Food Analysis and Techology Laboratory Notes (I&II)

EXPERIMENT 1: VISCOSITY MEASUREMENTS

In the food industry viscosity is important from the perspective of food processing, food acceptability and food handling. Rheology investigates the flow and deformation of foods and their transition between solid and fluid (Tabilo-Munizaga and Barbosa-Ca´novas, 2005). Viscosity is defined as the internal friction of a fluid or its tendecy to resist flow (Bourne, 2011). Figure 1 shows some food materail's flow behaviour.

Stress (τ) is always a measurement of force per unit of surface area and is expressed in units of Pascals (Pa). The direction of the force with respect to the impacted surface area determines the type of stress. Normal stress occurs when the force is directly perpendicular to a surface and can be achieved during tension or compression. Shear stress occurs when the forces act in parallel to a surface (Tabilo-Munizaga and Barbosa-Ca´novas, 2005).

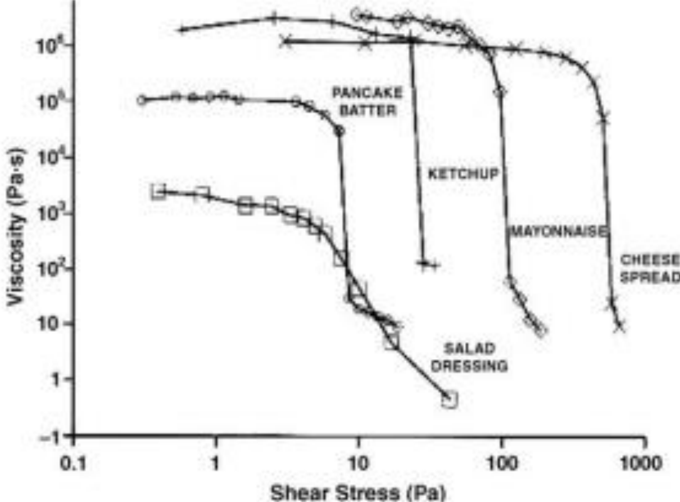

Figure 1. Viscosity of some food materials (Tabilo-Munizaga and Barbosa-Ca´novas, 2005)

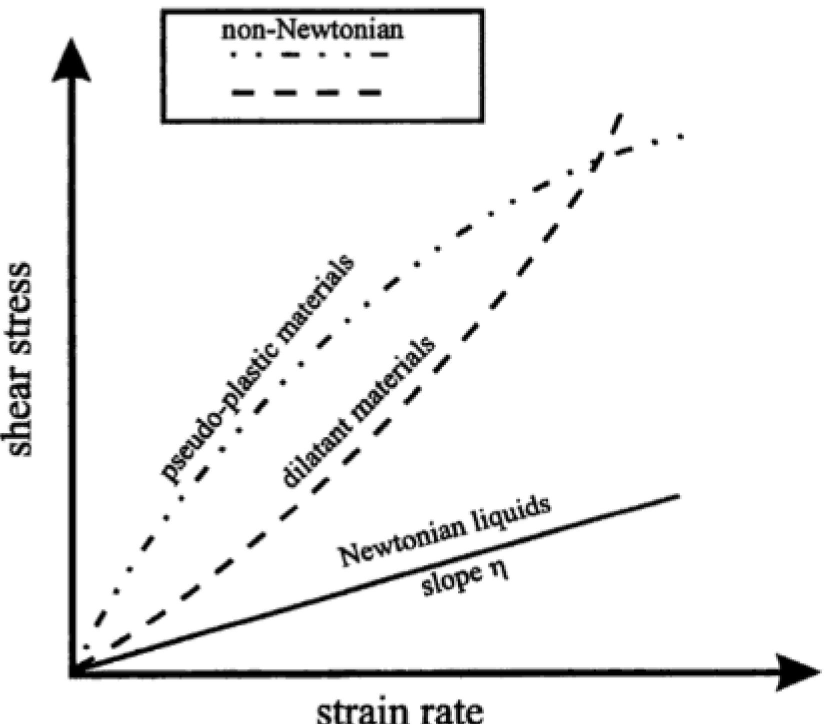

Figure 2. Flow curves that shows fluid behavior (Tabilo-Munizaga and Barbosa-Ca´novas, 2005)

Aim: Investigating viscosity behavior of different types of foods

Principle: Determining the stress response of food material under shear rate using air-bearing rheometer

Device: Anton Paar MCR-302 Air-Bearing Rheometer attached with paralel-plate geometry measuring equipment

Experimental Procedure:

1. Take a little amount of sample and put the plate of rheometer.
2. Take prob to the measuring position.
3. Start the test.

Results: Power Law model is used to define the type of flow. Shear rate and shear stress data that are acquired from the rheometer are used for regression analysis.

POWER LAW MODEL

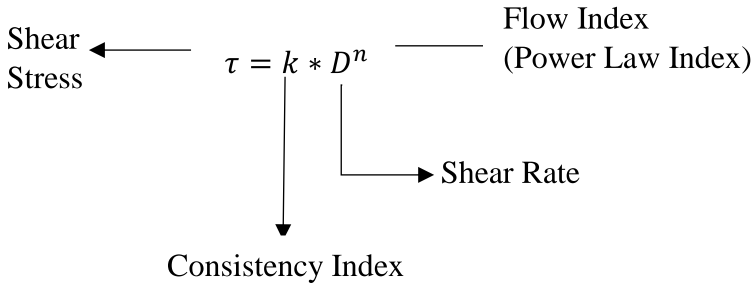

Figure 3. Viscosity of some food materials (Macosko, and Krieger ,1996)

According to Table.1, using n (flow index) value we can define the flow type of the material.

Table 1. Flow types according to flow index

n=1	Newtonian Flow
0<n<1	Psedoplastic Flow
n>1	Dilatant Flow

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EXPERIMENT 2: SOLUTION PREPARATION AND CALIBRATION CURVE

1. Preparation of reagent solutions

Every analytical method involving wet chemistry begin with the preparation of reagent solutions. This usually involves dissolving solids in a liquid or dilution of stock solutions. The concentration of analyte in the solution is expressed in the weight (kg, g, mg, ug etc.) or amount of substance (mol) per unit volume. Other some common ways to express reagent concentration are given in Table 1. For example, to express very small concentrations like in residue analysis, part per million (µg/mL or mg/L) or part per billion (µg/L) were preferred. Concentrated acid and bases are labeled in percent mass by mass (w/w) or percent mass per volume (w/v). For instance, a 28 % wt/wt solution of ammonia in water contains 280 g ammonia per 1000 g of solution. % 37 % wt/vol solution of NaOH contains 370 g NaOH per 1 L. The preparation of the solutions in correct concentrations is crucial for validity and reproducibility of any analytical method. The basic preparation of solid-liquid solution were shown in Figure 1. The aim of the experiment is to have basic knowledge about how to prepare reagents of specified concentrations.

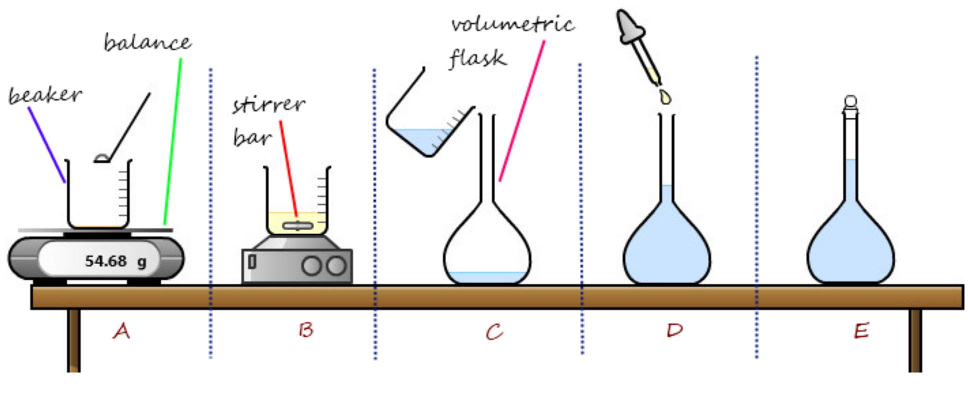

Figure 1. Preparation of a solution

A: Weighing solute, B: Dissolving solute in enough amount of solvent, C: Transfer of the solution to volumetric flask, D: Adding solvent until bringing the bottom of the meniscus to the mark, E: Inserting the stopper and shaking thoroughly

Table 1. Concentration expression terms

Unit	*Symbol*	*Definition*	*Relationship*
Molarity	*M*	Number of moles of solute per liter of solution	$M = \frac{mol}{liter}$
Normality	*N*	Number of equivalents of solute per liter of solution	$N = \frac{equivalents}{liter}$
Percent by weight (parts per hundred)	wt %	Ratio of weight of solute to weight of solute plus weight of solvent × 100	$wt\ \% = \frac{wt\ solute \times 100}{total\ wt}$
	wt/vol %	Ratio of weight of solute to total volume × 100	$wt / vol\% = \frac{wt\ solute \times 100}{total\ volume}$
Percent by volume	vol %	Ratio of volume of solute to total volume × 100	$vol\ \% = \frac{vol\ of\ solute \times 100}{total\ volume}$
Parts per million	ppm	Ratio of solute (wt or vol) to total weight or volume × 1,000,000	$ppm = \frac{mg\ solute}{kg\ solution}$

Adapted from Food Analysis laboratory Manual, 3rd ed. by S. Suzanne Nielsen, 2017, p. 23, Copyright 2016 by Springer.

Chemicals and devices

- Sodium chloride (NaCl)
- Hydrochloric acid (HCl)
- Gallic acid
- Spectrophotometers
- Mechanical pipettes (1000 uL, 5 mL)
- Beaker (100 mL), volumetric flask (100 mL), graduated cylinder (50 mL), test tubes

The preparation of the solutions

Solution 1: Prepare 250 mL of 25 mM NaCl solution using NaCl crystalline powder.

Solution 2: Prepare 250 mL of a 0.1 M hydrochloric acid solution from HCl stock solution 37 % wt/wt.

The molecular weight is 36.46 g/mol, and the manufacturer states that it has a density (d) of 1.2 g/mL.

Solution 3: Prepare 100 mL 0.03 M HCl solution using 0.1 M HCl.

Solution 4: Prepare 100 ppm (w/v) 100 mL gallic acid (mg solute/ L solution) from gallic acid crystalline powder.

References

Tyl, C. & Ismail, B.P. (2017). Preparation of Reagents and Buffers. In S. S. Nielsen (Ed.), Food analysis Laboratory Manual (3rd ed.). New York Dordrecht Heidelberg London: Springer.

2. Calibration Curve

Aim: Main objective of this experiment is make students to gain basic capabilities and knowledge for preperation of calibration solutions.

Principle: Instrument calibration is an essential stage in most measurement procedures. It is a set of operations that establish the relationship between the output of the measurement system (e.g.,the response of an instrument) and the accepted values of the calibration standards (e.g., the amount of analyte present). A large number of analytical methods require the calibration of an instrument. This typically involves the preparation of a set of standards containing a known amount of the analyte of interest, measuring the instrument response for each standard and establishing the relationship between the instrument response and analyte concentration. This relationship is then used to transform measurements made on test samples into estimates of the amount of analyte present, as shown in Figure 1.

Experimental Procedure:

Plan the experiments;

- Make measurements;
- Plot the results;
- Carry out statistical (regression) analysis on the data to obtain the calibration function;
- Evaluate the results of the regression analysis;
- Use the calibration function to estimate values for test samples;

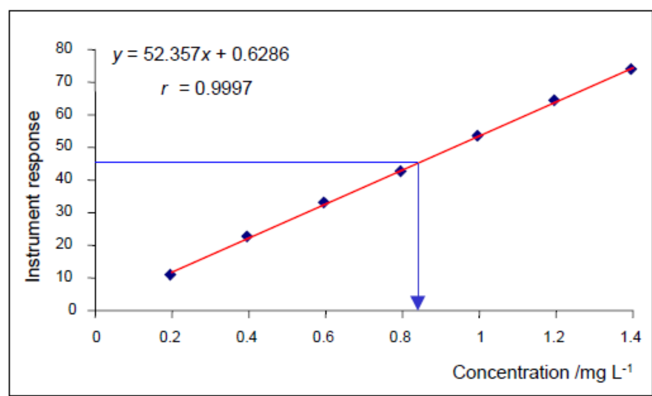

Figure 1: Typical calibration curve

Chemicals: Gallic acid (HPLC grade), distilled water, glass measuring cylinders, flask, spatula)

- automatic pipette, 10-100μL, 100-1000 μL, 1000-5000 μL
- vortex
- ultrasound water bath

Used Solutions:

- 100 mg/L (ppm) of gallic acid stock solution
- Ethanol | Water 10|90
- Gallic acid standard
- Folin reagent
- Na_2CO_3 solution

Dilution:

Concentrated Solution + Solvent Diluted Solution

The key fact to remember when diluting a concentrated solution is that the number of moles of solute is constant; only the volume is changed by adding more solvent. Because the number of moles of solute can be calculated by multiplying molarity times volume, we can set up the following equation: [1,2]

Moles of solute (constant) = Molarity × Volume

Mi × Vi = Mf × Vf

where Mi is the initial molarity, Vi is the initial volume, Mf is the final molarity, and Vf is the final volume after dilution (University of California, 2013)

Preperation of Standards:

Concentration of standards mg/L	100 mg/L (ppm) stock gallic acid	Final volume mL
0 mg/L	0	10
10 mg/L	1	10
20 mg/L	2	10
30 mg/L	3	10
40 mg/L	4	10
50 mg/L	5	10

Preparation of Folin-Ciocalteu Reagent

• 10 ml of Folin reagent is taken into a measure glass and finished in 100 ml volume with pure water and stored in an amber bottle.

Preparation of Na_2CO_3 Solution

• 7.5 g of Na_2CO_3 chemical is weighed to 100 ml volume with pure water. Kept it in the ultrasonic water bath for 15 minutes, complete dissolution is achieved.

Total Phenolic Component Determination

Gallic acid was taken into 500 µL of separate test tubes from each concentration of standard solutions. Then 2.5 ml of Folin-Ciocalteu reagent (solution) was added to each tube. Then 2 ml of 7.5% Na_2CO_3 solution was added to each tube. The mixture was allowed to stand at room temperature in the dark for 30 minutes and then absorbance was measured at 760 nm. All spectrophotometric measurements were performed using the Shimadzu UV-1800 spectrophotometer.

Results:

Preperation of Calibration Curves

To prepare standard solution series;

- Measure the standard solution series,
- Convert the measured values to a graph against the concentration of standards
- The result is to determine the amount of unknown sample using this calibration curve.

- If the calibration curve is generated on the computer, the graph of the sample will be calculated as the resulting graph will automatically give regression equation.

Regression Equation: Y = bx + a.

In the formula;

Y: Absorbance

x: Concentration

a: intercept

b: The slope of the line

References

[1] CHEMIASOF. (2011). Guide To Preparation of Stock Standard Solutions First Edition, (May).

[2] University of California. (2013). Solution Preparation. http://doi.org/10.1109/CIES.2013.6611722

EXPERIMENT 3: MOISTURE AND ASH ANALYSIS

MOISTURE ANALYSIS

Introduction: Water is the major ingredient in all foods, reacting chemically and physically with all components and is a major facilitator of microbiological decay (Lillford, 2016). Water is a constituent of food, which affects food safety, stability, quality and physical properties. Range of water concentrations in food is very broad and begins with a fraction of a per cent and reaches even more than 98%. Fresh products and liquid food contain usually large amounts of water while baked and dry products are poor in water (Lewicki, 2004).

Important properties and functions of water:

1. Universal solvent (salt, vitamins, sugar, gases, pigment)
2. Capable of ionizing ($H3O^+$, OH^-)
3. Affects the texture
4. Enters chemical reactions (hydrolysis of protein, lipids)
5. Stabilizes the colloids by hydration
6. Necessary for micro-organisms growth

Moisture analysis is needed for:

1. Material balance
2. Meeting the standards of product
3. Product stability (prevent deterioration, mold, bacteria, insect damage)
4. Express the composition on dry Weight Basis
5. Economic importance (H2O is cheap)

Forms of water in foods and their properties: Depending on the form of the water present in a food, the method used for determining moisture may measure more or less of the moisture present (Bradley, 2010).

1. Free water: This water retains its physical properties and thus acts as the dispersing agent for colloids and the solvent for salts.

2. Adsorbed water: This water is held tightly or is occluded in cell walls or protoplasm and is held tightly to proteins.

3. Water of hydration: This water is bound chemically, for example, lactose monohydrate; also some salts such as $Na_2SO_4 \cdot 10H_2O$.

Methods used for moisture determination are categorized into two groups: Direct methods, indirect methods (Bradley, 2010).

Direct methods: These are based on separation of water from food solids and measuring the loss in weight or the volume of water removed (Bradley, 2010).

A. Oven Drying Methods: In order to determine moisture content, a sample of known initial weight may be heated in specified conditions and the loss of weight determined. The results are dependent upon the type of oven used, the time and temperature of drying, and the characteristics of the product. Such methods are simple and direct, and can allow for simultaneous analysis of a large number of samples (Reid, 2006). The level of drying that can be achieved is then dependent upon the drying temperature. At higher temperatures, more moisture can be removed. However, as the drying temperature increases, loss of other volatile components also increases, somewhat negating the advantage in terms of moisture removal. Also, at higher temperatures the extent of thermal degradation increases (Reid, 2006).

1. Air-oven Method: Air oven method is based on putting the sample in flat, tarred dish - specified time and temperature (105^0C for 1 hr) - measuring the loss of water. Drying time periods for this method are 0.75–24 h (Bradley, 2010).

2. Vacuum oven Method: Moisture determination by drying in a vacuum oven is a dose and reproducible estimate of true moisture content. The rate of drying can be increased through lowering the vapour pressure in the air by using vacuum. (Pomeranz, 2013). Vacuum oven method can be used when if you do not want to expose to high temperature. By drying under reduced pressure (25–100 mm Hg), one is able to obtain a more complete removal of water and volatiles without decomposition within a 3–6-h drying time (Bradley,2010).Food rich in levulose (fruits) must be dried at 70 ^{0}C or below (Pomeranz, 2013).

3. Microwave: Microwave drying provides a fast, accurate method to analyze many foods for moisture content. There are some considerations when using a microwave analyzer for moisture determination: the sample must be of a uniform, appropriate size to provide for complete drying under the conditions specified; and the sample must be centrally located and

evenly distributed, so some portions are not burned and other areas are under processed (Bradley, 2010). 6-8 minutes are sufficient for drying of 10 g meat sample in a microwave oven with a power input of 100 W (Risman, 1978).

4. Infrared: Infrared drying provides more rapid heat transfer, thus shortens time, but may be too fast and burn sample. In infrared drying, a 250- to 500-W lamp is used, the filament of which develops temperature of 2000 to 2500 K. The distance of the infrared source from the dried material is a critical parameter because dose proximity may cause substantial decomposition. A distanee of about 10 cm is recommended.The thickness of the dried material should not exeeed 10-15 mm. Drying times under optimum conditions are up to 20 min for meat products and up to 25 min for baked products(Pomeranz, 2013). .

Principles governing moisture loss:

*Heat transfer rate (seldom a limiting factor)
*Temperature
*Surface area of product, particle size
*Diffusion of water through product
*Vapor pressure differences (RH at surface vs. product), number of samples in oven, air exchange rate in oven, vacuum applied or not, air movement in dryer,

Factors influencing results (the rate of evaporation of water in ovens).

a-Diameter or surface area of containers.

b-Depth of containers

c-Material of containers (Al; porcelain etc. Drying rate is highest in Al dishes)

d-Position and number of containers in the oven.

e-Drying is a function of time, temperature and water vapor pressure,

Drying methods have advantages of being:
- Simple
- Fast(infrared method),
- Handling large number of samples
- Inexpensive (equipment)

Disadvantages:

- Precautive measures have to be taken to avoid decomposition of solute components:

Example: Fructose containing foods may decompose with uncontrolled heating since fructose is destroyed above the 100°C,

- Solution: Apply vacuum, reduced temperature and longer drying time. e.g. Also may place in vacuum desiccator over concentrated sulfuric acid, phosphorus pentoxide, or magnesium perchlorate).

- Oven methods are not good for products that have a lot of bound water.

B. Distillation Techniques: Co-distilling water with a high boiling point solvent that is immiscible with water. This type of method is used for samples which contain other volatile components (Pomeranz, 2013).

Ex: Spices

Indirect methods:. These are based on properties of the food that are related to the presence of water, such as capacitance, specific gravity, density, refractive index, freezing point, and electromagnetic absorption (Bradley, 2010).

I. Methods based on chemical reactions of water:

Karl Fischer Method: The Karl Fischer titration has been found to be the method of choice for determination of water in many low-moisture foods such as dried fruits and vegetables, candies, chocolate, roasted coffee, oils, and fats. The method is superior to others for determining moisture in sugar-rich foods (sugars, honey) and in foods rich both in reducing sugars and proteins. The procedure has been applied also to foods with intermediate moisture levels (bakery doughs, baked products, fat-rich cake mixes) and to foods with high levels of volatile oils. The method is seldom used in the determination of water in structurally heterogeneous, high-moisture foods such as fresh fruits and vegetables(Pomeranz, 2013).

Principle: Reduction of iodine ($I^\circ \rightarrow I^-$) to iodate by SO_2 in presence of H_2O.

$$2\ H_2O + SO_2 + \mathbf{I_2} \rightarrow \mathbf{2HI} + C_5H_2SO_4$$

Without water in the medium, this redox reaction will not occur.

II.Methods measuring some physicalor colligative property that is correlated to moisture content .

Dielectric Method: The electrical properties of water are used in the dielectric method to determine the moisture content of certain foods, by measuring the change in capacitance or resistance to an electric current passed through a sample (Bradley, 2010).

Hydrometry: Hydrometry is the science of measuring **specific gravity** or **density**, which can be done using several different principles and instruments (Bradley, 2010).

Hydrometer**:** It is used for detection of specific gravity. A hydrometer is a standard weight on the end of a spindle, and it displaces a weight of liquid equal to its own weight. Lactometer , Baumé hydrometer, Brix hydrometer, alcoholometers, Twaddell hydrometer are most common used hydrometer (Bradley, 2010).

Pycnometer: One approach to measuring specific gravity is a comparison of the weights of equal volumes of a liquid and water in standardized glassware, a **pycnometer** (Bradley, 2010).

Refractometry: The refractometer has been valuable in determining the soluble solids in fruits and fruit products (AOAC Method 932.12; 976.20; 983.17). The **refractive index** (RI) of an oil, syrup, or other liquid is a dimensionless constant that can be used to describe the nature of the food. While some refractometers are designed only to provide results as refractive indices, others, particularly hand-held, quick-to-use units, are equipped with scales calibrated to read the percentage of solids, percentage of sugars, and the like, depending on the products for which they are intended. All chemical compounds have an index of refraction. RI varies with **concentration** of the compound, **temperature**,and **wavelength of light** (Bradley, 2010).

Moisture content can be expressed either as a wet basis percentage (g water/100 g

food) or a dry basis percentage (g water/100 g dry)(Young and Leonard, 1996).

1 Wet basis: expressed on untreated samples (also sometimes as “fresh weight basis” (g/100g tissue).

2. Dry matter basis: expressed on contents excluding water. Since moisture contents can vary greatly, it is best to report it in dry matter basis in order to be able to make comparisons.

Example: Sample composition is 10% H_2O; 40% fat on (wet) basis. To calculate how much fat this will correspond to on "dry matter" basis:

$Fat_{dm} = \frac{40 \times 100}{(100-10)} = 44.44$

Considerations in selecting the methods of moisture analysis (Bradley, 2010):

1. Expected moisture content.
2. Form of water present (free vs. bound water).
3. Nature of other food constituents: Volatile compounds , Unsaturated fat.
4. Speed necessary.
5. Accuracy and precision required.
6. Availability and cost of equipment

Aim: Determination of the total moisture content of food sample.

Principle: Moisture content is defined as the weight loss of mass that occurs as the material is heated. The sample weight is taken prior to heating and again after reaching a steady-state mass after drying.

Chemicals: None

Apparatus: Covered aluminum dishes, Mechanical convection oven, Analytical balance

Experimental Procedure:

- Accurately weigh 1 to 2 g of sample into a pre-weighed covered aluminum dish .
- Dry, with cover removed, for 2 hours ± 10 minutes at 135 ± 1 °C in a mechanical convection oven.
- Remove moisture dishes from oven, cover dishes, let cool to room temperature and weigh.

Results:

Moisture (%) $= \frac{100\times(B-C)}{A}$

A = sample weight (g)

B = weight of dish + sample prior to drying (g)

C = weight of dish + sample after drying (g)

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<u>ASH ANALYSIS</u>

Introduction: Ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter in a foodstuff (Harris and Marshall, 2010). Two major types of ashing are available:

- Dry ashing: Dry ashing is primarily used for proximate composition and for some types of specific mineral analysis. Dry ashing requires very high temperatures (500-600^{0}C), which may be achieved by conventional or microwave heating. Dry ashing refers to the use of a muffle furnace capable of maintaining temperatures of 500-

600°C. Water and volatiles are vaporized and organic substances are burned in the presence of oxygen in air to CO_2, and oxides of N_2. Most minerals are converted to oxides, sulfates, phosphates, chlorides, and silicates. Elements such as Fe, Se, Pb, and Hg may partially volatilize with this procedure, so other methods must be used if ashing is a preliminary step for specific elemental analysis.

Advantages of dry ashing:

- Safe method
- Requires no added reagents
- Requires little attention
- Can hundle large number of samples at once
- Can use ashed sample for several other analyses

Wet ashing: Wet ashing (oxidation) for samples with high fat content (meats and meat products) as a preparation for elemental analysis. Wet ashing uses lower temperatures than dry ashing and relies on strong acids and chemical oxidizers to rid samples of organic material. Wet ashing is often preferable to dry ashing as a preparation for specific elemental analysis. Nitric and perchloric acids are preferable, but a special perchloric acid hood is essential. This procedure must be conducted in a perchloric acid hood and caution must be taken when fatty foods are used.

Advantages of wet ashing:

- Minerals will usually stay in solution
- Little or no loss from volatilization
- Does not require muffle oven

Importance of ash in food analysis:

- Represents the total mineral content in foods.
- Parts of proximate analysis for nutritional analysis.
- Important from nutritional, toxicological and food quality standpoints.

Sources of error in the preparation of samples for ash analysis:

- Sample not representive
- Microelement contamination (use deionized water)
- Loss of sample in drying before ashing
- Ashing fat-extracted sample before ether removal-evaporate ether before ashing.

Aim: Determination of the total ash content of sample.

Principle: The method involves incinerating the dry matter at 525±25 ^{0}C in a muffle furnace and weighing the residue obtained (Schuck et al., 2012).

Chemicals: None

Apparatus: Porcelain crucible, Desiccator with an effective desiccant (e.g. silica gel), Analytical balance , Crucible tongs, Muffle furnace

Experimental Procedure:

AOAC International has several dry ashing procedures(e.g., AOAC Methods 900.02 A or B, 920.117, 923.03) for certain individual foodstuffs. The general procedure includes the following steps(Harris and Marshall, 2010).

1. Weigh a 2-5 g sample into a tared crucible. Pre-dry if the sample is very moist.

2. Place the crucible in the cold muffle furnace and gradually heat to 525 ±25 ^{0}C. Regularly monitor the sample in the crucible by slightly opening the furnace door.

3. Heat samples for 12–18 h (or overnight) at about 550 °C.

4. Turn off muffle furnace and wait to open it until the temperature has dropped to at least 250 °C, preferably lower. Open the furnace door slowly to avoid losing the powdery ash that may be disturbed by air movement.

5. Using safety tongs, place the crucible in a desiccator using the crucible tongs and leave to cool to room temperature. Weigh the crucible with the ash to the nearest 0.1 mg) using the analytical balance.

Results:

The ash content is calculated as follows:

%Ash $=\frac{(WAA-TWOC)}{OSW}\times 100$

where:

WAA = weight after ashing

TWOC = tare weight of crucible

OSW = original sample weight

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EXPERIMENT 4: PROTEIN DETERMINATION

Introduction: Proteins are polymers of amino acids. Twenty different types of amino acids occur naturally in proteins. Proteins differ from each other according to the type, number and sequence of amino acids that make up the polypeptide backbone. As a result they have different molecular structures, nutritional attributes and physiochemical properties. Proteins are important constituents of foods for a number of different reasons. They are a major source of *energy*, as well as containing essential amino-acids, such as lysine, tryptophan, methionine, leucine, isoleucine and valine, which are essential to human health, but which the body cannot synthesize. Proteins are also the major structural components of many natural foods, often determining their overall texture, *e.g.,* tenderness of meat or fish products. Isolated proteins are often used in foods as ingredients because of their unique functional properties, *i.e.,* their ability to provide desirable appearance, texture or stability. Typically, proteins are used as gelling agents, emulsifiers, foaming agents and thickeners. Many food proteins are enzymes which are capable of enhancing the rate of certain biochemical reactions. These reactions can have either a favorable or detrimental effect on the overall properties of foods. Food analysts are interested in knowing the total concentration, type, molecular structure and functional properties of the proteins in foods.

Kjeldahl method

The Kjeldahl method was developed in 1883 by a brewer called Johann Kjeldahl. A food is digested with a strong acid so that it releases nitrogen which can be determined by a suitable titration technique. The amount of protein present is then calculated from the nitrogen concentration of the food. The same basic approach is still used today, although a number of improvements have been made to speed up the process and to obtain more accurate measurements. It is usually considered to be *the* standard method of determining protein concentration. Because the Kjeldahl method does not measure the protein content directly a *conversion factor (F)* is needed to convert the measured nitrogen concentration to a protein concentration. A conversion factor of 6.25 (equivalent to 0.16 g nitrogen per gram of protein) is used for many applications, however, this is only an average value, and each protein has a different conversion factor depending on its amino-acid composition. The Kjeldahl method can conveniently be divided into three steps: digestion, neutralization and titration.

Principles:

Digestion

The food sample to be analyzed is weighed into a *digestion flask* and then digested by heating it in the presence of sulfuric acid (an oxidizing agent which digests the food), anhydrous sodium sulfate (to speed up the reaction by raising the boiling point) and a catalyst, such as copper, selenium, titanium, or mercury (to speed up the reaction). Digestion converts any nitrogen in the food (other than that which is in the form of nitrates or nitrites) into ammonia, and other organic matter to $C0_2$ and H_20. Ammonia gas is not liberated in an acid solution because the ammonia is in the form of the ammonium ion (NH_4^+) which binds to the sulfate ion (SO_4^{2-}) and thus remains in solution:

N(food) □ $(NH_4)_2SO_4$ (1)

Neutralization

After the digestion has been completed the digestion flask is connected to a *recieving flask* by a tube. The solution in the digestion flask is then made alkaline by addition of sodium hydroxide, which converts the ammonium sulfate into ammonia gas:

$(NH_4)_2SO_4$ + 2 NaOH □ $2NH_3$ + $2H_2O$ + Na_2SO_4 (2)

The ammonia gas that is formed is liberated from the solution and moves out of the digestion flask and into the receiving flask - which contains an excess of boric acid. The low pH of the solution in the receiving flask converts the ammonia gas into the ammonium ion, and simultaneously converts the boric acid to the borate ion:

NH_3 + H_3BO_3 (boric acid) □ NH_4^+ + $H_2BO_3^-$ (borate ion) (3)

Titration

The nitrogen content is then estimated by titration of the ammonium borate formed with standard sulfuric or hydrochloric acid, using a suitable indicator to determine the end-point of the reaction.

$H_2BO_3^-$ + H^+ □ H_3BO_3 (4)

The concentration of hydrogen ions (in moles) required to reach the end-point is equivalent to the concentration of nitrogen that was in the original food (Equation 3). The following

equation can be used to determine the nitrogen concentration of a sample that weighs *m* grams using a *x*M HCl acid solution for the titration:

$$\% N = \frac{x \text{ moles}}{1000 \text{ cm}^3} \times \frac{(v_s - v_b) \text{ cm}^3}{m \text{ g}} \times \frac{14 \text{ g}}{\text{moles}} \times 100 \quad (5)$$

Where vs and vb are the titration volumes of the sample and blank, and 14g is the molecular weight of nitrogen N. A blank sample is usually ran at the same time as the material being analyzed to take into account any residual nitrogen which may be in the reagents used to carry out the analysis. Once the nitrogen content has been determined it is converted to a protein content using the appropriate conversion factor: %Protein = F� %N.

Advantages and Disadvantages

Advantages. The Kjeldahl method is widely used internationally and is still the standard method for comparison against all other methods. Its universality, high precision and good reproducibility have made it the major method for the estimation of protein in foods.

Disadvantages. It does not give a measure of the true protein, since all nitrogen in foods is not in the form of protein. Different proteins need different correction factors because they have different amino acid sequences. The use of concentrated sulfuric acid at high temperatures poses a considerable hazard, as does the use of some of the possible catalysts The technique is time consuming to carry-out

EXPERIMENT 5: FAT CONTENT DETERMINATION

Background

Lipid means a class of compounds which are sparingly soluble in water but demonstrates various solubility in a number of organic solvents such as ethyl ether, hexane, ethanol, acetone, methanol, benzene etc. Solvent extraction is most frequently used method to determine fat content but nonsolvent wet extraction (e.g., Babcock, Gerber) and instrumental methods exploiting physical and chemical characterisitics of lipids (e.g., infrared, density, X-ray absorption) are also utilized (Ellefson, 2017). Even though ether (ethyl and/or petroleum) and hexanes are common solvents, they are not able to extract polar lipids efficiently (Christie, 2003). Solvent extraction methods are simple and need no expertise (De Castro & Priego-Capote, 2010). Before evaporation, it is not required to filtrate the organic solvent. However, large volumes of solvents are used, which are costly to dispose of and hazardous to the enivironment (Srigley & Mossoba, 2017).

Objective

Determination fo the lipid contents of various snack foods by the Soxhlet method.

Principle

Fat is extracted, semicontinuously, with an organic solvent. Solvent is heated and volatilized, then is condensed above the sample. Solvent drips onto the sample and soaks it to extract the fat. At 15–20 min intervals, the solvent is siphoned to the heating flask, to start the process again. Fat content is measured by the weight loss of sample or weight of fat removed.

Chemicals

- Petroleum ether: Harmful, highly flammable, dangerous for environment

Supplies and Devices

- 3 weighing pans
- Cellulose extraction thimbles, predried in 70 °C vacuum oven for 24 h
- Desiccator
- Glass boiling beads
- Glass wool, predried in 70 °C vacuum oven for 24 h
- Graduated cylinder, 500 mL
- Mortar and pestle

- Plastic gloves
- Snack foods (need to be fairly dry and able to be ground with a mortar and pestle)
- Spatula
- Tape (to label beaker)
- Weighing pan (to hold 30-g snack food)
- Precision scale
- Soxhlet extraction unit

Procedure

1) Record the fat content of your snack food

2) Slightly grind 30 g of sample with mortar and pestle

3) Label the thimbles on the outside with your initials and a number (use a lead pencil), then weigh accurately on an analytical balance.

4) Place about 2–3 g of sample in the thimble. Place the three samples in a Soxhlet extractor.

5) Weigh pre-dried round-bottom flask, add around 200 ml of solvent with several glass boiling beads.

6) Extract for 6 hours.

7) Evaporate the solvent in round-bottom flask by rotary evaporator.

8) Reweigh the round bottom flask remaining fat inside.

Results and Calculations

$$\textbf{\% Fat}= \frac{(\text{Weight of round−bottom flask with fat} - \text{Weight of round bottom flask})}{\text{Sample weight}} \times 100$$

Compare the results with the fat amount on the package label.

What is the the percentage of fat in wet and dry basis?

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EXPERIMENT 6: CHEESE MAKING

Introduction: Feta is a soft brined white cheese with small or no holes, a compact touch, few cuts, and no skin. It is usually formed into large blocks, which are submerged in brine. Its flavor is tangy and salty, ranging from mild to sharp. Its maximum moisture is 56%, its minimum fat content in dry matter is 43%, and its pH usually ranges from 4.4 to 4.6 (Tavantzis, 2005).

The main and important raw material in cheese is milk. Milk and dairy products are important sources of protein, vitamins, minerals, and essential fatty acids. Water (86-88%), milk fat (3-6%), lactose (5%), protein (3-4%), and minerals (ash) (0.7%) are the major constituents of milk. Breed, season, stage of lactation, genetic factors, health status of cow and feed of the cow affect the composition of milk (Oztop, 2014).

Milk contains caseins and whey (serum) proteins. The primary group of milk proteins are the caseins. Casein which is divided to four major types which are α_{S1}-, α_{S2}-, β-, κ- caseins. All other proteins found in milk are grouped together under the name of whey proteins. The major whey proteins in cow milk are beta-lactoglobulin and alpha-lactalbumin. whey is also sometimes called the serum phase of milk and it contains the water, lactose and soluble non-casein proteins ("Milk Composition Proteins," n.d.).

In cheese making, for milk to separate into curds and whey (curdling), the process requires the addition of rennet. Rennet contains the enzyme chymosin. Rennet can be sourced from the abomasum (fourth stomach) of newly-born calves where the chymosin aids digestion and absorption of milk. When it is added in appropriate dilute form to our milk, the enzyme rapidly sets about removing the kappa-casein hairs from the casein micelles in the milk. When they are gone, the micelles can no longer resist their compulsion to group together, and so they come out of precipitation, coagulating the milk into more of a gel than a liquid. We cheese makers then cut that gel to form curds and whey ("How rennet works," 2015; Walstra, Wouters, & Geurts, 2006).

Calcium chloride is often needed when the milk used for cheesemaking has been pasteurized and/or homogenized. During processing, the chemical structure of milk is changed, sometimes drastically. Those changes include a slight decrease in calcium levels within the milk. Calcium is necessary for proper curd formation and development of the properties of milk coagulation. By adding calcium chloride to the milk before adding the coagulant,

calcium levels are restored ("Cheese Production," n.d.; "Common Additives in Home Cheesemaking," n.d.).

Aim of Experiment: The aim of experiment is learning the theory and practicing the cheese making process, while process parameters, the yield and quality of the final product have been analyzing and evaluating, for a selected method is the aim of the experiment.

Principle of Method: Feta cheese is made by coagulating or curdling the milk. In the industry, cheese making includes some extensive steps listed below. In addition, the production of milk and milk products includes quality control parameters and methods. Cheese making experiment involves all production steps of feta cheese and its important quality control methods.

Chemicals/Materials Used:

For Cheese Making:

- Milk (avoid UHT or ultra-pasteurized milk)
- Starter Culture
- Rennet
- Calcium Chloride ($CaCl_2$)
- Salt

For Titratable Acidity measurement:

- 0.1 N NaOH solution
- 1 % Phenolphthalein solution (as indicator)

Experimental Procedure:

Feta Cheese Production Steps:

1) Storage Tank:

Raw milk which is used for cheese production should be stored smaller than 24 hours at <4°C in order to prevent growth of psychrotroph bacteria. Presence of antibiotics, pesticides and mycotoxins, pH & acidity values, and storage time must be tested before cheese-making. Antibiotics must be absent in milk.

2) Filtration:

In order to remove particular contaminants, mechanical filtration through stainless steel mesh, or a filter cloth are used. Filtration is necessary for visual perception of the integrity.

3) Homogenization:

Homogenization should be conducted before pasteurization in order to reduce the surface area of the fat globules and obtain more uniform product in terms of fat content.

4) Pasteurization:

Pasteurization is one of the most important and critical steps in cheese making since it is vital to destroy all pathogenic microorganisms in milk. Primary objective is to kill pathogenic microorganisms. There are two ways for the pasteurization. During HTST (high temperature short time) process, milk is kept at 72˚C for 15 seconds. During LTLT (low temperature long time) process, milk is pasteurized at 62˚C for 30 minutes. For the white cheese production, milk is generally kept at 72-73˚C for 15 seconds and this is the most common method for white cheese production.

5) Cooling:

After pasteurization step, milk is cooled to 30-35 °C in order to reach the temperature at which starter bacteria has grown.

6) $CaCl_2$, Starter Culture and Rennet Addition:

Ca^{+2} improves elastic behavior of curd and so whey can be easily removed from the curd. $CaCl_2$ can be also added to milk to fasten enzyme coagulation.

Starter cultures are thermophile and mesophile lactic acid bacteria. These bacteria are Lactobacillus bulgaricus and Streptococcus thermophilus. They are added to pasteurized milk and incubated between at 32 and 35 ºC until acidity increases which means that pH decreases. This pH drop important since it directly affects the curd formation.

After obtaining sufficient acidity, rennet should be added in order to ease the coagulation of casein which is an important process during cheese making. If the rennet is added, coagulation can take place in a very short time and curd formation occurs.

7) Settling:

After rennet addition, settling takes between 60 and 90 minutes to form firm coagulum which means that curd is not disturbed.

8) Curd Cutting:

Curd is cutted into cubes of approximately 1 cm^3 by using vertical and horizontal knives. After curd cutting, heating can be applied depending on the type of the cheese produced.

9) Draining (Whey out):

The whey is drained from the vat for stability of cheese.

10) Pressing:

Compression is applied to solid form by steel weights for 2-5 hours. Remained whey is drained from the cheese.

11) Cutting:

Cheese is cut into 8x8x8 cm. Sizes can be change according to packaging.

12) Brining:

Dry salt or salt solution (20% NaCl) are added to the cheese due to ripening, control of microbial growth and its activity, control of enzyme activity. Addition of salt also leads to syneresis of the curd and moisture reduction.

13) Packaging:

Feta cheese is packaged with 10% NaCl solution.

14) Cold Storage:

Feta cheese is stored in cold storages at 2-4 °C for 2 months until desired age is reached.

Brix Measurement:

Brix value of milk will be measured with a refractometer.

pH Measurement:

pH will be measured in every 5 min.

Titratable acidity Measurement:

1) Fill the burette with 0.1 N NaOH solution.
2) Mix the milk sample thoroughly by avoiding incorporation of air.
3) Transfer 10 ml milk with the pipette in conical flask.
4) Add equal quantity of glass distilled water.
5) Add 3-4 drops of phenolphthalein indicator solution and stir with glass rod.
6) Take the initially reading of the alkali in the burette.
7) Rapidly titrate the contents with 0.1 N NaOH solution continue to add alkali drop by the drop and stirring the content with glass rod till first definite change to pink colour which remains constant for 10 to 15 seconds.
8) Note down the final burette reading. . ("Titrable Acidity of Milk," n.d.)

Note: Titratable acidity will be measured in every 15 min

Calculation & Discussion:

1) $\% \, Yield = \frac{Mass \; of \; cheese \; produced \; (g)}{Mass \; of \; milk \; used \; for \; cheese \; (g)} x \; 100$

2) Titratable acidity and pH calculations

$$\% \, Titratable \; acidity = \frac{Volume \; of \; NaOH \; solution \; used \; (ml) x \; Notmality \; of \; NaOH \; solution \; x \; 90 \; x \; 100}{Volume \; of \; milk \; (ml) \; x \; 1000}$$

Time (min)	pH	% Titratable Acidity	Production stage
0			
5			
10			
15			
20			
25			
30			
35			
…			
80			
85			
90			

3) Calculation of cheese content (%) in terms of protein, fat, lactose, mineral and water by using the calculation of cow milk and whey content and comparison with literature.
4) Observation of cheese made.
5) Comparison of process steps of the feta cheese with another cheese type.

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EXPERIMENT 7: ACIDITY ANALYSIS IN MILK

Introduction: Food acids are usually organic acids, with citric, malic, lactic, tartaric, and acetic acids being the most common. However, inorganic acids such as phosphoric and carbonic (arising from carbon dioxide in solution) acids often play an important and even predominant role in food acidulation

The organic acids present in foods influence such as;

- ✓ flavor (i.e.,tartness),
- ✓ color (though their impact on anthocyanin and other pH-influenced pigments),
- ✓ microbial stability (via inherent pH-sensitive characteristics of organisms),
- ✓ keeping quality (arising from varying chemical sensitivities of food components to pH).

Organic acids may present

1. Naturally,
2. By Fermentation
3. Added as part of a specific food formulation.

The importance of determining food acidity

1- determine the degree of maturity of fruits and vegetables . If the determination of organic acids contained in the grape is malic acid, the description is not yet ripe grapes, ripe grapes contains a lot of tartaric acid.

The titratable acidity of fruits is used, along with sugar content, as an indicator of maturity, generally the higher the maturity, the lower the acid content. e.g. in the ripening process, such as tomatoes from green to mature stage , there is an increase in sugar content.

2.To determine the freshness of foods for example in milk, the more the lactic acid levels, means that milk is rotten.

3. Acidity indicators reflect the quality of food the amount of organic acids in food directly affects the food flavor, color, stability, and the level of quality.

4. Determination of acid on the microbial fermentation process such as: fermentation products in soy sauce, vinegar and other acids is an important indicator of quality.

Food acidity determination is made two ways;

1)Titratable acidity

2)Hydrogen concentration pH

Titratable acidity

Titratable acidity is determined by neutralizing the acid present in a known quantity (weight or volume) of food sample using a standard base. The endpoint for titration is usually either a target pH or the color change of a pH-sensitive dye, typically phenolphthalein.

Titratable acidity**;**

- ✓ Simple estimate of the total acid content of food.
- ✓ Also called total acidity.
- ✓ Better predictor of acid impact on flavor

Aim: Determine the amount of acid in the sample by neutralizing with base.

1-Determination of Milk Acidity (Titratable Acidity):

The acidity of fresh milk is due to :phosphates, casein and whey proteins, citrates and carbon dioxide dissolved during the process of milking. Developed acidity which is due to: lactic acid produced by the action of bacteria on lactose in milk.

Generally the acidity of milk means the total acidity (Natural +developed) or titrable acidity.

The acidity of fresh milk is ranged 0,16-0,18%. If acidity is found to be 0.30% or more, it means that the milk will be cut when heated.

The acidity of cow milk ranges from 0.10 to 0.26 %. Acidity is expressed as percentage of lactic acid.

To determine whether the milk sample is fresh

To determine whether a neutralizing agent is added

To determine whether the milk is mastitis

To determine the conformity to the standard, regulation and codec

Principle: The acidity of milk is neutralized using a base of known normality (generally 0.1N sodium hydroxide) in the presence of phenolithinate. **S**imple direct titration with 0.1N sodium hydroxide, using phenolphthalein as an indicator.

Chemicals:

a) 0,1 N NaOH solution

b) % 1 Phenolphthalein indicator (1 g phenolphthalein is dissolved in 96% alcohol and volume is completed to 100 ml)

Experimental Procedure:

1. Mix the milk sample thoroughly by avoiding incorporation of air.

2. Transfer 10 mL milk to conical flask or beaker .

3. Add equal quantity of distilled water .

4. Add 3-4 drops of phenolphthalein indicator and stir

5. Rapidly titrate the contents with 0.1 N NaOH solution, continue to add alkali drop by the drop and stirring the content till first definite change to pink colour .

6. Note down the final burette reading.

Result and Calculation:

$$\text{Lactic acid \%} = \frac{N \text{ x } V \text{ x } 0.009 \text{ x } 100}{W}$$

- ✓ *N* = normality of titrant, usually NaOH (mEq/mL)
- ✓ *V* = volume of titrant (mL)
- ✓ Eq. wt. = equivalent weight of predominant acid (mg/mEq) (0.009 for lactic acit)
- ✓ *W* = mass of sample (g-mL)

0.009 gram of lactic acid is found in 1 ml of 0.1 N lactat

Spent each 1 ml of 0.1 N NaOH neutralizes 0.009 g of lactic acid

!!! 0.25 N NaOH is used as the base to determine the acidity as SH (Soxhlet-Henkel)

% Acidiyt = SH x 0,0225

pH Analysis

Introduction:

pH is defined equal to the negative logarithm of the molar concentration of hydrogen ions in solutions (Tyl and Sadler, 2017) . A definition of pH is the measurement of the acidity or alkalinity of a solution commonly measured on a scale of 0 to 14. pH 7 is considered neutral, with lower pH values being acidic and higher values being alkaline or caustic. pH is the most common of all analytical measurements in industrial processing and since it is a direct measure of acid content [H+], it clearly plays an important role in food processing. pH and titratable acidity are not the same values .

pH analysis

- ✓ Depend on the strength of acid condition,
- ✓ Also called active acidity.
- ✓ Quantify only the free H_3O^+ concentration

pH=−log [H+]

The reasons for measuring pH in food processing ;

- ✓ To produce products with consistent well defined properties
- ✓ To efficiently produce products at optimal cost
- ✓ To avoid causing health problems to consumers
- ✓ To meet regulatory requirements
- ✓ To control process efficiency

Milk and Dairy Products: pH of milk is around 6.8 and it is tested for impurities and signs of infection upon collection as well as at point of delivery. In processes such as sterilization, pH is checked since a lower value helps to speed up the process. However, lower pH values can indicate that the cattle carried leukocyte infections such as mamites.

Milk used for cheese manufacturing must be of excellent quality and its pH value contributes to whether the cheese will be soft or hard. pH is also checked during cheese preparation, souring of milk and cream maturation. Pathogen multiplication of the fresh and soft variety, is slowed down considerably by ensuring that the pH stays in the 4.1 to 5.3 region.

Controlling the pH value is very important in butter manufacturing processes. For example, cream is cooled after pasteurization at pH value of 6.70 to 6.85 to generate sweet butter. In order to manufacture sour butter, citric acid extracts are added to acidify the cream to 4.6-5.0 pH. With butter having a high diacetyl content, a starter is added to bring the pH value to around 5. As with other products, a lower pH value enhances the shelf life of the product.

Aim: The concentration of dissociated hydrogen ions [H+] in the sample is determined by

pH meter**.**

Principle: The pH measurement is performed to express the degree of an acid or base in terms of the activity of the hydrogen ion.

- ✓ Depend on the strength of acid condition
- ✓ Also called active acidity.
- ✓ Quantify only the free H3O+ concentration

Experimental Procedure:

1. The first is to calibrate the ph meter using buffer solutions.
2. The electrode is washed is washed with distilled water.
3. The electrode of the pH meter is dipped into the milk sample and read pH value
4. The ph measurements are repeated with at least two parallel.
5. The sample temperature should be 20 centigrade degrees.

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EXPERIMENT 8: DETERMINATION OF STARCH IN YOGURT

Introduction: Starch can be used in yogurt production in order to make up low quality and to thicken the texture when producers want to reduce production cost (Scott-Thomas, 2011). Starch forms a typical blue color with iodine. This color is due the absorption of iodine into the open spaces of the amylose molecules (helices) present in starch. Amylopectin, which is the other type of molecules present in starch, form a red to purple color with iodine (Pavia, Lampman, Kriz, & Engel, 2005).

Aim of Experiment:

The aim is to determine the starch ingredient in yogurt taken from grocery.

Principle of Method:

The determination of apparent starch is based on the reaction of iodine with starch which results in the production of a blue color when the amylose component is present.

Chemicals/Solutions used:

- Lugol solution

  Preparation:

  - 1 g iodine
  - 2 g potassium iodure
  - 300 ml distilled water

  Iodine and potassium iodure is dissolved in 300 ml distilled water.

Experimental Procedure:

1) Put 2-3 ml mixed yogurt sample into a tube.
2) Add 2-3 ml distilled water to sample and mix them homogeneously.
3) Add 2-3 drops lugol solution and mix them gently.
4) Record the color change.

Evaluation of results: Starch gives a blue-black color. If blue color is observed, the presence of starch in yogurt become to be proved. A negative test or non-starch-containing samples gives the yellow color.

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EXPERIMENT 9: GERBER MILK FAT ANALYSIS IN DAIRY PRODUCTS

The Gerber method is used as a simple, cheap, rapid volumetric method for determining fat in raw milk and milky product such as butter, cheese, ice cream (Kleyn, Dick H. et al. 2001). The fat content of milk between 2.5% and 14.0% depending on the species, race, personage, lactation period, season and feeding of obtained of animals and milk types (skim milk, semi skim milk, whole milk (Miller et al.2000).

Gerber volumetric method that uses chemical reagents (sulfuric acid, detergents) to achieve the breaking of the emulsion and the fat separation. Volumetric methods give quick results and are widely used in practice. Gravimetric methods are used when very sensitive results are needed.

1. Determination of Fat by Gerber Method
2. Babcock Method
3. Rose-Gottlieb Method
4. Photometric Method (Milko-Tester)

Aim: the determination of milk fat which is the most valuable component of milk in emulsion and in varying proportions.

Fat content determination is of great importance because

- ✓ This parameter is used to determination of adulteration by adding water in milk
- ✓ This parameter impacts on the price paid per liter of milk.
- ✓ It is used to determine if a sample of milk or cheese complies with established legal values.
- ✓ It is necessary to know its value to classify the milk for the preparation of derivatives.

Principle:

The basic principle of fat determination by Gerber method; separating the free oil by centrifugation and reading the amount of milk fat (%) from the scale of the butyometer after dissolving the protein and hard soluble salts of a certain volume of milk by the addition of concentrated sulfuric acid and amyl alcohol (MEGEP, 2012).

Chemicals:

1) Sulphuric acid 90-91% (density:1.812-1.815)

2) Amyl alcohol 98.5% (density:0.82-0.83)

The different density and consantrations of reagents and butyrometer involved in these analysis depending on the type of sample (milk, cheese or ice cream)

Experimental Procedure:

1. Put the clean and dry butyrometer in a butyrometer stand with open mouth upwards.

2. Run 10 ml of sulphuric acid with the tilt measure in the butyrometer.

3. Pipette out 11 ml of milk sample gently by the side of butyrometer, whose temperature is about 15-21 degrees C.

4. Pour 1 ml. of amyl alcohol with tilt measure.

5. Stopper the butyrometer with the help of lock stopper using regulating pin/guiding pin.

6. The tube is well (mixed) shaken till mahogany red colour is obtained. Keep the butyrometer in hot water bath till it attains 15-21 degrees C and the butyrometer are placed in the centrifuge at 1100 rpm for 4 minutes.

7. Take out the butyrometer in an upright position with the stopper end down wards.

8. Keep the butyrometer in hot water bath at (65 degrees C) for some time.

9. Note the reading. Reading should be taken from bottom of the fat column to lower border of meniscus on the scale

Results:

The amount of milk fat obtained from the sample is expressed as % milk fat.

References

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EXPERIMENT 10: DETERMINATION OF TOTAL AND REDUCING SUGAR IN FOODS (LANE-EYNON METHOD)

A large proportion of the solids of fruits was formed sugar, and generally all of the sugar in fruits consists of hexoses (glucose and fructose). These sugars are named as "reducing sugars". In addition, very variable amounts of sucrose are included in the composition of the fruits. Peach (57-79%); while apricot (66-87%) and plum (44-71%) contain sucrose, whereas cherry and sour cherry contain only fructose and glucose. Sucrose, which is found in the structure of fruits and vegetables, undergoes more or less inversion depending on the ambient temperature during processing and/or storage (No, 2009).

The sum of the reducing sugars and sucrose determined by any method in fruit-vegetables and their products indicates the total sugar content of that food. The basic principle of all methods of sugar determination is based on the reducing properties of sugars. All monosaccharides have reducing properties. Disaccharides and oligosaccharides may have reducing properties (such as maltose and lactose) depending on the monosaccharides that compose themselves. Sucrose has no reducing properties. Therefore, in the determination of sugar by chemical methods, sucrose is first converted to invert sugar and together with glucose and fructose it is determined as invert sugar. Since the reducing property of sugar is used in the determination of sugar, other substances which have reducing properties in the medium must be removed. For this purpose, sugar analysis is applied clarification by using Carrez I and Carrez II solutions ("Gıda Teknolojisi Gidalarda Şeker Tayini," 2011).

Although there are different methods for the determination of sugar, the Lane-Eynon method is often preferred because of its practical and easy use. Luff-Schoorl and high performance liquid chromatography (HPLC) methods can also be used to determine the total sugar contained in fruits and vegetables and their products. Sugar determinations in foods are carried out for the following reasons:

- ✓ To determine the total amount of sugar and invert sugar in the composition of foods
- ✓ To direct the process (production processes)
- ✓ To find the amount of energy they have

- ✓ To determine if it complies with the standard (“Gıda Teknolojisi Gidalarda Şeker Tayini,” 2011).

General Information:

Reducing sugar: Glucose, with its aldehyde group, can reduce some substances. Sugars, which have reducing properties are named as “reducing sugars”. When the sucrose is formed, the reactive groups of glucose and fructose combine with each other so that there is no free reactive group. Therefore, sucrose has no reducing properties. If sucrose breaks down into glucose and fructose, which forms in any way, it becomes reductive again.

Inversion: It is called the decomposition of sucrose. D-glucose and D-fructose is formed by hydrolysis of sucrose.

Invert sugar: It is the product obtained from the decomposition of sucrose by enzyme or acid and its reduction to glucose and fructose. Sugar obtained by inversion process is called invert sugar.

Factor: It is the amount of sugar reduced by 5 mL of Fehling-I and 5 mL of Fehling-II solutions.

Determination of Reducing and Total Sugar with Lane-Eynon Method

In this method, the copper-2 oxide of invert sugar in the Fehling solution is based on the principle of reduction of insoluble copper-1 oxide.

Since copper oxide is insoluble in water, the water-soluble complex salt of copper (senyet salt) is used. The alkalinity of the medium is provided with NaOH. Soluble copper tartrate ion in the solution is blue. Copper ions formed by the reduction of copper do not form complex ions by tartrate, and so separate and precipitate as copper-1 oxide.

Methylene blue used as indicator is blue in basic medium and colorless in sugar. Therefore, it becomes colorless at the end of the titration and the medium is copper red.

Sample Preparation and Factor Determination:

Tools & Materials:

- Bunsen burner
- Erlenmeyer
- Pipette

- Funnel
- Beaker
- Burette
- Tongs
- Wire mesh

Chemicals:

- Carrez-I solution
- Carrez-II solution
- Fehling A solution
- Fehling B solution
- 1% aqueous methylene blue solution

Procedure:

- Homogenize the sample.
- Weigh 2.5 g of the homogenized sample.
- Dilute the sample with distilled water upto 100 ml.
- For clarification, add 10 ml of Carez I and 10 ml of Carez II solution and mix well. Complete to 250 ml with distilled water.
- Wait for 30 min.
- Filter the solution to obtain a clear solution/filtrate.

  Factor Determination:
- Take 5 ml of both Fehling A and Fehling B solutions and add 20-25 ml of distilled water in erlenmeyer.
- Take 15 ml of the standard invert sugar solution in the burette into the conical flask.
- Boil the solution in the burner flame.
- After boling for 2-3 minutes, add 2-3 drops of methylene blue.
- Titrate the solution with the standard invert sugar solution in the burette until the color turns red.
- Record the consumption by ending titration as soon as the color changes from blue to copper red.
- Calculate the factor from the formula.

Calculation:

Multiplying the amount of solution spent in titration (V) by mg sugar per ml of spent sugar solution gives us the factor (F or K).

$$F\ (K) = V\ x\ mg\ of\ sugar\ contained\ in\ ml\ of\ the\ standart\ sugar\ solution\ spent$$

F (K) = The amount of invert sugar in mg which reduces 5 ml of Fehling A and 5 ml of Fehling B solution,

V = Standard amount of sugar solution spent in titration + 15 ml (amount of sugar solution added before titration)

The mg of sugar contained in ml of the standard sugar solution consumed should generally be between 2-3 mg.

Determination of Invert Sugar

$C_{12}H_{22}O_{11} + H_2O \longrightarrow C_6H_{12}O_6 + C_{12}H_{22}O_{11}$

sakkaroz +su ⟶ glikoz + fruktoz = İnvert şeker

Tools & Materials:

- Bunsen burner
- Erlenmeyer
- Pipette
- Funnel
- Beaker
- Burette
- Tongs
- Wire mesh

Chemicals:

- Carrez-I solution
- Carrez-II solution
- Fehling A solution
- Fehling B solution
- 1% aqueous methylene blue solution

Procedure:

- Homogenize the sample.
- Weigh 2.5 g of the homogenized sample.
- Dilute the sample with distilled water upto 100 ml.
- For clarification, add 10 ml of Carez I and 10 ml of Carez II solution and mix well. Complete to 250 ml with distilled water.
- Wait for 30 min.
- Filter the solution to obtain a clear solution/filtrate.
- Take 50 ml from the clear filtrate and put it in the burette.
- 5 ml of both Fehling A and Fehling B solutions, 10 ml of water, 5 ml of the test solution in burette are placed in the erlenmeyer and allowed to boil within 3 minutes.
- During boiling for 2 minutes, 2-3 drops of methylene blue are added dropwise and titrated with the solution in the burette.
- When the color becomes red, the titration is stopped to determine the approximate amount spent. (Amount spent in titration + 5 ml).

Calculation:

The amount of invert sugar present in the sample is determined as g/L in terms of the amount spent. The amount of invert sugar is calculated according to the following formula:

$$Invert\ Sugar = \frac{V_2\ x\ F}{V\ x\ V_1}$$

V2 = Diluted volume (ml)

V1 = Amount of sample taken (ml)

F = Factor

V = Amount spent from the burette in titration (consumption)

Determination of Total Sugar

Multiplying the difference between total sugar and invert sugar by 0.95 gives the amount of sucrose. The reason for multiplying by 0.95 is that 1 mole of sucrose absorbs 1 mole of water during inversion and 100 g of invert sugar is produced from 95 g of sucrose.

The total amount of sugar is found to determine the amount of energy contained in the food.

The method is based on the principle that the invert sugar reduces the copper-2 oxide in the Fehling solution to the water-insoluble copper-1 oxide.

Tools & Materials:

- Bunsen burner
- Erlenmeyer
- Pipette
- Funnel
- Beaker
- Burette
- Tongs
- Washing bottle
- Wire mesh
- Fume cupboard

Chemicals:

- Clear filtrate
- Fehling A solution
- Fehling B solution
- Methylene blue solution
- Phenolphthalein
- HCl

Procedure:

- Homogenize the sample.
- Weigh 2.5 g of the homogenized sample.
- Dilute the sample with distilled water upto 100 ml.
- For clarification, add 10 ml of Carez I and 10 ml of Carez II solution and mix well. Complete to 250 ml with distilled water.
- Wait for 30 min.
- Filter the solution to obtain a clear solution/filtrate.
- Take 50 ml of clear filter prepared for invert sugar and put into 100 ml measuring flask.
- 5 ml of HCL is added slowly by rotating the flask.
- Close the flask and kept in a water bath at 65-67˚C for 5 minutes. Cool quickly.
- Then, add 1-2 drops of phenolphthalein to the solution and neutralized with 4 N NaOH until the color becomes pink. In the meantime, the balloon will be heated and should be kept under water during titration. Complete with distilled water up to volume line and shake.
- Fill the sample prepared into the burette.
- Add 5 ml of both Fehling A and Fehling B solutions into the erlenmeyer, and then add 20-25 ml of distilled water and allow to boil within 3 minutes.
- Before 2 minutes for boiling, add 2-3 drops of methylene blue dropwise and titrate with the solution in the burette.
- When the color becomes red, stop the titration for determination the approximate amount spent.
- Write the consumption. Calculate the total amount of sugar from the formula (V).

Calculation:

The total amount of sugar is calculated using the amount spent. When the dilution ratio is as above, the result is found with the following equation. The total amount of sugar is calculated according to the following formula:

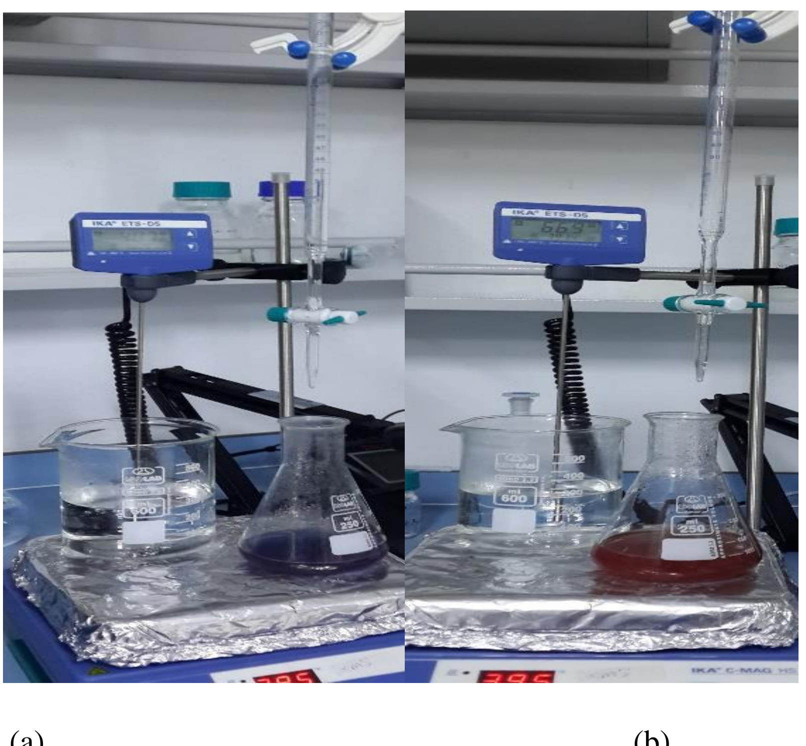
$$Total\ sugar = \frac{V_2\ x\ F}{V\ x\ V_1}\ x\ 2$$

In this formula, the values are the same as in the invert sugar formula. Only difference is '2 value'. The reason for using 2 here is that the sample prepared for invert sugar is taken and diluted again.

Calculation of the Amount of Sucrose:

The amount of sucrose is obtained by multiplying the difference between total sugar and invert sugar by 0.95. The reason for multiplying by 0.95 is that 100 g of invert sugar is produced from 95 g of sucrose.

$$Sucrose = (Total\ Sugar - Invert\ Sugar)\ x\ 0.95$$



(a) (b)

Figure 1. Boiling of fehling solutions with filtrate prepared for sugar determination (a) and transformation of boiled solution to copper red color (b)

References:

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No, W. (2009). Method 3 . 9 – Juices : reducing sugars by the Lane and Eynon method, (91), 4–7.

EXPERIMENT 11: ACIDITY & PEROXIDE NUMBER IN OILS

1.INTRODUCTION:

Acidity Determination: Determination of acidity in edible oils includes determination of acidity number (degree) and free fatty acids. This analysis provides insight into the degradation (rancidity) of fats and is considered as a measure of quality classification of oils.

The number of acids in fats is indicated as "a weight in mg of potassium hydroxide or sodium hydroxide required to neutralize 1 gram of oil". This is also called acid percentage.

This method can be applied to crude and refined vegetable oils, fish oils and animal oils.

Number of Peroxide: It is the measure of the amount of active oxygen present in oils and the amount of peroxide oxygen in miliequvalent grams per 1 kg of oil.

Oils degrade during storage for the following reasons;

- Oxygen intake,
- Temperature,
- Moisture,
- The amount of air in contact with oil,
- Light (especially ultraviolet and near-ultraviolet light),
- Presence of antioxidants and peroxidants and so on.

According to oxygen intake oil starts to get bitter and those are considered as related with oil unsaturation. Number of peroxides; is a parameter indicating the degree of oxidation. It also shows whether the deodorization process is performed effectively. Determination of the amount of peroxide in the oil gives an idea of the degree of degradation of the oil and how long it can be stored.

2. PRINCIPLE OF THE EXPERIENCE:

Acidity Determination Principle: It is based on the principle of titration of free fatty acids in the oil which dissolved in alcohol-ether mixture with a calibrated alkali solution using phenolphthalein as an indicator and by calculation of the amount of alkali spent.

Determination of Peroxide Number Principle: It is based on the principle of saturated iodide solution which is weighed and dissolved in acetic acid - chloroform solution, is allowed to react in the dark and at room temperature and released to the free amount of iodine peroxide oxygen and titration of the remaining iodine with thiosulfate.

3. EXPERIMENTAL PROCEDURE:

Process steps of determination of acidity in edible vegetable oils;

1. Weigh 5 or 10 g of the oil sample into a 250 ml flask with a sensitivity of 0.01 g.
2. Add 50 - 150 ml of 1/1 (v / v) ethyl alcohol- di ethyl ether solution to dissolve the oil.
3. Shake with 2 -3 drops of phenolphthalein solution.
4. Titrate with 0.1 N potassium hydroxide solution which dissolved in ethyl alcohol placed in burette until permanent light pink color is formed (This color should remain for 30 seconds).
5. Calculate the spent 0.1 N potassium hydroxide dissolved in ethyl alcohol.
6. The results of both experiments are averaged by working in parallel from the same sample.

Process steps of determination of peroxide number in edible vegetable oils;

1. Sample shall be prepared in the amount specified in tables according to the estimated number of peroxides. Weigh the sample to the flask.
2. After 10 ml of chloroform is added, the flask is shaken rapidly and the oil is dissolved.
3. Add 15 ml of acetic acid and 1 ml of potassium iodide solution and immediately shake glass for one minute.
4. Wait for 5 minutes in a dark place.
5. After this time, 75 ml of distilled water and 1 ml of starch solution are added.
6. If the expected number of peroxides is less than 12.5, use 0.002 N, if it is 12.5 or higher titrate with 0.01 N sodium thiosulfate solution.

Note: At the same time, the amount of free iodine should not be present as a result of the test without using a sample.

4.CALCULATION OF THE RESULTS:

The calculated result is compared with the values in the related communiqué. Analysis report is prepared.

Every ml of spent 0.1 N KOH is equal to 0.028 g oleic acid.

$$\%Free\ Fatty\ Acids = \frac{V}{m} * 0.028 * 100(\%oleic\ acid)$$

$$\%Free\ Fatty\ Acids = \frac{V}{m} * 2.8(\%oleic\ acid)$$

V=Amount of volume of 0.1N potassium hydroxide dissolved in ethyl alcohol (ml) m=Weight of sample (g)

NOTE: The equivalence of potassium hydroxide and sodium hydroxide is the same. You can use the same formulas. Blind tests will be taken in 0 ml.

0.1 N potasium hydroxide solution's 1 ml equals to 56.1 mg potasyum hydroxide so,

$$Acid\ Number = \frac{V}{m} * c * 5.61\ KOH/g(\%oleic\ acid)$$

$$Peroxide\ Number = \frac{10 * (V_1 - V_2) * F}{m} meq\ g\ O_2/kg$$

V_2= 0.01 N sodium thiossulfate solution that spent at titration (ml)
V_1=0.01 N sodium thiossulfate solution that spent at blind test (ml)
F=Factor of 0.01 N sodium thiossulfate solution***(Assumption=1)***
C; assumption=1
m= Weight of sample (g)

EXPERIMENT 12: ASCORBIC ACID DETERMINATION

1. INTRODUCTION:

Vitamins are important organic substances for normal growth and maintenance of life. If any of the vitamins are not taken into the body, there may be disruptions in growth and functions of organism due to the chemical reaction that the vitamin helps.

The effects of vitamins in functions of body are related to the regulation of biochemical reactions. The effects of vitamins on human health can be divided into three groups;

- Helping grow
- Help create healthy generations
- Normal functioning of the nervous and digestive systems, proper use of nutrients, and assistance to body resistance.

Vitamins are required in very low amounts in all metabolic activities. Today there are 15 compounds identified as vitamins. These compounds:

- Active vitamin structure from the body can be taken from food
- Provitamins (vitamin precursors) after being taken into the body undergoes a series of chemical changes into one or more compounds that show vitamin activity.

Vitamins are present in different amounts in foods and show different distributions. Although some foods are quite rich in one or several vitamins, they contain some vitamins in trace levels.

- These compounds, which are involved in many functions such as growth, development and repair in the body, should be taken regularly in certain amounts daily. For this reason, one should create a balanced diet from food.
- Inadequate intake of vitamins with food causes absorption disorders or some metabolic disorders.
- Disease caused by vitamin deficiencies is defined as hypovitaminosis (taking vitamins below the normal minimum). For example; without fresh fruit and vegetables, sailors who had to manage dry foods for a long time had scurvy due to vitamin C deficiency. Since vitamin pills could not be made with the technology of the time, vitamin C had to be obtained from foods other than fresh fruits and vegetables. Vinegar, pickles, rum

and lime raw lime began to be included in the list of supplies on the ships for this purpose.

The amount of nutrients taken into the body differs from the amount used in the body. This is related to the bioavailability (bioavailability; absorption of any nutrient in the body and the level of its use in the organism). The need for vitamins in very low amounts increases the importance of bioavailability. Bioavalibility of vitamins varies depending on;

- The composition of the individual's diet,
- Whether the vitamin is in active vitamin form or coenzyme form,
- How the vitamin interacts with other nutrients.

In addition, the bioavailability of vitamins may be affected by the way foods are processed and may also vary from individual to individual.

Vitamins are generally classified according to their physical properties as follows:

- Fat-soluble vitamins: A, D, E, K.
- Water soluble vitamins: B1 (Thiamine), B2 (Riboflavin), B6 (pyridoxine), B12 (cobalamin), Niacin, Folic acid, Biotin, Pantothenic acid, Vitamin C (Ascorbic acid).

Since vitamins are generally unstable substances, they can be damaged during processing of foods. In addition, care must be taken when preparing the extract in vitamin analysis. The amount of vitamins in foods can be expressed as gr /100gr, USP unit or I.U.

Vitamin analysis in foods can be determined by volumetric titration and instrumental analysis. Vitamin analysis in most foodstuffs can be performed by spectroscopic methods. For this purpose, vitamins in foodstuffs are extracted with suitable solvents (mostly in acidic medium) and taken into solution. The solution is passed through a special chromatographic column to separate the vitamins and quantify using fluometry or other optical methods.

The most valid method for accuracy is HPLC. However, it is not preferred because it is very expensive.

The most common vitamin analyzes in foods;

- Determination of vitamin A (beta carotene) in soda and fruit and vegetable products
- Determination of vitamin A and D in vegetable margarines
- Vitamin C determination in fruit and vegetable products.

Vitamin C: Vitamin C, composed of colorless crystals with a melting point of 192°C and a molecular weight of 176, is an antiscorbut factor. It contains a dienol group which has both reducing power and acidic properties.

- Easily soluble in water, methanol and ethanol.
- Insoluble in benzene, ether, petroleum ether, chloroform and oil.
- Vitamin C (Ascorbic acid) is the most vulnerable of vitamins.
- Very sensitive to alkalis and oxidation, especially when catalysts such as Cu and Fe are present.
- More resistant as dry crystals.
- Very resistant to acid solutions (below pH 4).
- Ascorbic acid is also oxidized by the oxygen of the air. As a result of this oxidation, the molecule loses its vitamin activity.
- Available in nature as reduced ($C_6H_8O_6$) and oxidized ($C_6H_6O_6$).

Deficiency:

- Capillary vessels can have a weak structure,
- Teeth gums can bleed easily, teeth can be lost
- It causes joint diseases.
- It is also necessary for the normal formation of collagen and protein, an important element of the skin and connective tissues.
- Vitamin C increases the body's resistance to many diseases. Daily vitamin C requirement is 30–40 mg in adults.
- Vitamin C is the main vitamin contained in foods of plant origin.

Richest resources:

- Fresh fruits, especially citrus fruits such as oranges, tangerines, rose hips, strawberries
- Fresh vegetables are dark green leafy vegetables, tomatoes, green and red peppers, and potatoes.

Vitamin C is very easily broken down by oxidation and also thermally, especially at high temperatures. Vitamin C is the most lost during processing, storing and cooking foods. As it is so sensitive to various processes, the amount of loss in ascorbic acid is used as a criterion of determining the negative effect of many processes applied to foods. In the products made

from vegetables and fruits, vitamin C is determined to investigate whether vitamin C is present, how much is the amount, and whether it will contribute to nutrition.

Some products may contain vitamin C or ascorbic acid to facilitate processing or to prevent oxidation. In this case, it is investigated whether the quantity in the product is as much as the allowable amount. The findings are compared with the standards (TS 6397).

2. PRINCIPLE OF THE EXPERIMENT: It is a pink color produced by dehydro ascorbic acid formed by titration with 2,6 dichlorophenolindophenol dye solution of ascorbic acid which is a strong reducing agent.

3. EXPERIMENTAL PROCEDURE:

1. Preparation of Sample (Extraction):

If the amount of ascorbic acid in the foodstuff to be determined is solid:

- Approximately 200 - 300 g of sample is weighed and put into a blender,
- Add 6% metaphosphoric acid solution up to the amount of sample weighed,
- Blender is turned into a homogenous crush by operating. (It is enough to run the blender for 2 minutes) Metaphosphoric acid is used as stabilizer to prevent enzymatic oxidation of ascorbic acid during this process.
- After weighing 10 - 40 grams of the mixture in the blender, add 3% metaphosphoric acid solution to a 100 ml measuring flask and fill the line with the same solution and filter through filter paper.

For the preparation of fruit and vegetable cannes or similar products:

- All can or jar contents are poured into blender,
- It only disintegrates for 5 - 10 seconds,
- Weigh up to 20 g of this (in an amount containing 1 to 5 mg of ascorbic acid) and transfer to a 100 ml flask,
- The volumetric flask is completed to the marking line with 3% metaphosphoric acid, the sample is thoroughly mixed and filtered.
- Treated foods such as canned food contain high amounts of Fe^{+2}. It reduces paint and gives incorrect results. To prevent this, 8% acetic acid is added as extracting solution.

If the sample to be examined is a homogeneous liquid such as fruit pulp, juice or tomato paste:

- Take 20 g or 20 ml directly and transfer to a 100 ml flask. Make up to volume with 3% metaphosphoric acid solution.
- The sample is thoroughly mixed and filtered. In this way, the sample can be stored without oxidation until titration.

If the raw material of the fruit or vegetable to be analyzed consists of large grains:

Care should be taken to represent the entire mass. For example, if it is to be made in fruits such as tomatoes and peaches, only 1 - 2 of them can weigh 200 - 300 grams. In such cases, the sample must be crushed in the blender by taking at least eight or ten tenths of each of at least 20 fruits to represent the whole group. However, the time it takes to cut such a large number of fruits with a knife and take pieces from them is very likely to cause loss of ascorbic acid, particularly enzymatically. In order to eliminate or limit this possibility;

- First weigh between 200 - 300 g of 6% metaphosphoric acid solution in the blender chamber,
- Then the sample pieces are cut from the fruits even though the hopper is on the scale. Each cut is immediately thrown into the blender chamber containing metaphosphoric acid.
- After reaching the prescribed weight, the blender is started.
- For these operations, a weighing scale with a precision of 0.2 gr is required. In this way, the homogenized sample is weighed 10-40 grams with a more sensitive balance and transferred to a 100 ml flask.
- Volumetric flask is completed with 3% metaphosphoric acid or oxalic acid solution up to the mark,
- Filter after shaking well. This filtrate is then titrated with the dye solution.

As described above, the sample of the same weight is crushed by mixing with the same weight of metaphosphoric acid solution. Thus, half of the weight weighed from this paste gives directly the weight of the sample.

The best way to prepare samples for the determination of ascorbic acid in citrus fruits is;

- Weigh 250 g of 6% metaphosphoric acid solution in a liter beaker.
- The number of fruits that will represent the whole mass of citrus fruits to be analyzed is selected and pressed on this solution. For this purpose, the determined number of fruits is divided into two parts in order, and only one half of each is pressed directly

onto the solution. The juice thus obtained is taken up directly into the metaphosphoric acid solution.

- Then weigh the beaker and determine the amount of juice taken up on 250 g metaphosphoric acid.
- After thoroughly mixing with a glass baguette, a certain amount of this mixture is weighed directly.
- 100 ml with 3% metaphosphoric acid. is completed and the contents of the flask are filtered.

2. Blind trial:

- 25 ml of MFA solution and the amount of water which spent dye solution for standardization is put into a flask.
- Titrate to pink color with dye solution in the burette.
- The amount spent is saved. (V_0) =… .m

The aim of the witness trial is to eliminate the effect of other substances which can reduce the dichlorophenolindophenol solution except ascorbic acid. Calculate how many mg ascorbicity of each 1 ml dichlorophenolindophenol solution is subtracted from the average amount spent for the blind trial from the average amount of dichlorophenolindophenol solution spent during the adjustment of the dichlorophenolindophenol solution (blind trial).

3. Titration of the sample:

- Take 20 ml of the test sample and place 20 ml of MFA solution on top.
- Mix well and filter.
- 5 - 10 ml is taken from the filtrate and titrated to the light pink color with the dichlorophenolindophenol solution in the burette. (The resulting color should be permanent for at least 15 seconds and the titration should be completed as soon as possible.)
- The amount spent is saved. (V_1) =… .ml

4. CALCULATION OF THE RESULTS:

$$Dye\ solution\ factor\ (F) = \frac{1}{Spent\ dye\ solution\ amount\ (ml)}$$

$$Ascorbic\ acid \left(\frac{mg}{100g}\right) = \frac{V_1 * F}{m_2} * 100$$

V_1:Spent dye solution amount (ml)

F: Factor

M_2: Amount of original sample in titrated filtrate (g)

$$m_2 = \frac{m_1 * V_t}{V}$$

M_1: Amount of sample taken (g)

V_t: Amount of filtrate taken for titration

V: Completed flask volume (ml)

EXPERIMENT 13: PHYSICAL ANALYSIS IN WHEAT GRAIN

Composition of wheat kernel

Wheat quality can be defined in terms of physical characteristics of the kernel including size, weight, and hardness (physical) and intrinsic properties such as protein contents and quality.

Wheat Kernel Quality:

1. Hectoliter Weight or Test Weight
2. Vitreous Kernel Content
3. Weight per 1000 Kernels
4. Kernel Size
5. Kernel Damage
6. Shrunken and Broken Kernels
7. Foreign Material
8. Protein Content
9. Falling Number
10. Moisture
11. Grade

Wheat grains include water, carbohydrates, protein, fat, cellulose, minerals, enzymes and vitamins. The amount of these substances in the grain of wheat varies according to the variety and the region in which it grows. On average, the amounts of these substances are as follows:

	Whole grain	Mealy endosperm	Bran	Germ
Proteins	16	13	16	22
Fats	2	1.5	5	7
Carbohydrates	68	82	16	40
Dietary fibers	11	1.5	53	25
Minerals (ash)	1.8	0.5	7.2	4.5
Other components	1.2	1.5	2.8	1.5
Total	100	100	100	100

The moisture content of lots of commercial wheat may vary between 12 and 18%, depending on the weather during harvest. Wheat is an important source of proteins since large amounts of wheat is often included in the diet, and wheat contains 8-20% proteins but generally wheat grains contain approximately 12% protein. This ratio varies according to the factors such as soil, climatic conditions and wheat varieties. Wheat proteins are classified into several groups on the basis of their solubility properties, genetic background and amino acid composition etc. The most well known classification system classifies the wheat proteins into albumins, globulins, gliadins and glutenins on the basis of solubility.

It is one of the important factors in determining the economic value of wheat grain. Wheat with high protein content has higher economic value because high protein, flour is preferred in bread making. It should be remembered that protein quality as well as protein quantity is an important factor in bread quality.

Table 1: Wheat proteins are classified into groups on the basis of their solubility (Hussain, 2009).

Proteins		**Soluble in**	**Location in**
Non-gluten protein	Albumins	Water	Embryo (metabolic proteins) and endosperm cells (cytoplasmic proteins)
	Globulins	Dilute salt solutions (0.5 M NaCl)	Embryo and aleurone layer (storage proteins) and endosperm cells (cytoplasmic proteins)
Gluten proteins	Gliadins	70-80% ethanol	Endosperm (storage proteins)
	Glutenins	Dilute acids or alkali solutions (0.05 M acetic acid)	

Starch is a major carbohydrate which found in wheat grain and present in its endosperm. Wheat grain contains about 63-66% of starch, figures being higher for soft wheats than for hard wheats. The major components of starch are amylose and amylopectin. The contents of amylose and amylopectin are significantly different among varieties of cereals. The ratio of amylose and amylopectin differs among starches. The level of amylose and amylopectin in wheat flour is 25–28 and 72–75%, respectively (Hussain, 2009).

Wheat grain lipid content is low; they contain healthy polyunsaturated fatty acids. They are sources of vitamins; vitamins B and E and mineral; selenium, zinc, copper. Phytic acid– found in bran chelates the minerals.

Structure of wheat grain

Wheat grain is constituted by three distinct parts: the germ (embryo), the bran and the mealy endosperm. Wheat grains contain 2–3% germ, 13–17% bran and 80–85% mealy endosperm (all constituents converted to a dry matter basis). The outer layer of kernel is removed as “bran” during milling. Bran is rich in “cellulose”, “hemi cellulose” and “lignin”. “Endosperm” of the kernel is like an energy depot .Endosperm is generally composed of starch particles and cytoplasm. Germination of the grain is managed by embryo (germ) and also supplies energy. About 80% of the daily energy and protein demand of population is provided by cereal grains in Turkey.

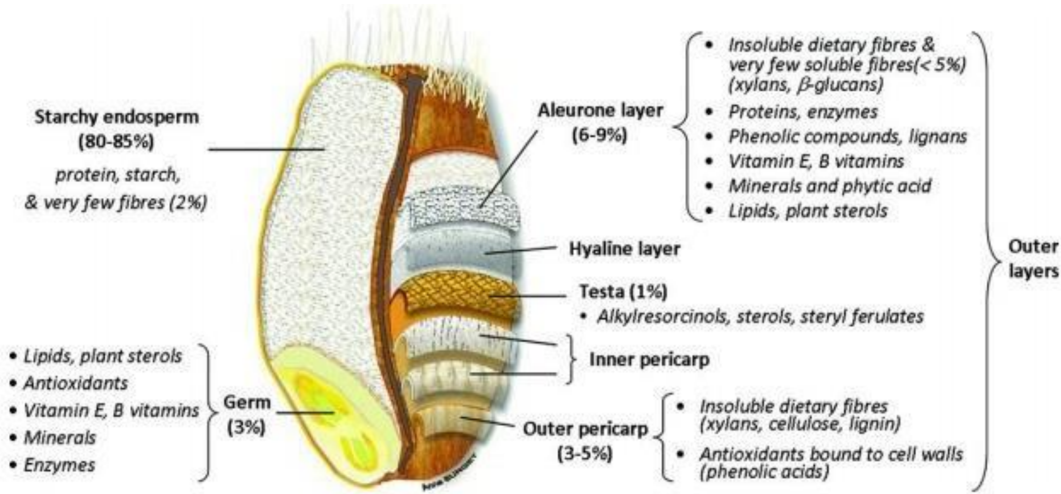


Figure 1: Wheat grain structure (Onipe et al., 2015)

Wheat Grain Quality

Analysis Foreign Matter

Analysis

Foreign matter in wheat contains all the elements in the wheat sample except the main variety. These are organic and inorganic material other than wheat, broken kernels, other grains and filth. Organic matter is material of plant origin e.g. stalks, chaff, weed seeds, etc., while inorganic matter includes plastics, stones, glass, metals, etc. Foreign matter is a very important qualitative criteria for milling. Grains affected by diseases and pests before

harvesting reduce the yield of flour. In addition to the decrease in flour yield in frozen grains, ash content increases, and in grains stored in places with high moisture content, some problems are encountered. Some impurities adversely affect the storage stability; commercial value and flour yield of wheat and may be harmful to health. Moreover foreign materials such as metal, stone and sand damage the machines during grinding and cause dust explosion.

Procedure: Place a clean dry basin (Bottom Pan) in place to receive any materials that may go through the sieve. Weigh 200 grams of the representative sample. Put the Wheat in the sieve provided (1.6 mm slotted-hole sieve).Shake the sieve horizontally for 30 times for about 15 seconds. Collect all the foreign organic matter that has passed through the sieve. Weigh all the foreign matter collected from the bottom.

Foreign matter =	$\frac{Weight_2}{Weight_1} * 100$

Testing for Foreign Odour

Wheat should not contain any odours other than its own odour. Odours found in wheat can be grouped as follows:

Normal grain odor

- Fresh wheat smell
- Warehouse smell

Disordered grain smell

- Damp smell
- Mold smell

Foreign smells:

- Grass smell
- Gas

- Onion
- Garlic

Procedure: Determination of odour in wheat is done by two methods:

As the first method; obtain a representative sample. A handful of wheat is taken and a strong breath is given to the wheat in the hand, and then it is smell. The sensed odour is detected. If no odour is detected, return the sample into the container and seal it. The smell should be typical of Wheat without other smells e.g. chemicals, mouldiness', earthy, rotten, musty smell, etc. As the second method; put a small quantity of ground or un-ground wheat kernels in a container and pour some warm water (60 – 70 ˚C) (1:10 ratio) into the wheat and boiled and resulting steam is smell and note whether foreign odour is present.

Colour Analysis

Wheat comes in different colours: red, white, and amber. As the hardness of the grain increases, the colour darkens. Colour also varies according to nature conditions and harvest season. Improper storage conditions and diseases have a detrimental effect on color.

Figure 2: Colours of wheat

The colour determination is done by using the standard colour charts (scales). As the determination is subjective, the results differ slightly from the determinants.

Determination of Test Weight-Hectoliter Weight

Hectoliter mass, also referred to in some countries as bushel-, specific- test-, or hectolitre weight is the weight of a standard volume of grain and is generally believed to be a measure

of the bulk density and soundness of grain (Manley et al., 2009). It is one of the oldest specifications used in wheat grading and serves as a guide to a combination of characteristics. Hectoliter weight is an important indicator of the physical quality of wheat and has long been recognised as an indicator of the flour yield of wheat. An increase in hectolitre weight results in a higher allocated grade and subsequently in a higher price per ton of wheat unless other grade determining factors such as protein (in countries such as Australia and South Africa), weather or insect damage negatively impact the grade. The hectolitre weight values of sound wheat normally vary from 70 to 85 kg/hl , but can be higher or lower due to environmental conditions and insect damage. Several factors could influence the hectolitre weight value of wheat. The presence of impurities such as chaff or large weed seeds can lower the hectolitre weight value. This is the primary grading factor. It can also, in general, be an indicator of flour extraction rate. It is related with variety and environmental conditions. A high linear correlation coefficient was reported between test weight and flour yield in the range from 53 to 84 kg/hl. It is affected from the shape and uniformity of the kernel. Hectoliter weight is changing according to biological structure, moisture content and chemical composition.

Hectoliter weight of Turkish wheat is between 72-83 kg/hl. The average is 78 kg/hl.

Table 1 – Illustration and a short description of the respective HLM devices

	Country	Description of HLM devices
	Australia	Aluminium 500 ml measure with filler and cutter bar.
	Canada	Ohaus 500 ml measure with Cox Funnel and round wooden striker. 500 ml measure supplied with certificate of calibration (calibrations performed traceable to national standard).
	France	Niléma Litre with filling hopper and cutter bar (1000 ml receiving cup). Designed in accordance with the AFNOR NF V 03-719 (1996) standard and standardised to a 50 l French reference.
	Germany	Kern 220/222 Grain Sampler with filler and cutter bar (1000 ml measuring cup). Compliant to ISO 7971-2:1995 standard.
	South Africa	South African two-level HLM device with funnel and 500 ml measuring container and wooden scraper.
	United Kingdom	Easi-Way Portable Hectolitre Test Weight Kit with cutter bar (500 ml measuring cup). Matched to EC 20 L volume (Directive 71/347/EC) and conforms to ISO 7971-2: 1995 and BS 4371 Part23 standards.
	USA	Seedburo 151 Filling Hopper with quart cup (1100 ml) and strike-off stick.

http://megep.meb.gov.tr/mte_program_modul/moduller_pdf/Un%20Ve%20Unlu%20Mamull erdeki%20Analizler%201.pdf

Thousand kernel weight

1,000 kernel weights is the weight in grams of 1,000 seeds. It will vary with seed. Some people regard this as a better indicator of flour extraction than test weight. Related to variety and environmental conditions. European prefer to use this criteria. The measurement of the thousand kernel weight includes weighing and counting the kernels. Weight data is obtained from scales and traditional methods based on manual counting are used to count

the kernels. As manual counting is time-consuming and labor-intensive with subjective results, electronic counting devices are also commonly used. The ratio of endosperms of large and dense grains to non-endosperms is higher than that of small grains. For same variety, a thousand grain weight is directly proportional to the amount of starch and inversely proportional to the amount of protein. It is important to give an idea about the grain size, fullness, ricidity and flour yield. It is generally higher in hard wheat. The weight of a thousand grains varies according to variety, climate and soil conditions. It is a healthy measure for estimating the flour yield of wheat grain.

Procedure:

- First, the impurities in the sample are removed.
- The weight of a thousand grains is then determined by counting 500 or 1000 grains and weighing weight of these 500 or 1000 grains or weighing 15 grams of grains and then counting them.

During analysis studied with at least three parallel and then the average of the parallel is taken. Calculation is done according to the following formula:

$$\textit{Thousand kernel weight} = \frac{\textit{Sample weight}(g)}{\textit{Number of counted grain}} \times 1000$$

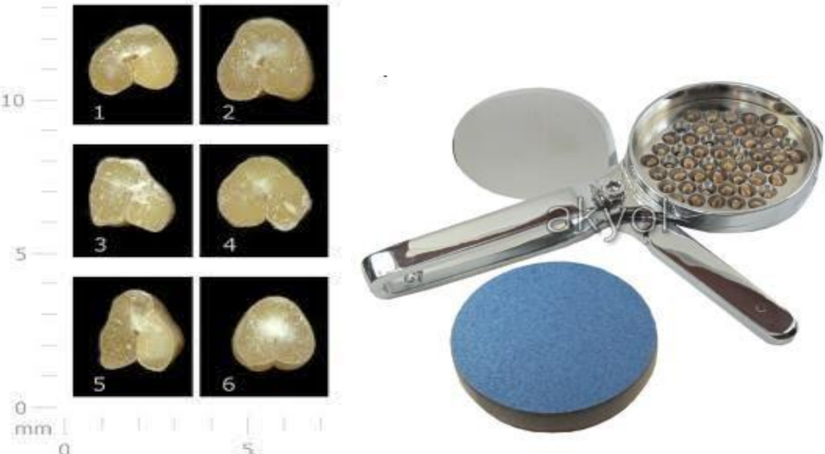
It is stated that thousand kernel weight varies between 24-51 g for soft wheat and 26-58 g for hard wheat.

Grain hardness analysis

Grain hardness is important for the flour industry because it has significant impacts on milling, baking and qualities of wheat. The farmer generally makes more profit with harder textured wheat, due to the higher protein content. It is one of key determinant for classification of wheat and end product quality. Wheat hardness, defined as the force needed to crush the kernels, is, next to its protein content, a major quality trait. Bread wheat (Triticum aestivum L.) endosperm texture ranges from very soft to hard, whereas durum wheat (T. turgidum L. ssp. durum) has the hardest kernels of all wheat cultivars. Grain hardness is normally influenced by various environmental, physical and chemical factors like kernel protein, vitreousness of grain, kernel size, water-soluble pentosans, moisture content and lipid content. Differences in wheat hardness result probably from adhesion between starch granules and storage proteins. This is a grading factor and is also generally an indicator of protein content. In general, glassiness is related to hardness and high protein content; opacity is considered to be associated with softness and low protein content. The light transmission property of the endosperm causes glassy or floury appearance of the grain. While wheat grain loses water during drying, air gaps may form as a result of compression and rupture in protein structure and the endosperm appears opaque. In the vitreous grains, the protein is compressed, there are no breaks in the structure so that reason, no air gaps occur. Starchy durum wheat tends to be lower in protein content than vitreous durum wheat, imparting weak breaking strength to dried pasta and poor cooking quality. It has been concluded in some studies that starchy durum wheat is softer than vitreous durum wheat, and gives a lower yield of coarse semolina and a higher yield of flour, thereby reducing milling potential. In general, vitreous kernels are associated with higher protein content and water-absorption capacity and with greater loaf volume potential in breadmaking. Therefore, in the production of bread, it is desirable for hard wheat to contain a high percentage of vitreous kernels (Baasandorj et al., 2015). Kernels that are glasslike and translucent in appearance are referred to as vitreous, whereas kernels that lack translucency or are light colored opaque are called nonvitreous (Starchy or piebald).

Wheat section:

- If it looks like glass, it's hard wheat (glassy, vitreous)
- If it looks floury, soft is soft wheat (starchy, mealy)
- If there are local starch spots on glassy region, it is piebald (Dönmeli buğday)



Procedure: The Grobecker cutting tool has a bottom plate with 50 holes on which the wheats are placed, a blade over it and a perforated plate on the blade.For the determination of the hardness, the blade between the two plates is taken out in the position to cut the grains. The wheat is placed on the top plate of the hole and gently shaken to fill all the holes and the tool's special wood is pressed from the top. Then the grain is cut from the center of the wheat by a single action transversely with the blade and the cross-sectional surfaces of crosscut half- grains in the holes in the bottom plate are examined one by one by pouring the remaining portion on the blade. Since the Grobecker cross-section tool can take 50 cross-sections, the result is multiplied by two and the hard and soft grain rates are expressed as a percentage.

Determination of falling numbers

Falling numbers is an indicator of the extent of sprouting of wheat. Wheat germination released enzymes that cause digestion of the endosperm (starch). This is a measure of alpha- amylase activity. Amylases: enzymes that are hydrolyzing starch. Alpha-amylase; is an endoenzyme attacking to the bonds in the molecular structure of starch.(alpha 1-4 bonds).

Fermentability of the dough is affected from the activity of this enzyme so the activity should be monitored. Falling number test is the rapid method that can analyze alpha amylase activity. Low FN value= high alpha-amylase activity (60-600). High activity: is seen in germinated grains after rainy harvesting period. But should not only related with germination. For an acceptable bread, desired value for wheat : 225-275

- If <150 bread crumb is wet inside and sticky

- If >350 small volume, dry crumb.

Activity can be regulated by using flour mixes or for increasing activity malt or amylase enzyme can be added. A falling number of 250 units or higher is usually desired.

Related to amount of sprouted wheat kernels in a parcel of grain. Apart from the three major types determining factors (hardness, gluten strength, protein content), soundness is another important wheat quality factor influencing baking quality. Sound wheat contains very low levels of an enzyme (α-amylase) that attacks and liquefies starch. Germination is associated with a rapid increase in enzyme activity and a severe sprouted kernel may be subject to several thousand times much activity than sound kernel.

High enzyme activity can cause severe problems in baking (lower absorption, sticky dough and in extreme cases, sticky crumb). In general, the following values are indicative of the flour's baking qualities:

A FN value of 250 sec. indicates flour with a normal α-amylase activity and good baking quality.

A value of 65 sec. indicates a high α-amylase activity and production of sticky breads.

High FN values in the range of 400 sec. indicate too low α-amylase activity for bread baking.
This test required specialised equipment. The basis of the test is to measure the time it takes for a stirrer to fall through gelatinised slurry in a test tube, made from flour of the wheat sample.

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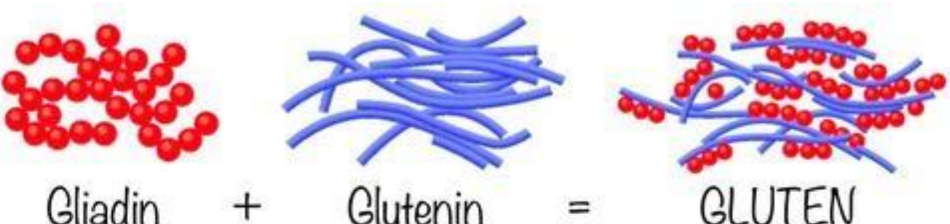
EXPERIMENT 14: PHYSICOCHEMICAL ANALYSIS OF WHEAT FLOUR

1) Determination of Wet Gluten Content

Gluten is the main storage protein of wheat grains. The wheat kernel contains 8%–15% of protein, from which 10%–15% is albumin/globulin and 85%–90% is gluten . Gluten is a complex mixture of hundreds of related but distinct proteins, mainly gliadin and glutenin. Different wheat varieties vary in protein content and in the composition and distribution of gluten proteins (Biesiekierski, 2017). Collectively, the gliadin and glutenin proteins are referred to as prolamins, which represent seed proteins insoluble in water, but extractable in aqueous ethanol and are characterized by high levels of glutamine (38%) and proline residues (20%). The gluten matrix and its resulting functions are essential to determining the dough quality of bread and other baked products such as pasta, cakes, pastries, and biscuits. Gluten is heat stable and has the capacity to act as a binding and extending agent and is commonly used as an additive in processed foods for improved texture, flavor, and moisture retention. When water is added to wheat flour and kneaded gluten proteins; gliadin and glutenin absorb water and form viscoelestic dough. Gluten ensures that the CO_2 gas produced by the yeast during fermentation in bread production is retained in the dough and a high volume of bread is formed. Gluten can only be obtained from wheat in cereals by simple washing. The



amount and quality of wet gluten are very important quality criteria for bread making.

Figure 1:Gluten

Wet gluten is viscoelestic structure which is formed by the swelling of gliadin and glutenin with water. Wet gluten amount give indication about quality of wheat proteins rather than their quantity. High wet gluten amount is an indication of good bread quality. Wet gluten content refers to the amount and properties of gluten protein which found in wheat flour.

Amount of wet gluten varies according to wheat type, growing conditions, air flow during the death period. Increasing the amount of nitrogenous substances and soil

conditions also increases the amount of wet gluten.

Figure 2: The relationship between the amount of wet gluten and bread volume

The wet gluten test provides information on the quantity and estimates the quality of gluten in wheat or flour samples. Gluten is responsible for the elasticity and extensibility characteristics of flour dough. Wet gluten reflects protein content and is a common flour specification required by end-users in the food industry.

Principle: It is the determination of the amount of insoluble material (gluten) remaining by washing wheat flour with dilute salt solution by removing starch, water soluble proteins (albumin) and proteins (globulin) dissolved in dilute salt solutions. Preparation of dough from a sample of flour and a buffered solution of sodium chloride. Isolation of the wet gluten by washing this dough with a buffered solution of sodium chloride, followed by removal of excess washing solution and weighing of the residue.

The amount of wet gluten can be determined in flour and semolina by hand washing or by using automatic washing process. However, gluten obtained from both methods is not pure and contains ash, fat and some starch.

Materials:

- Porcelain mortar,
- Burette,
- Spatula,
- Balance,
- Separation funnel

Procedure:

- Weigh, to the nearest 0.01g, 10.00g of the test sample and transfer it quantitatively to the mortar
- Add, drop by drop 5.5ml of the sodium chloride solution from the burette while continuously stirring the flour with the spatula.
- After adding the sodium chloride solution, compress the mixture with the spatula and form a dough ball, taking care to avoid loss of flour. Dough residues adhering to the wall of the vessel or to the spatula shall be collected with the dough ball.
- To homogenise, roll out the ball to a length of 7 to 8 cm with the flat of the hand on the roughened glass plate then fold it.
- Take the dough ball in the hand and allow the sodium chloride solution to drip onto it from the container at a rate such that 750ml flow in 8 min. During this time, successively roll out the dough ball, flatten it, stretch it to make two pieces, then mould them together into one piece.
- After the washing process is finished, squeezed the wet gluten or press between two glass plates to remove excess water and weigh.
- Then, three times in the centrifuge or between the fingers is vigorously dehydrated and weighed. During determination of wet gluten; iodine solution (N / 1000) is used for detecting starch is washed completely.
- By multiplying the value in the weighing by 10, the amount of wet gluten is found as%. The result is calculated on the basis of the following formula and given on a dry matter basis.

$$\text{Wet gluten content (Dry basis \%)} = \frac{Wet\ gluten\ amount}{(100 - \%moisture\ of\ flour)} \times 100$$

Table 1: Evaluation of results

%Wet Gluten Amount	**Gluten quality**
Flour	
>35	High
28-35	Good
20-27	Intermediate
<20	Low

2) Determination of Dry Gluten Amount

Dry gluten is obtained by drying the wet gluten at a certain temperature and time. The amount of dry gluten can be determined in the oven or in special instruments developed for rapid drying of wet gluten . When drying in the oven, the oven temperature is set to 105^0C. Wet gluten obtained by hand or machine washing is dried in oven for 3 hours and spread into a tared petri box or drying containers as a thin layer. The containers are placed in an oven and kept for 24 hours. At the end of the period, it is taken to the desiccator and after cooling it is weighed

When drying with glutork, the instrument is heated and wet gluten which are obtained by hand or machine wash are placed in the gluten device for 5 minutes. At the end of the period the dry gluten is taken to the desiccator and after cooling it is weighed.

The dry gluten values obtained in the oven or Glutork are multiplied by 10 to obtain the percentage amount of dry gluten. In general, the amount of dry gluten is about 1/3 of the amount of wet gluten. The amount of dry gluten is calculated on the basis of dry matter.

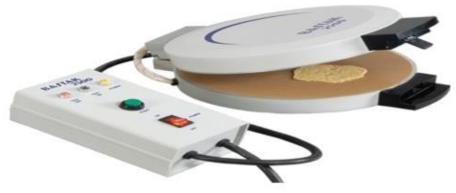


Figure 3: Dry Gluten Device (Glutork)

3) Gluten Index Value:

Gluten index value gives information about gluten quality in wheat flour. The gluten index test has gained wide acceptance as a method of determining gluten strength and is used in international trade specifications (Oikonomou et al., 2015). Many countries indicate that flours for the production of bakery products have the gluten index values of 60 to 90 and a high gluten index, exceeding 95, indicates strong gluten, while index values lower than 60 indicate flours too weak for bread production (Lonescu et al., 2010).

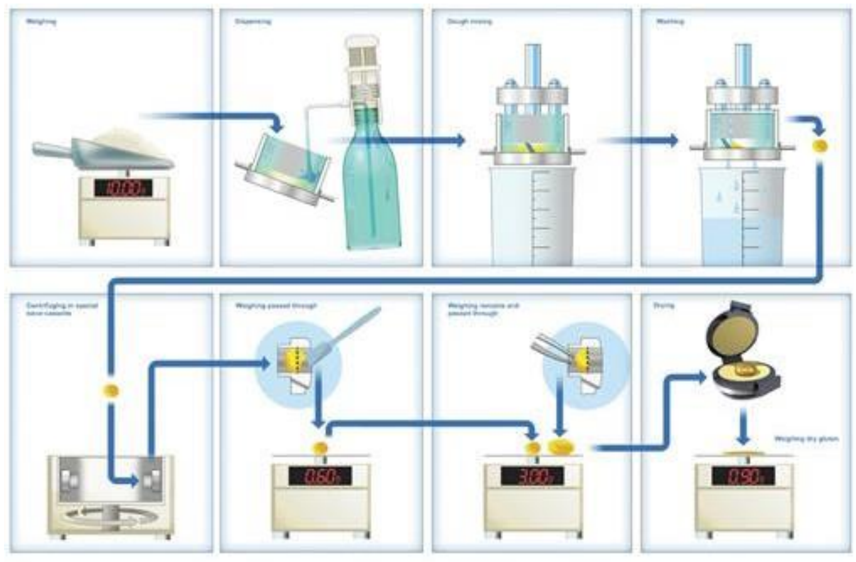
Principle: Gluten index value analysis is based on the determination of amounts of gluten that have passed through a special sieve during centrifugation at a constant rate for 1 minute. The ratio of the amount of gluten not passed through the sieve to the total amount of gluten gives the gluten index value. The higher the gluten index value, the higher the gluten quality of the flour.

Gluten index is determined by the Glutomatic system (Perten Instruments AB, Sweden) and is a measure of gluten strength regardless of the quantity of gluten present.

Procedure: 10.0 g ± 0.01 g of whole meal or flour is weighed and put into the

Glutomatic® wash chamber with an 88 micron polyester sieve. When vital wheat gluten is measured, 1.5 ± 0.01 g is weighed. 4.8 ml of salt solution is added to the meal or flour samples. No salt solution is added to vital wheat gluten samples. Meal or flour and the salt solution are mixed to form a dough during 20 seconds. After termination of the mixing phase, the washing automatically starts and continues for five minutes. For wheat meal the sample is transferred to a chamber equipped with a coarse 840 micron sieve allowing bran particules to be washed out. Exactly 30 seconds after completed washing, the undivided wet gluten piece is transferred to the special sieve cassette and centrifuged one minute at 6000 ± 5 rpm in centrifuge. The fraction passed through the sieves is scraped off with a spatula and weighed. The fraction remaining on the inside of the sieve is collected and added to the balance. The total wet gluten weight is obtained.

The gluten index value of commercial bread flours is generally between 60-90. Gluten index value should be around 70 for flour suitable for Turkish bread making. Flours with a gluten index of less than 40 are not suitable for baking.



$$luten\ index\colon \frac{(Total\ gluten - Gluten\ which\ is\ passed\ from\ the\ sieve)}{total\ gluten} \times 100$$

Figure 4: Gluten index device

4.) Determination of sedimentation value

Sedimentation value is a practical method that gives information about gluten content and quality of flours. It also serves to estimate protein amounts of flours with the same gluten quality. It is used to determine the bread quality of wheat flour. The sedimentation value varies according to the type of flour grinding method. Wheats should be ground in a standard way for sedimentation analysis. Bread quality of flour is detected by the volume of flour particles collapsed in a suspension which is prepared with flour and lactic acid.

Principle: It is based on increasing the volume of flour particles by swelling in weak acid solution according to gluten quality and measuring the amount of precipitation of these particles in a certain time. Flours with high gluten content and quality have high sedimentation value.

Materials:

- Pipettes: 25 ml and 50 ml
- Sedimentation cylinders: 100 ml
- Shaker
- Balance
- Isopropyl alcohol
- Distilled water
- Bromophenol blue solution
- Lactic acid stock solution: Take 250 ml of 85% lactic acid and add 1 liter of water. The solution was refluxed for 6 hours.
- Sedimentation test solution: 200 ml of isopropyl alcohol is added to 180 ml of lactic acid stock solution. After 48 hours, the solution is adjusted to 0.1 ± Na with 0.1 N NaOH or KOH solution.

Procedure:

- Weigh 3.2 g of flour and put it in a 100 ml graduated cylinder.
- 50 ml bromophenol blue is added.
- Close the lid and shake 12 times horizontally in 5 seconds to mix the flour and the solution thoroughly.

- Then the graduated cylinder is placed in the sedimentation device and shaken for 5 minutes.
- At the end of the period, 25 ml of lactic acid solution is added.
- The mouth is closed and put into the sedimentation device to shake again for 5 minutes.
- At the end of the procedure, graduated cylinder is taken from the device and placed on a flat surface for 5 minutes.
- At the end of the waiting period, the settling part is read in ml (value on the graduated cylinder). This value is the sedimentation value.

It is assumed that moisture content of flour is 14%. If it is different than 14%, the following correction should be made.

$$\text{orrected sedimentation value: } \frac{Sedimentation\ value \times (100-14)}{100 - moisture\ content\ of\ flour}$$

Table 2: Evaluation of flour according to sedimentation values

Sedimentation value	**Gluten Quality**
36 ml and above	Very good
25 ml- 36 ml	Good
15 ml- 24 ml	Moderate
15 ml ve below	Weak

5.) Delayed Sedimentation Analysis

Delayed sedimentation is an analysis to determine whether the flour is made from wheat which is damaged by the sunn pests.

Principle: It is based on the reduction of the amount of precipitate formed by flour particles in weak acid solution over a period of time as a result of hydrolysis of gluten

proteins. Gluten breaks down as a result of proteolytic enzyme activity in sunn pest-damaged flours and the delayed sedimentation value of these flours is lower than normal sedimentation value. As the difference between delayed sedimentation and normal sedimentation value increases, it is understood that sun damage is increased.

Procedure: Delayed sedimentation is almost identical to sedimentation analysis. Unlike normal sedimentation, the sedimentation tube with bromophenol blue was shaken in the apparatus for 5 minutes and immediately the sedimentation solution (18% lactic acid + 20% isopropyl alcohol) was added and the tube was placed on a flat surface for 2 hours. At the end of the time 25 ml of sedimentation solution are added and shaken in the apparatus for 5 minutes. After standing for 5 minutes on a flat surface, the amount of precipitation is read.

The result is compared with normal sedimentation value, the presence of sunn pest damage and it is degree is detected and the necessary measures are taken. The smaller the difference between normal sedimentation and delayed sedimentation value is the indication of presence of less sunn pest damage.

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EXPERIMENT 15: TBA ANALYSIS

Spontaneous autoxidation reactions occur between the fats in the food and the oxygen of the air. Such reactions are always undesirable for the food industry, causing more or less noticeable quality degradation. The decrease in quality is in the form of changes in color, odor and taste, degradation of some compounds and even formation of toxic compounds. It has been shown that oxidation products of meat give color reaction with TBA. Malondialdehyde is formed during the autoxidation of polyunsaturated fatty acids. The 2-thiobarbituric acid solution, prepared with glacial acetic acid, causes the formation of color with malondialdehydes and provides the determination of oxidative rancidity level.

Table 1. TBA values in meat and some meat products

Product	TBA value	Product	TBA value
Bologna Salami	0,226	Beef fat	0,491
Fresh sausage	0,211	Rabbit meat	0,254
Bacon	0,278	Ground beef	0,212
Salami	0,153	Roasted meat	2,109
Italian salami	0,351	Pastrami	0,070
Corned beef	0,273	Ground beef + nitrate	0,080
Pepperoni	0,343	Ground beef + red pepper	0359

Samples: Homogenized meat or meat product sample

Chemicals:

- **2-thiobarbituric acid (0.02 M):** 0.2883 g TBA is heated in a 90% glacial acetic acid over a boiling water bath to form a 100 mL solution.
- **Glacial acetic acid**
- **Hydrochloric acid – HCl (4 N)**

- **Sulfanilamide:** 1 g of sulfanilamide is dissolved in 40 mL of concentrated HCl and 160 mL of purified water.
- **1,1,3,3-Tetraethoxypropane (TEP) Standard:** 10^{-8} M TEP is dissolved in pure water. This solution is stable for 1 week in the cold.

Materials:

- Balance
- Distillation apparatus
- Spectrophotometer
- Glass beads
- Volumetric flask
- Antifoaming agent
- Capped tubes

PROCEDURE:

A. Uncured Meats; 10 g of sample is homogenized with 50 mL of purified water for 2 min. Transfer to the distillation flask and add 47.5 mL of distilled water. 2.5 mL of 4 N HCl is added to make the pH of the medium around 1.5 and the total volume is made to 100 mL. Paraffin as an antifoaming agent and boiling stones to facilitate boiling are added to the distillation apparatus and connected to the distillation apparatus. Distillation is continued until about 50 mL of the distillate is collected.

Take 5 mL of distillate into the capped tubes and add 5 mL of TBA reagent. For blank, 5 mL of purified water and 5 mL of TBA reagent are added. After the tubes are mixed well, they are put into boiling water bath and left for 35 minutes. It is then cooled within 10 min and the absorbance read at 538 nm.

B. Cured Meats; 10 g of the sample is blended with 49 mL of purified water and 1 mL of sulfanilamide reagent. Wash with 48 mL of distilled water and transfer to a distillation flask. Add 2 mL of HCl solution. Other processes are the same as for uncured meats.

Obtaining Standard Curve; For calibration, a solution containing 10^{-8} M TEP in 1 mL of water is used. The solution should be stored at 4 ° C. It is warmed to room temperature just before use. Transfer 1,2,3,4,and 5 mL of 10-8 M TEP solution into flasks. Pure water is added until approximately 10 mL of space remains. Pure water is added instead of TEP solution in blank.

5 mL of TBA reagent is added to the volumetric flasks and filled to the line with distilled water. It is kept in a boiling water bath for 35 minutes, and in a cold water bath for 10 minutes and absorbance read at 538 nm. In 10^{-8} M moles of malonaldehyde are found.

There are many factors affecting the TBA value in meat and meat products. The presence of carbohydrates, nitrite, ascorbic acid, etc. Therefore, it is useful to know the average TBA table which contains important factors in the interpretation of the results.

Calculation; If no standard calibration graph is obtained, the TBA value (mg malondialdehyde / kg sample) is calculated as follows;

TBA value (mg malondialdehyde / kg sample) = 7.8 * A

A = absorbance at 538 nm.

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EXPERIMENT 16: DPPH FREE RADICAL SCAVENGING ACTIVITY ANALYSIS BY SPECTROPHOTOMETRIC METHOD

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron-transferthat produces a violet solution in methanol. This free radical, stable at room temperature (purple colour), is reduced in the presence of an antioxidant molecule, giving rise to colorless methanol solution. The use of the DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry so it can be useful to assess various products (Garcia et al., 2012).

Aim: The antioxidant activity of the plant tor fruit extracts and the standard is evaluated on the basis of the radical scavenging effect by using a spectrophotometric procedure.

Principle: The ability of the extracts to annihilate the DPPH radical (1,1-diphenil-2-picrylhydrazyl) was investigated.

The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as:

(DPPH) + (H-A) ⟶ DPPH-H + (A)

(Purple) (Yellow)

Antioxidants react with DPPH, which is a stable free radical and is reduced to the DPPHH

and as consequence there is decreased from the DPPH radical to the DPPH-H form. The

degree of discoloration indicates the scavenging potential of the antioxidant compounds or

extracts in terms of hydrogen donating ability (Killedar, More, Shah, & Gaikwad, 2013).

Chemicals:

- DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) Reagent
- Methanol (Analytical Grade)

Experimental Procedure:

0.1mM of DPPH was prepared in methanol and 4.9 mL of DPPH (0.1mM) solution was mixed with 0.1 mL of sample solution (fruit or plant extract) and standard solution in test tubes separately in triplicates. These solution mixtures shanked vigorously, then were allowed to stand at dark for 30 min and optical density was measured at 517 nm using UV-VIS Spectrophotometer. Methanol (0.1 mL) with DPPH solution (0.1mM, 4.9 mL) was used as blank.

DPPH Reagent: Methanolic solution of DPPH (0.1 mM): 39.4 mg of DPPH was dissolved

in one liter of analytical grade methanol.

Results:

Antiradical Activity:

$$DPPH\ Scavenged\ (\%) = \frac{Acontrol - Atest}{Acontrol} x100$$

$Acontrol$=Absorbance of the blank sample

$Atest$= Absorbance of the test sample (eg: fruit or plant extracts)

$DPPH\ Scavenged\ (\%)$= % DPPH radical scavenged

EXPERIMENT 18: Nitrite and Nitrate Determination in Meat Products

Introduction

Sodium nitrate, $NaNO_3$, and sodium nitrite, $NaNO_2$, are commonly added to meat products which are kept for an extended period in a cold, but not frozen state (e.g. bologna, hot dogs, salami, ham, sausage, etc). These additives have two purposes. One is "cosmetic", they give the meat a pinkish-reddish color and prevent it from turning brown. The other is that they prevent the growth of clostridium botulinum, the microorganism that produces the deadly botulism toxin. The addition of nitrates and nitrites to foodstuffs are a mixed blessing. It has been found that feeding sizeable amounts of sodium nitrite to animals results in formation of compounds called nitrosamines which are suspected of being carcinogenic (i.e. cancer causing). Additionally, large amounts of nitrate or nitrite in the diet may induce the disorder known as methemoglobinemia, which can be fatal. In methemoglobinemia the ferrous ion, Fe^{2+} , in hemoglobin (red) is oxidized by nitrite to the ferric ion, Fe^{3+}, converting the hemoglobin to methemoglobin (brown) which does not transport oxygen as efficiently.

The reaction of nitrite with protein to produce a red/pink color is interesting. Muscle (meat) contains a red pigment, myoglobin, that is similar in many respects to hemoglobin in that it contains Fe 2+ , and transports oxygen. Myoglobin and oxygen combine to form oxymyoglobin which reacts with nitrite in the presence of a reducing agent such as Vitamin C to yield nitrosomyoglobin, a stable red pigment. In the absence of this protection the Fe^{2+} in red myoglobin is converted to the brown methemoglobin. Nitrate effectively produces the same red color because it is reduced to nitrite in the tissues. In order to produce the red color in meat, 10-20 times more nitrite is needed than is required for preservative effects. Thus, the quantity of these compounds in meats can be drastically reduced with only the loss of the "cosmetic" effects. Salami, hot dogs, ham, bologna, etc. might be slightly brown, but they would be safer. The potential danger of nitrates and nitrites has lead to the development of a number of analytical methods that measure their concentrations in foodstuffs. In this experiment a colorimetric method will be used to determine the amount of nitrite in processed meat. Since sodium nitrite is soluble in water it is easy to extract it from meat with hot water. After extraction, the nitrite is reacted with two reagents, sulfanilamide (sulfa) and naphthylethylenediamine (NEDA). These compounds react with nitrite to produce purple colored dye. The amount of dye formed is dependent on the concentration of nitrite ion present. The more intense the color of the solution the greater the quantity of nitrite present.

$$\underset{\text{NEDA}}{C_{12}H_{14}N_2} + \underset{\text{sulfa}}{C_6H_8O_2N_2S} + \underset{\text{nitrite}}{NO_2^-} + \underset{\text{acid}}{H^+} \longrightarrow \underset{\text{purple color}}{C_{18}H_{19}O_2N_5S} + 2\,H_2O$$

Reagents

For seperation and precipitation;

- Carrez I (10.6 g Potassium hexacyanoferrate(II) trihydrate in 100 L water)
- Carrez II (21.9g zinc acetate dihydrate in 3g glacial acetic acid and 100ml with water
- Saturated borax solution

For reaction;

Reagent I (100 mg sulfanilamide in 10 ml HCL and 90 ml water)

Reagent II (25 mg N-(1-Naphthyl)ethylenediamine dihydrochloride in 25 ml water)

Reagent III (a mixture of 44.5 ml HCL and 55.5 water)

For Standard

10 -5-2.5- 1 µg/ml nitrite solutions

Experimental

- Accurately weigh about 10 g of well homogenized sample into a 100 ml beaker, add about 5 ml of saturated borax solution and 50 ml of boiling redist. water, mix and boil on a water-bath for about 15 min.
- Allow to cool to +15 to +25°C and successively add 2 ml each of concentrated Carrez I solution and concentrated Carrez II solution (mixing well after each addition)
- Transfer the contents of the beaker quantitatively to a 200 ml volumetric flask, make up to the mark with redist. water, mix, filter and if necessary centrifuge.
- Pipet 2.00 mL of the filtrate (liquid portion) into a clean 100 ml beaker and then 60 ml water is added.
- 10 ml of reagent I and 6 ml of reagent III are added, respectively, and kept in a dark place for 5 minutes.
- Pipet 2 mL reagent II into the beaker . mix and store in a dark place for 3-10 min. It is filled to the 100-mL mark with water
- Absorbance of samples and standards measured at 538 nm

Calculation

The amount of nitrite in the sample (mg nitrite / kg sample) is calculated using the formula below.

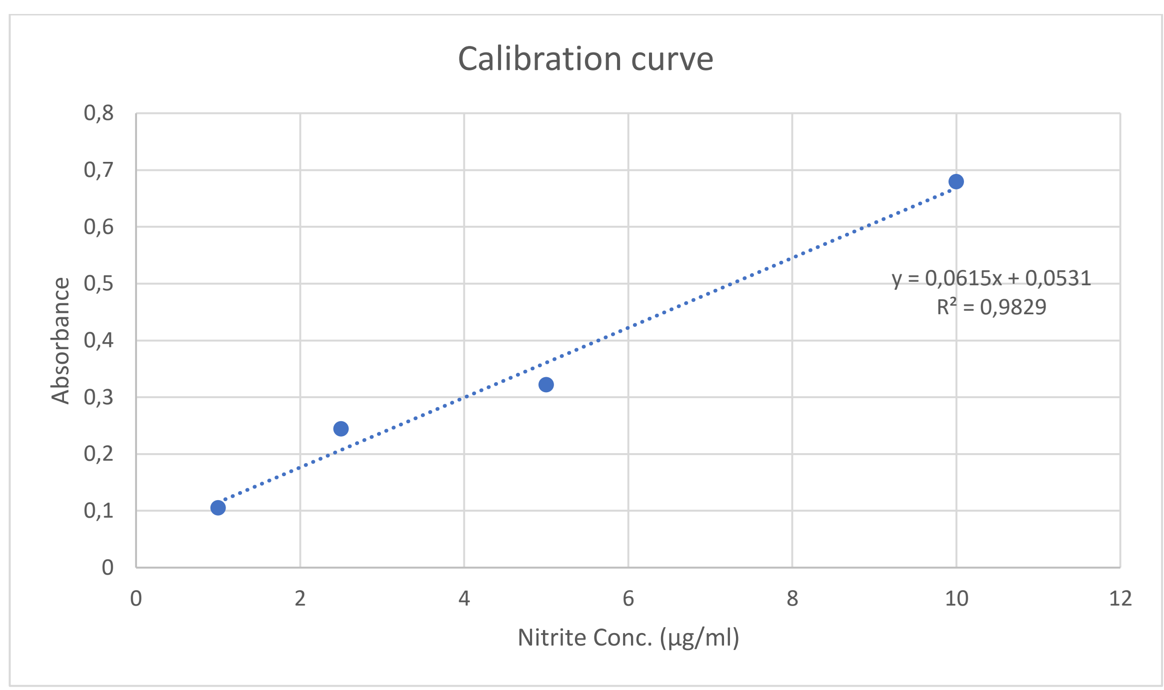
N=[(Cx20000)/(mxV)]

N: Amount of Nitrite in the sample (mg/kg)

m: Amount of sample to be tested (g)

V: sample filtrate volume taken for spectrophotometric measurement (ml)

C: The amount of nitrite corresponding to the absorbance of the sample from the curve prepared with nitrite standards (µg/ml)



EXPERIMENT 19: Oxidation Stability Test On Fish Oil

Introduction:

The auto-oxidation of the lipids contained in foods and animal eeds is recognized as on ofe the most important factors conditioning negatively the shelf-life.

The oxidation test reactor allows the sample to be submitted to highly oxidative conditions (high temperature and pure oxygen over-pressure) in order to know, whitin a short time , the product stability against the oxidation of the limits (rancidity).

During the sample oxidation, the instrument records the oxygen pressure drop inside the oxidation chambers due to the chemical reactions. Oxitest is provided with two seperated oxidation chambers, in order to perform the oxidation test in dublicate on the same food (solid, liquid or doughy), to conpare the oxidative stability of different formulations of the same food or to test at the same time different samples, using the same or different operative conditions.

Experimental:

Mix accurately the packing content, avoiding as much as possible the contact between sample and atmospheric oxygen. Distribute homogeneously on 1 sample holder 3-5 g of oil. Introduce 2 spacers inside the oxidation chamber and place the sample holder on top.

When the sample has been introduced inside the oxidation chambers, close firmly the 2 covers using the screws, after placing the o-rings and filters inside their sites. Leave the discharge valves in open position.

For oxidative stability : 110 ºC at 6 bar

For shelf life : 100 ºC -90 ºC- 80 ºC-70 ºC at 6 bar

Test results

File name	Set point T (ºC)	Set point P (bar)	IP (h:m)	InIP
Test at 70 ºC	70	6	18:27	2,91
Test at 80 ºC	80	6	6:07	?
Test at 900 ºC	90	6	2:32	?
Test at 100 ºC	100	6	0:50	?
Test at 4 ºC	4	6	?	?
Test at 25 ºC	25	6	?	?

EXPERIMENT 20: DETERMINATION OF COLOR, SPECIFIC VOLUME AND TEXTURE PROPERTIES OF BREAD

1. DETERMINATION OF COLOR

Color, flavor and texture appear as three important features in terms of acceptability of a food However, the first judgment about food quality is usually based on the color of the product. In this sense, manufacturers must carefully handle the color characteristics of the product and the changes in color during the process. In this context, food processing, storage and so on. factors are used in the analysis of quality changes, the determination of the compliance of food quality with standards, quality control of raw and processed foods are used as color measurements.

Color is a perception that occurs when different wavelengths of light reach the retina of the eye. This perception differs due to the impact and partial reflection of light on substances, which are called color tone or color.

If all wavelengths reach the eye suddenly, it is perceived as white and if no light is reached, it is perceived as black. The human eye can see wavelengths between 380 nm and 780 nm, so this part of the electromagnetic spectrum is called visible light. Color; a feature of the visual perception of light reflected or emitted by objects.

Color measurement systems have been developed in order to recognize and compare colors since people have very low memory and cannot express colors in numerical values.

Color measurement in food industry;

- Munsell system
- Lovibond tindometer
- Hunter colorimeter, spectrophotometer
- Pigment analysis
- With color measurement systems such as Color Comparison Solutions, Colored Glass Filters, Standard Colored Plastics, Color Scales based on comparison principle,
- CIE (International Lighting Commission) system.

1.1. Hunter Colorimeter; hunter colorimeter is photoelectric colorimeter that is cheaper, simpler and faster than spectrophotometric system.

The Hunter colorimeter has three color values;

* a value of red or greens,

* b value yellow or blue,

* The L value measures the degree of brightness between 0 (black) and 100 (white).

The spectrophotometer is instrument which measures color, concentration, chemical composition and molecular structure of that substance by measuring the wavelength of light reflected from a substance.

1.2. Measurement color intensity of bread

Color analysıs in bread is made with 2 steps as shell and inner layer

- ➢ The color of the bread crust should not be too light or dark.
- ➢ Each side should be same brightness and fried.
- ➢ Bread crust should be thin, bright, dark in color, easy to break.
- ➢ The shell should not be thick and hard.
- ➢ There must be no puffiness and cracks on the shell.

1.3. Process steps of determinatıon of color in bread

- ✓ After the color device is turned on, it is first calibrated.
- ✓ After slicing the bread, the color device is placed in upright position on the bread slice.
- ✓ The button on the device is pressed and the color values are determined by averaging of the three readings.

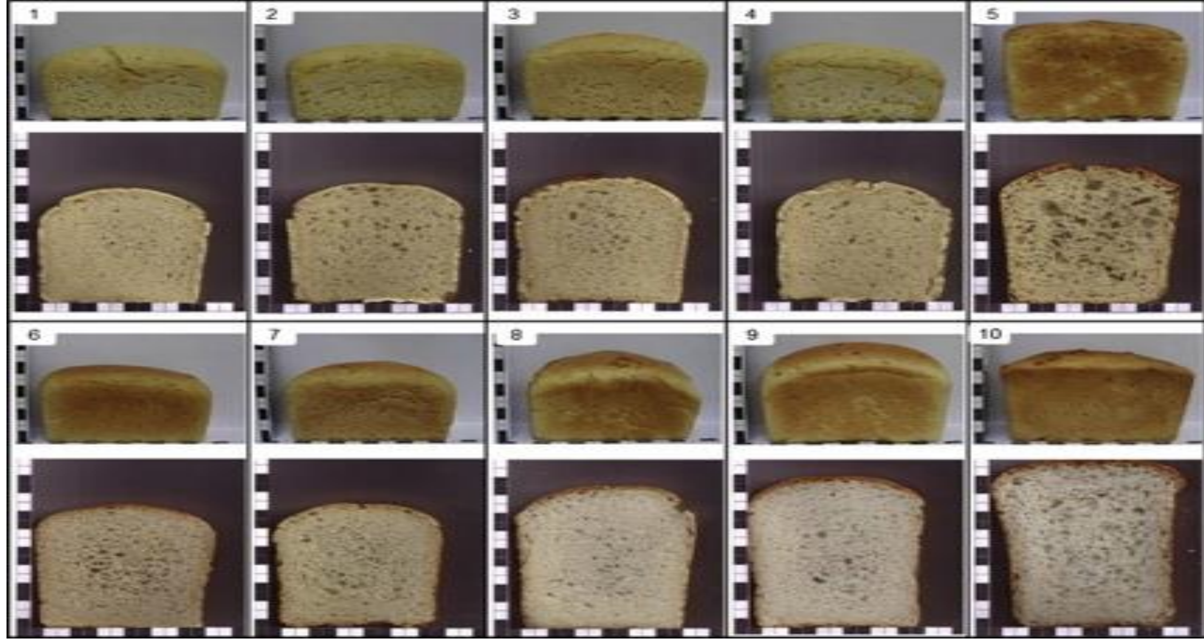


Figure 1. Color measurement surfaces in bread

2. MEASUREMENT OF BREAD VOLUME WITH NEUMAN TOOL

The principle of analysis is to find the specific volume of the bread from the amount of seed carried by putting the bread into the seeds of certain volume. It cause to overflow seeds as much as the volume of bread placed in neuman appliance filled with rapeseed. The overflow volume is measured in a cylinder to determine the specific volume of bread.

2.1. Process steps of determinatıon of specific volume

- ✓ For filling the glass container with seeds, first place the seeds in the funnel
- ✓ The slider in the section is filled into the funnel even though it is covered.
- ✓ The seeds (rapeseed or millet seeds) are poured into the glass bowl.
- ✓ After that, the seeds of the glass bowl are taken with a straight ruler are rubbed
- ✓ Seeds up to the volume of the glass container by closing the slide on the funnel's pipe
  back to the funnel.
- ✓ Put the bread on the seeds and transfer the seeds from the funnel
- ✓ Spill ceases after the glass container is full and overflowed.
- ✓ Excess seeds are taken from the glass bowl with the ruler, if any, together with the hopper volume
- ✓ The volume of these seeds gives the volume of bread.
- ✓ Volume measurements are averaged by repeating 4 times.

Specific volume(ml / g)= volume of bread made from 100 g of flour ⁄ bread weight

The bread volume is graded according to the specific volume of bread.

3. TEXTURE PROFILE ANALYSIS OF BREAD

3.1. Purpose and Importance

Texture is any kind of mechanical, geometric and surface properties of a product which can be detected by tactile, visual and auditory receptors.
Texture is a feature that can be perceived by mouth and tongue after consumption of a product and is closely related to density, viscosity, surface tension and other physical properties.

Textural properties are generally expressed in terms such as hardness, adhesive and cohesive adhesion, glue, brittleness, chewability, viscosity, elasticity, elasticity.
Texture Profile Analysis (TPA) is the most commonly used objective method for determining and measuring textural properties of foods.
The TPA test is based on the principle that a foodstuff is compressed twice by means of a piston, similar to jaw movement.

What is Texture Analyzer?

It is a device that measures the sample responses of a sample to the effects such as stretchıng, pressıng, drıllıng, bendıng.

3.2. Process steps of TPA

- ✓ The probe proceeds from the initial position to the sample with the Pre-Test Speed.

- ✓ When the probe reaches Trigger Power, the pre-test speed changes to Test-Speed and data begins to be recorded

- ✓ The probe moves with the test-speed, for example, until the specified force, distance or deformation point is reached.
- ✓ The probe returns to the starting point with the Post-Test Speed.
- ✓ When the probe returns to the point where it reaches trigger power, Surface Adhesion, if any, is measured.
- ✓ The results of the textural properties are obtained by making use of the Force-Time graph drawn during this compression.

Textural properties of foods are examined in two groups as primary (hardness, cohesive stickiness, elasticity, adhesive stickiness) and secondary characteristics (brittleness, chewability, guminess).

Texture Profile Analysis Graph

According to TPA graphic example;

- ✓ Hardness; the maximum force obtained at the time of the first compression,
- ✓ Fragility; first peak obtained at the first compression,
- ✓ Cohesive stickiness; It is a measure of how well the product can withstand the second deformation and is calculated by the ratio of Area 2 to Area 1, according to the figure.
- ✓ Elasticity; It is a value of how good the product is to return after deforming during the first compression and is expressed as the ratio of length 2 to length 1.
- ✓ Guminess can only be applied to semi-solid foods and is calculated as hardness * cohesive stickiness.
- ✓ Chewability; It is a value that can be applied only for solid foods and calculated as gumminess * elasticity (length 1 / length 2).
- ✓ Adhesiveness; It is defined as the work required to overcome the gravitational force between the food and the probe surface (Area 3).
- ✓ Flexibility; is a value that indicates the resistance of the product to restore its former state (Area 5 / Area4)

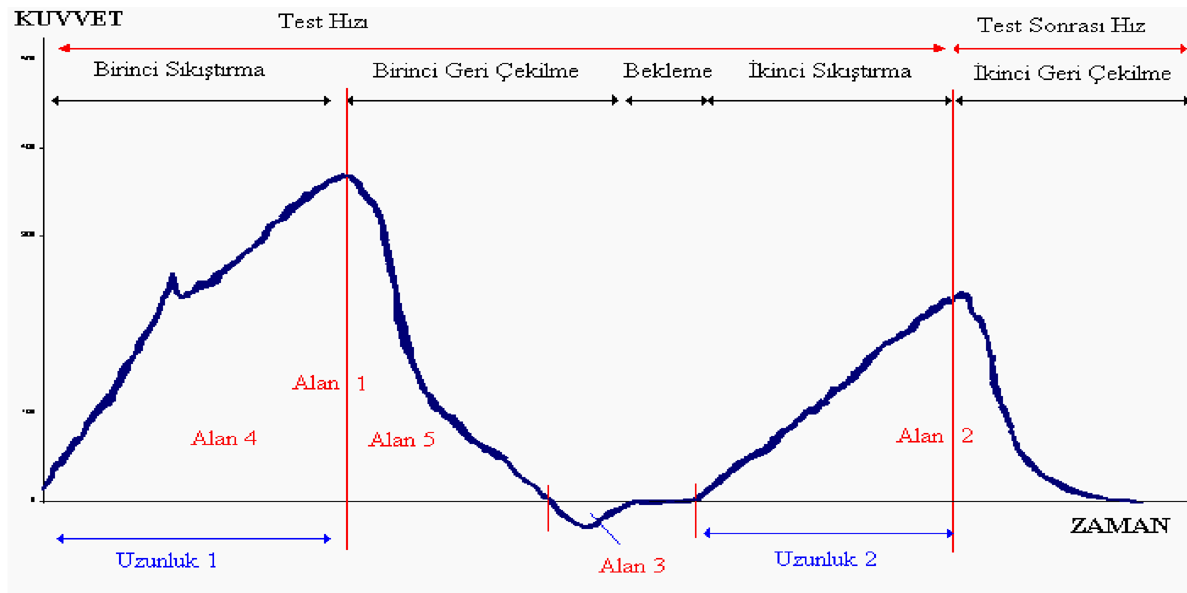


Figure 2.Texture profile analysis graphic

In texture profile analysis, it is important to obtain both compression plots under the same conditions and to ensure that the test speed and post-test speed are the same in order to fully compare the areas under the curve.

In bread and similar products, there is a lot of textural change, with a wide variety of sources. All baked products consist of an outer shell and an inner layer. The crust layer is darker in color and has low moisture content, causing the inner and outer parts of the product to exhibit different textural properties.

Texture, an indicator of freshness in bread, is an important feature in the acceptability of breads by consumers. The most commonly used method for determining the structural properties of bread is to measure the hardness of the bread, as well as sensory analysis.

Resources

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EXPERIMENT 21: DETERMINATION OF COLOR, SPECIFIC VOLUME AND TEXTURE PROPERTIES OF BREAD

1. INTRODUCTION

Rheology is a science that works on the deformation and fluidity of matter. The properties of a foodstuff such as shape and consistency are rheological properties and dough rheology is a science that examines dough properties.

- ✓ Water absorption,
- ✓ kneading resistance,
- ✓ elongation ability,
- ✓ resistance to elongation,
- ✓ development time,
- ✓ stability of the dough are rheological properties.

The rheological properties of the dough, which is an intermediate product in bread making, are important because they directly affect the quality of the bakery products and give information about the structure of the dough. Its composition and structure determine the rheological properties of the dough, an intermediate product in the conversion of flour into bread and other bakery products.

2. DETERMINATION OF FARINOGRAM PROPERTIES IN DOUGH

2.1. Purpose and Importance

Rheological properties of the dough are important in terms of processing the dough and affecting the final product quality obtained. Farinograph device is used to determine the amount of water required for flour to form dough and to indicate the resistance of dough to the kneading pallets graphically during kneading.

The water absorption % of flour is a parameter that depends on the first moisture content of the flour. It is expected that the development time and stability value is high; softening value is to be low in flour with high protein content and high quality.
The Farinograph measures the water absorption of the flour and the resistance of the dough prepared from this flour to kneading. Flour water removal, mainly the amount of gluten, mainly particle size and the amount of damaged starch is also effective. Resistance to kneading is related to gluten quality.

Figure 1. Farinograph device

2.2. Experimental Procedure

- ✓ The device is operated to ensure that the water bath and the circulating water are at 30 ° C.
- ✓ 300g of flour is weighed and put into kneading bowl.
- ✓ Burette is filled with water at 30 ° C.
- ✓ The tip of the printer is lowered onto the paper.
- ✓ When the end of the printer reaches the 0 line, water is started to be fed from the burette.
- ✓ After the dishes on the edge of the kneader are added to the base mass with a spatula, water is supplied from the burette until the curvature 500 is centered on the line of consensus.
- ✓ The amount of water consumed is determined. Continue to draw from 12 minutes after the peak of the curvature.

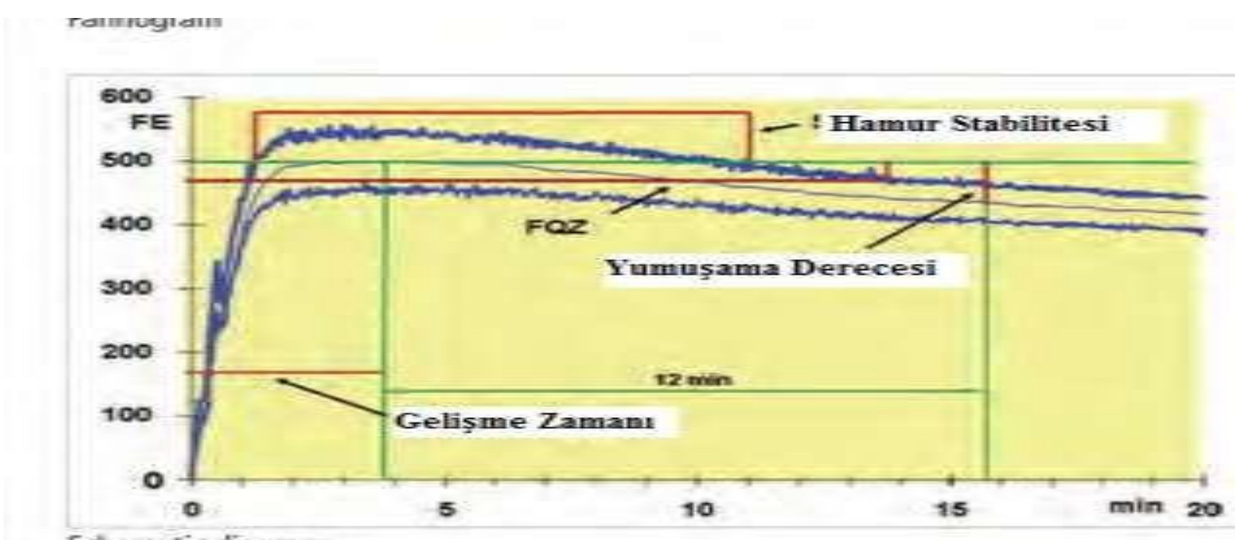

Figure 2. Farinograph chart

2.3. Evaluation of Farinogram

Duration of Development (D.T): It is the period from the beginning of the course to the point where the center averages the maximum height of the course. The unit is minute. It should be 1.6 minutes in bread flour.

Stability (S): The resistance of the dough to the pallets remains unchanged for a while depending on the quality of the flour during kneading. So the curve is drawn over the 500 line for a while. The time between the point at which the curve reaches the 500 line and the point at which it leaves the 500 consensus line is the stability value. The unit is minute. Stability value is minimum 6.5 in bread flour.

Kneading Tolerance (KT): The distance at which the peak of the curvature falls at the end of 5 minutes, the unit is BU. Kneading tolerance is maximum 40 BU in bread flour.

Softening Degree (S.D): The distance between the center of the curve and the 500 consensus line after 12 minutes from the peak of the curvature. The unit is BU. Bread flour should be maximum 60 BU.

Volarimeter Value(V.V): It gives information about the quality of the dough by evaluating the curv with a special template. In flour with good bread quality, the volarimeter value is high.

The higher the development time, the higher the stability and the lower the degree of softening cause the greater the volarimeter value. There is no unit. Volarimeters should be at least 48 in bread flour. Usually the curve width, stability, kneading tolerance number and development time is high, the degree of softening flours with high technological value and bakery quality is high.

3.1. DETERMINATION OF EXTENSOGRAPH PROPERTIES IN DOUGH

Wheat flour forms a viscoelastic form capable of holding gas necessary for the production of bakery products when dough is made. Gluten is responsible protein for dough formation, has important effects on the rheological properties of dough in fermentation and mixing processes.

Estimation and measurement of rheological properties such as elasticity, viscosity, extensibility are important for the bread industry.

PURPOSE AND IMPORTANCE

The extensograph determines the elongation ability and resistance to elongation of the 150 g pieces cut from the dough prepared with flour, water and salt after being kept in the fermentation cabinet at 30 ° C for a certain period of time.

Fıgure 3. Extensograph device

3.2. EXPERIMENTAL PROCEDURE

Process steps of determination extensograph of properties in dough;

- ✓ Dough is prepared using flour, water and salt.
- ✓ The dough is shaped in a standard form in the extensograph's rollers and cylindrical shape transmitters.
- ✓ After waiting for a while in the fermentation cabinet, the instrument is extended in the special part of the appliance and the applied force is recorded.
- ✓ Immediately after the first extension process, the same test is applied to the second dough piece, forming, holding and extending.

3.3 EVALUATION OF EXTENSOGRAPH

Energy: It is the planimetric area of the curvature and evaluated in cm². In bread flour, this area should be higher than 80 cm². This high value indicates that the gas holding capacity and fermentation tolerance of the dough is high.

Dough resistance: It is the height of the resistance to the elongation read from the curvature 5 cm from the point where the curvature starts to rise, expressed by BU. The average of the parallels is evaluated.

Elongation: The average length of the curve from the point at which the elongation begins to the point at which the dough breaks.

Maximum resistance (peak height): The average of the values of the convergence corresponding to the peaks of both parallel curves, expressed as the consensus (BU)

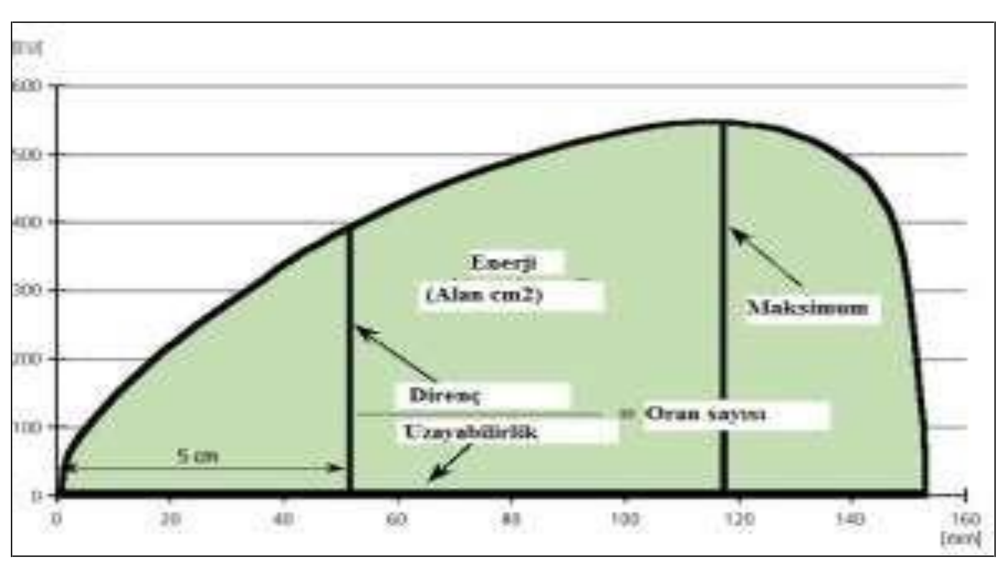

Figure 4. Evaluation of the extensograph graph

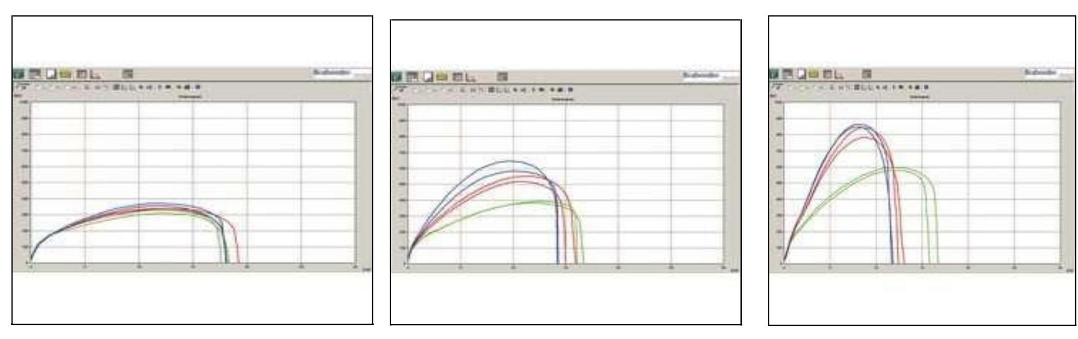

Figure 5. Soft flour **Figure 6. Normal flour** **Figure 7. Hard flour**

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