three fatty acid molecules. The high melting point of fats and the viscosity of oils are physical properties of fats and oils that are influenced by their molecular weight (BM) or by the short length of the carbon chain and by the degree of saturation and unsaturation of the fatty acids that make up the triglycerides. Solid fats in materials (e.g. cocoa beans) cause the need for heating when extraction is

carried out by pressing, different when in the form of liquid oil (e.g. peanut seeds, copra, sunflower seeds, etc.).

Triglycerides in wet/moist biological material tissues or as moist fats/oils will easily undergo hydrolysis to produce free fatty acids. Relatively high levels of free fatty acids are considered to reduce the quality because in addition to spurring further damage reactions, also when there are short-chain free fatty acids will cause a bad taste.

The relative BM can be estimated by determining the *saponification number*. Soap (sapon) is a salt that is the result of the reaction between alkalis (NaOH, KOH, LiOH) and fatty acids (RCOONa, RCOOK or RCOOLi). Saponification number is expressed as the number of milligrams of KOH required to saponify 1 gram of fat/oil. Fats/oils with relatively low BM means that they are composed of relatively short C-chain fatty acids so that the number of fatty acids per 1 gram is relatively more than in fats/oils with relatively high BM.

Fat melting point and oil viscosity are also affected by the unsaturation of the fatty acids that make up triglycerides. The more unsaturated the fatty acids that make up the triglycerides, the lower the melting point of the fat and the viscosity of the oil. The unsaturation of fatty acids can be measured by *Iodine Number* Determination, which is based on the fact that each double bond in the C chain of fatty acids can be charged (reacted) with 2 iodine atoms. The more unsaturated the triglyceride, the higher the Iodine number.

Unsaturated lipids have the disadvantage of being easily and quickly oxidized by air oxygen, and this will result in a decrease in quality. The initial oxidation will give rise to fatty acid peroxides, while further oxidation will produce compounds that smell bad (= rancid odor).

Procedures:

1. Determination of Free Fatty Acids (ALB/FFA) (Mechlenbecker, 1960):

- Weigh 28.2 ± 0.2 g of sample in an erlenmayer (the material should be liquid and homogeneous when taken). Add 50 ml of hot neutral alcohol and 1 ml of phenol-phthalein (PP) indicator.
- Mix for a few minutes then titrate with 0.1 N NaOH solution until a pink color is achieved which does not disappear for 30 minutes.
- Percent free fatty acids are expressed as oleic acid ($C18{:}1$ BM=282) in most oils, as palmitic acid ($C16{:}0$ BM=256) in palm oil and as lauric acid ($C12{:}0$ BM=200) in coconut oil and palm kernel oil, and as linoleic acid ($C18{:}2$ BM=278) in soybean oil.
- The Acid Number or % FFA is calculated as follows:

$$\text{Acid number} = \frac{(\text{ml x N})\text{NaOH x BM KOH}}{\text{sample grams}} = \frac{\text{mg KOH}}{\text{gram sampel}}$$

$$\%\ \text{FFA} = \frac{(\text{ml x N})\text{NaOH x BM dominant fatty acid}}{\text{sample miligrams}}\ \text{x } 100\%$$

2. Determination of Saponification Rate (AOAC, 1926):

- Weigh 1.5 - 5 g of fat/oil in a 200 ml erlenmayer, then add 25 ml of KOH-alcohol (dissolve 40 g of KOH in 1 liter of alcohol).
- Cover the erlenmayer with counter-cooling, boil carefully for 30 seconds, cool and add a few drops of PP indicator.
- Titrate the excess KOH with 0.5 N HCl standard solution. Also perform a blank titration which is the same work but without the oil sample.

$$\text{Aponification Rate} = \frac{\text{titration mililiters (blank} - \text{sample) x BM KOH}}{sample\ grams}$$

3. Determination of Rancidity by Peroxide Number (Iodometric Titrimetric Method):

- Weigh 5 ± 0.005 g of sample in 250 ml of dry closed erlenmayer and add 30 ml of acetic acid-chloroform solution (3:2). Shake until the material is completely dissolved, then add 0.5 ml of saturated Ki solution free of I2.
- Let stand for 1 minute with occasional shaking then add 30 ml of distilled water.
- Titrate with 0.1 N Na S O_{223} (Na-thiodisulfate) until the yellow color disappears. Add 0.5 ml of 1% amylum solution and continue the titration until the exact blue color disappears.
- The peroxide number is expressed as milliequivalents of peroxide in 1000 grams of sample.

$$\text{Peroxide number} = \frac{\text{(ml x N)Na} - \text{thiosulfat}}{\text{sample grams}}\ x\ 1000$$

V. IODOMETRIC VITAMIN ANALYSIS

The method of measuring the concentration of the solution using the titration method, which is the addition of a color indicator to the solution being tested, then dripped with a solution that is the opposite of the nature of the solution being tested. Measurement of Vitamin C levels with a redox reaction using iodine solution (I_2) as a titrant and starch solution as an indicator. In the titration process, after all vitamin C reacts with iodine, the excess iodine will be detected by starch which makes the solution dark blue. The reaction of Vitamin C with iodine is as follows:

$$C\ H\ O_{686} + I2\ C\ H\ O_{666} + 2I^{-} + 2H^{+} \text{ .....(2.1)}$$

Vitamin C analysis procedure:

1. Weigh 10 g of sample.
2. put in a volumetric flask and dilute with distilled water to the limit.
3. pipette 10 ml of our filtrate then put it in a 25 ml Erlenmayer.
4. Add 2 ml of 1% amylum and if necessary add 20 ml of distilled water.
5. Titrate with 0.01 N I_2 solution until a blue solution is formed.

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Carbohydrate Test

1. **Quantitative Test (Nelson Somogyi Method)**

A. Standard curve generation

Anhydrous glucose 0.01 g + 100 ml distilled water

	1	2	3	4	5	6
Standard sugar solution (ml)	0,0	0,2	0,4	0,6	0,8	1,0
Aquadest (ml)	1,0	0,8	0,6	0,4	0,2	0,0
Total volume (ml)	1,0	1,0	1,0	1,0	1,0	1,0
Glucose level (ml)	0,00	0,02	0,04	0,06	0,08	0,1

↓

Making glucose solutions with levels of 0; 0.02; 0.04; 0.06; 0.08; 1

↓

Take 1 ml each, put into a test tube

↓

Add 1 ml of Nelson's solution (A:B=25:1)

↓

Memvortex

↓

Heating in a glass beaker filled with boiling water for 20 minutes

↓

Cooling to tube temperature 25ºC

↓

Adding 1 ml Arsenomolybdate

↓

Vortexing

↓

Adding 7 ml of distilled water

↓

Vortexing

↓

Measuring OD with a wavelength of 540 nm

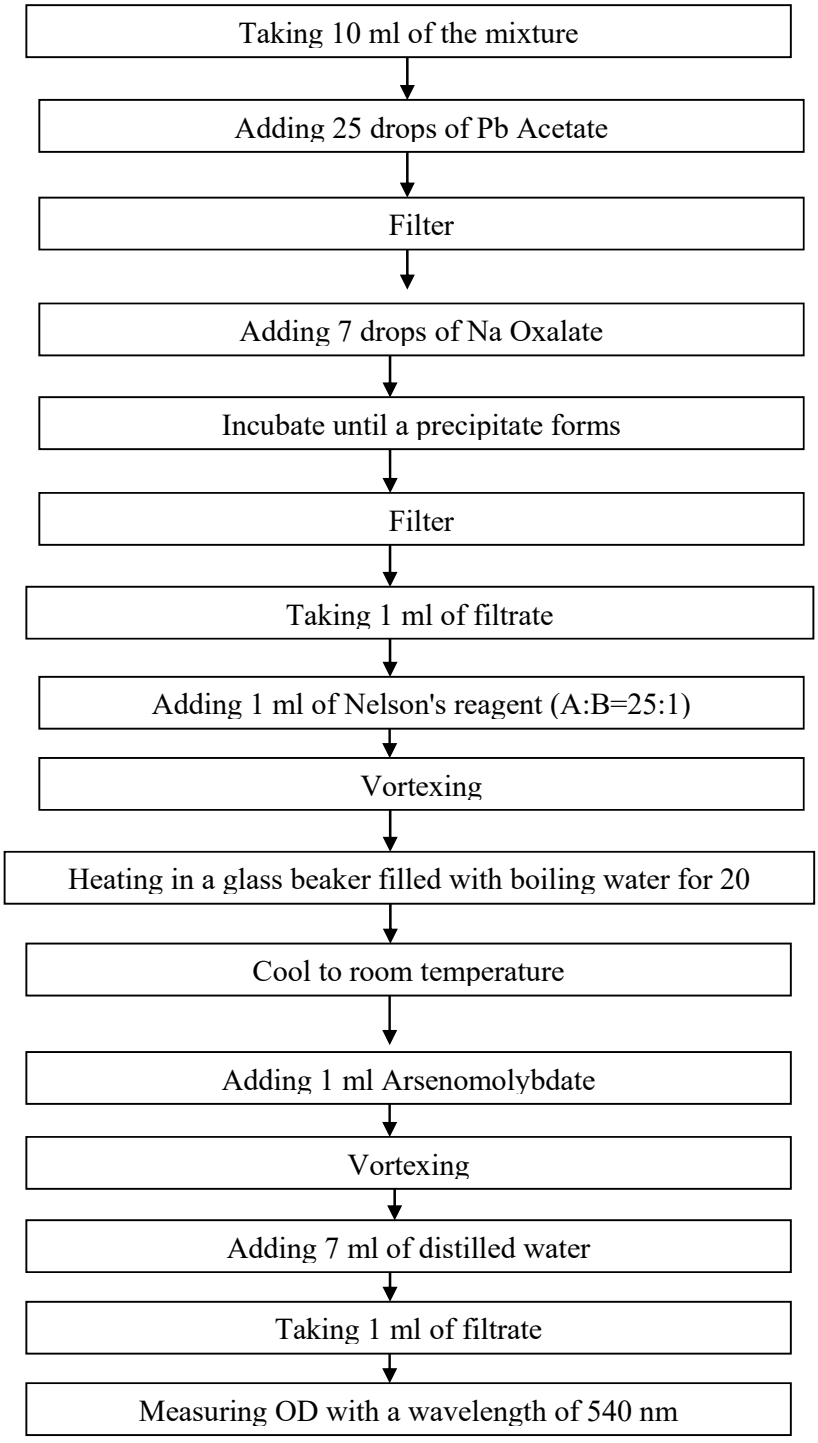
B. Reduced Sugar Analysis

1 g sample + 100 ml distilled water

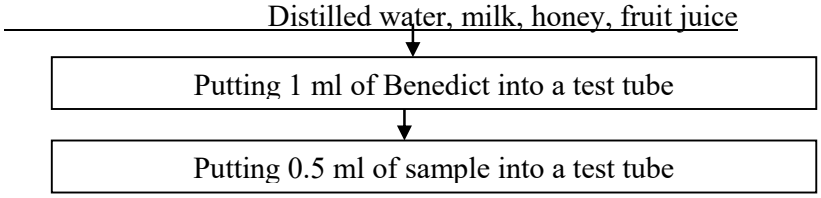
↓

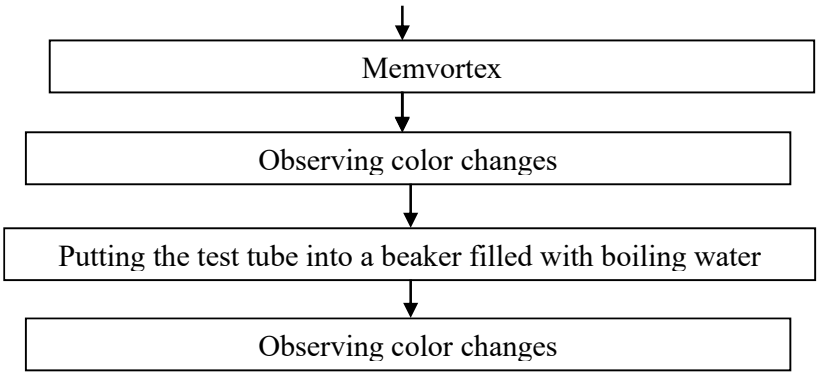
Vortexing

↓



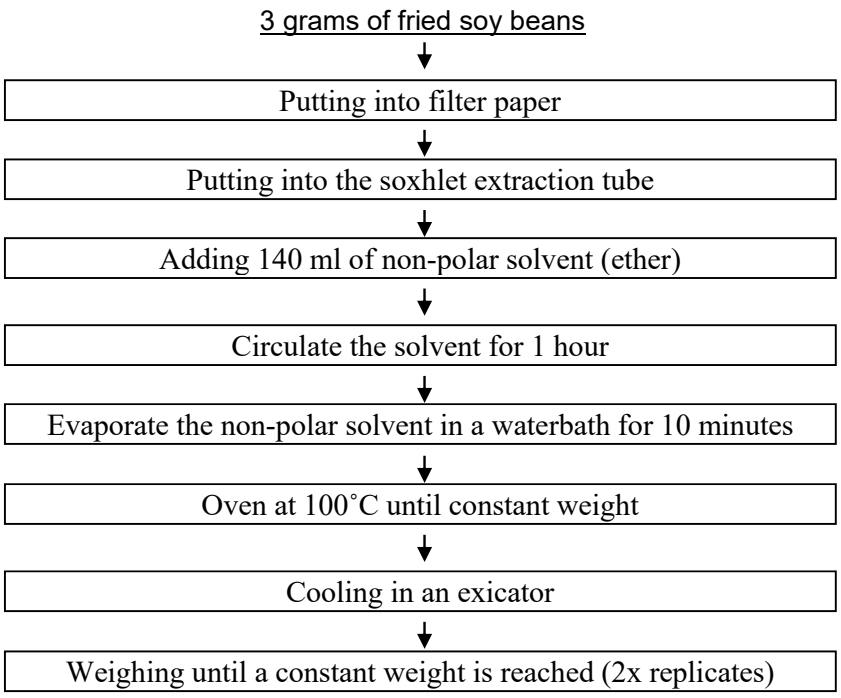
3.2 Qualitative Analysis



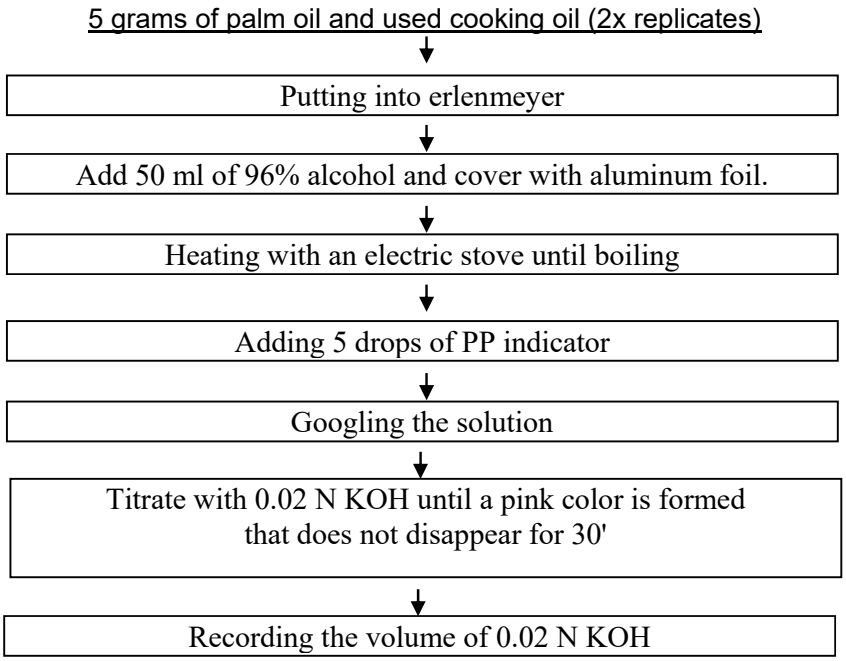

Memvortex
Observing color changes
Putting the test tube into a beaker filled with boiling water
Observing color changes

Fat Test

Soxhlet Method



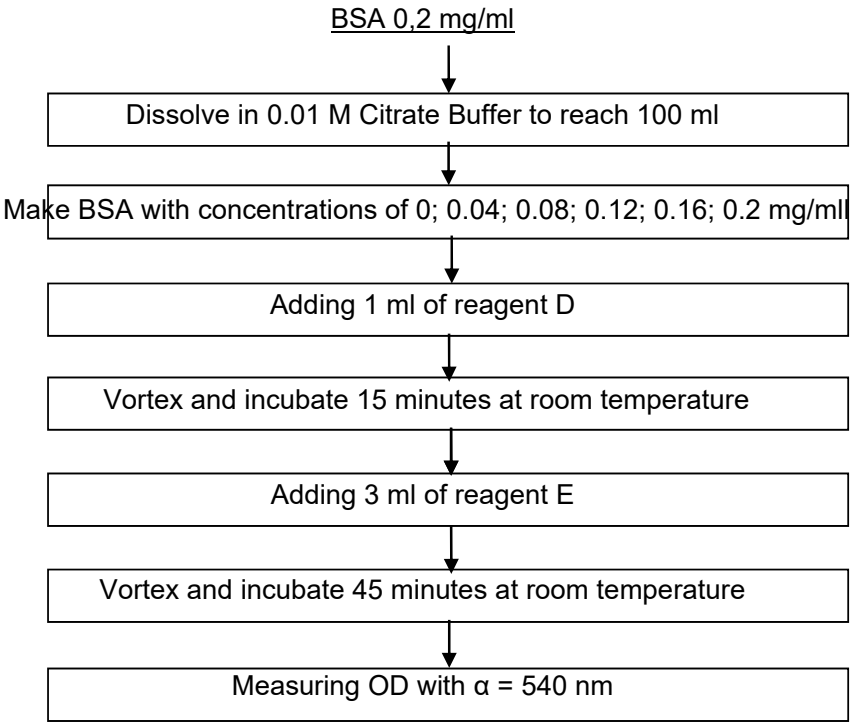
Titrimetric Method (Acid Number and %FFA)



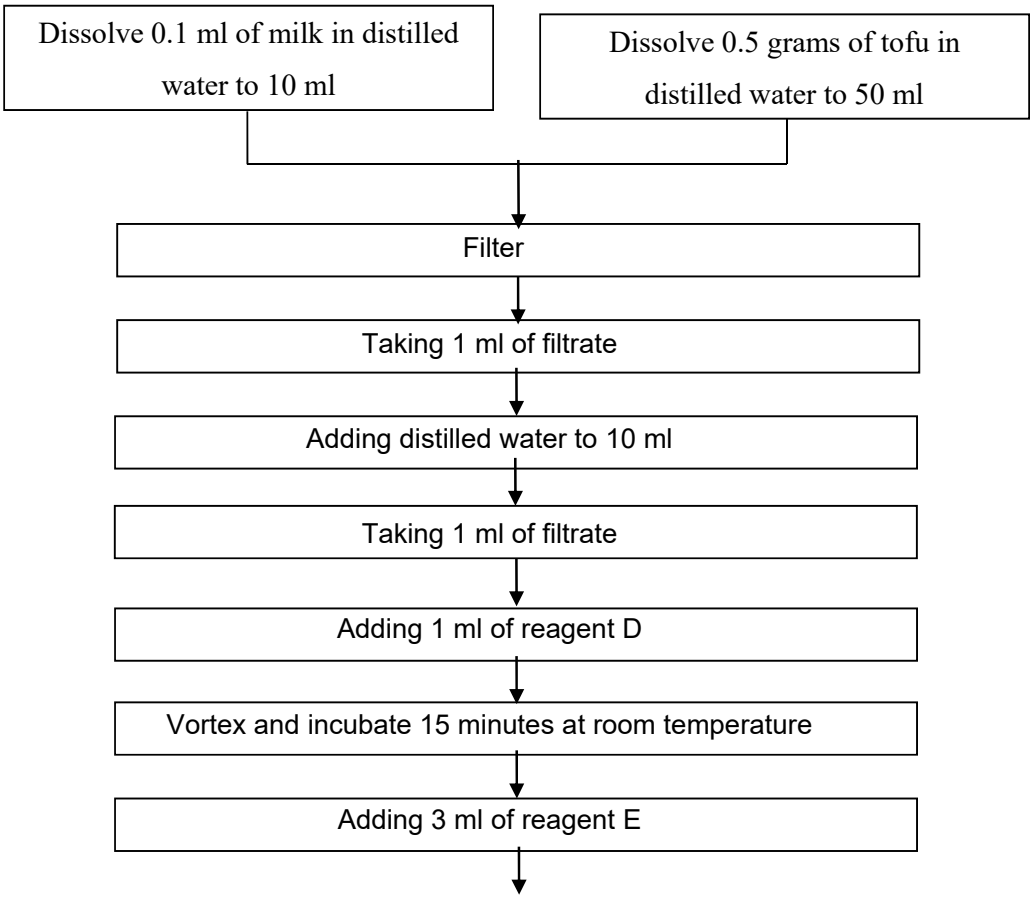
Protein Test

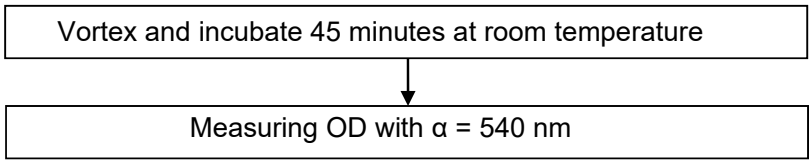
Soluble Protein Assay (Lowry Follin Method)

Preparation of Standard Solution

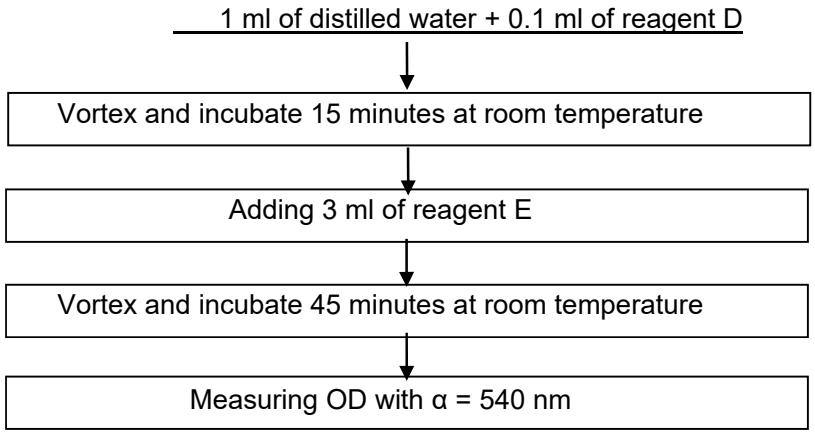


Determination of Soluble Protein

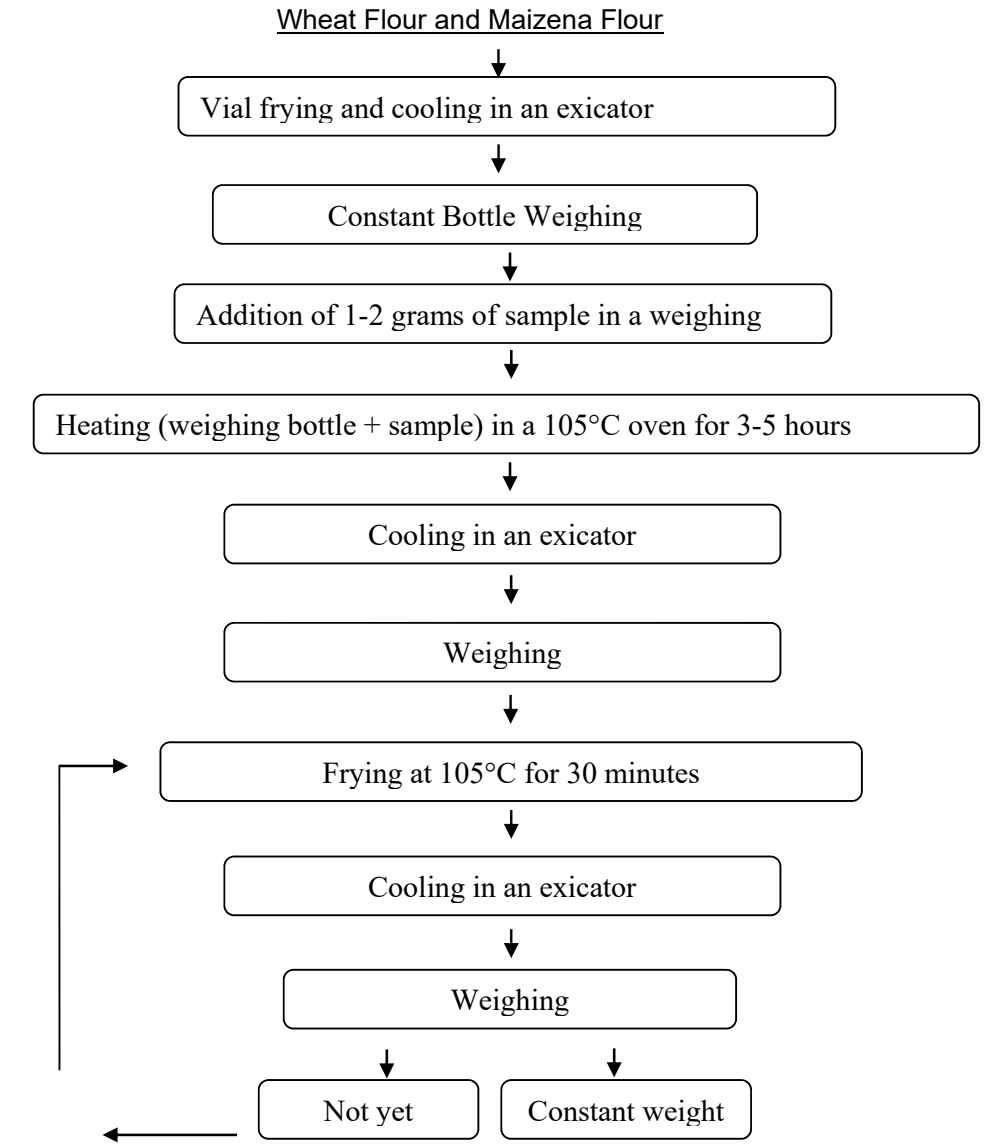




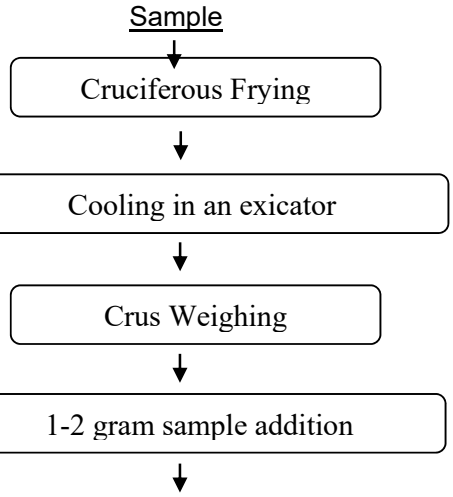
3.3.2. Blank Making

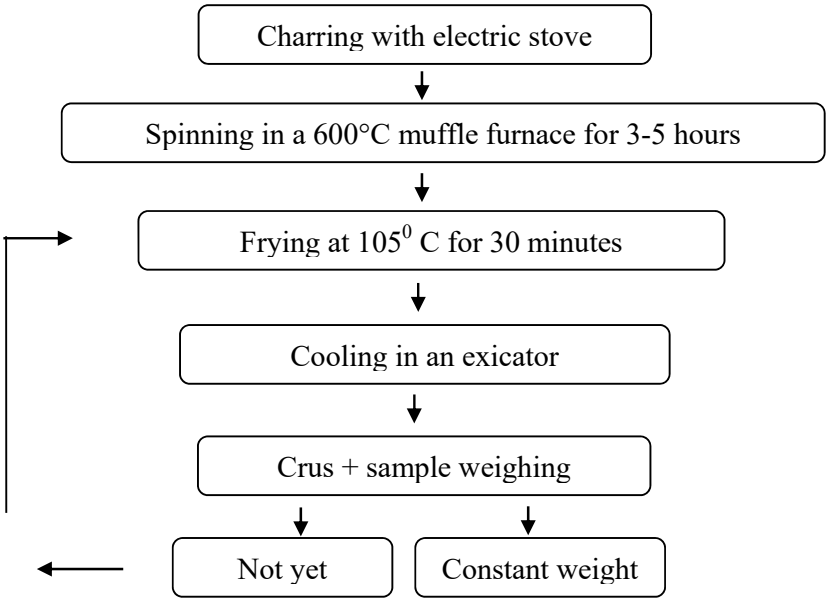


Moisture Content Analysis



Ash Content Analysis





Vitamin C Analysis