4. CHAPTER IV

4.1. ANALYSIS OF FLOUR

The routine analysis of flour may include the determination of moisture, ash, added chalk, SO_2, oil, protein, acidity, iron, thiamine and nicotinic acid, an examination for improves and bleaching agents and a microscopic examination.

Industrially, certain other types of analysis are of some importance, e.g., examination of the gluten, physical tests on the dough produced from the flour, determination of the particle size, maltose, color and grade figures.

4.1.1. Composition of Flour

The percentage of total product in any desired fraction of flour is called the extraction. In other words, a straight grade flour that is 72 % of the total product is a 72 % extraction flour. The composition of flour with various percent extraction is given in Table 4.1.

Table 4.1. Composition of flour (Pearson, 1976)

Component	Flour (72%)	Flour (80%)	Whole meal (95-100%)	Wheat Germ	Wheat Bran
Moisture (%)	13-15	13-14.5	13-14	9-12	14
Protein (%)	8-13	8-14	10-15	25-30	12-16
Fat (%)	0.9-1.4	1-1.6	1.5-2.5	8.5-11	3-4
Carbohydrate (%)	65-70	64-70	60-68	39-45	---
Fibre (%)	0.1-0.3	0.2-0.4	1.8-2.5	2-2.5	9-12
Ash (%)	0.3-0.5	0.6-0.9	1.2-2.0	4.0-4.5	4-6
Calcium (mg/100 g)	15	20	30	---	---
Iron (mg/100 g)	1.2	1.7	2.5	---	---
Vit. B_1 (mg/100 g)	0.1	0.25	0.4	---	---
Nicotinic acid (mg/100 g)	0.8	1.3	6	---	---

Table 4.2. Compositions of some cereals (Keskin, 1982)

	Rye	Barley	Oats	Corn
Water (%)	11	12.3	9.8	15
Protein (%)	10.2	10	12	10
Oil (%)	2.5	2.1	4.6	5
Carbohydrate (%)	72.3	68.3	58.6	67
Crude fibre (%)	2.1	4.6	11	2-3
Ash (%)	2.1	2.2	4	1-2

4.1.2. Standards of Flour (TS 4500)

Table 4.3. Turkish standard (TS 4500) values for flour

Type	% Ash (max.)*	% Raw cellulose (max.)*	% Dry gluten (max.)*	% Insoluble ash in 10 % HCl (max.)*	% Flour coarser than the sieve (No 70)	% Flour finer than the sieve (No 100)	% Acidity*
Type I	0.50	0.1	9.0	0.10	60.0	1.0	0.04
Type II	0.60	0.2	9.0	0.10	60.0	2.0	0.04
Type III	0.65	0.3	8.5	0.12	60.0	3.0	0.05
Type IV	0.88	---	8.5	0.15	60.0	3.0	0.06
Type V	1.20	---	8.5	0.20	---	5.0	0.08
Type VI	2.00	---	8.0	0.20	---	5.0	0.10

(--- not inspected)
* dry basis

4.1.3. Analysis

4.1.3.1. Determination of Ash in Wheat Flour

Procedure:

1. Weigh 5 g of flour into a well-dried platinum or porcelain evaporating dish.
2. Then add 1 ml of 50:50 ethanol-glycerol solution to the dish.
3. Heat the dish gently on a Bunsen burner and ash the dish at 550-570°C.
4. After two hour ashing transfer the dish to a desicator, cool, and weigh.
5. Calculate the total ash as a percentage of the sample.

4.1.3.2. Determination of Crude Gluten in Flour

The strength of flour depends to a considerable extent on the nature and amount of gluten present. Such properties can be assessed by forming a dough with water, washing out the starch and examining the elastic mass remaining. The crude gluten obtained is not pure protein, but contains starch, lipids and mineral, water.

Gluten is thought to be formed by a combination of glutenin and gliadin. Wheat is the only grain whose flour yields significant quantities of gluten, and damaged wheat kernels may yield little or non. For this reason bakers have used the gluten content as an index of quality of flour.

Procedure (Hand Washing Method):

1. Place 25 g of flour in a mortar, add about 25 ml water, work the mixture into a dough with a spatula and allow it to stand for 1 h.
2. Knead the dough gently under a water tap for 10-15 min So that soluble matter and starch are washed away.
3. Place the solid matter under water for 1 h (KI can be used for controlling the washing away of the starch).
4. Then compress it as dry as possible, roll it into a ball, weigh the moist gluten and express it as a percentage of the flour.

$$\%\ Wet\ gluten\ (in\ dry\ matter) = \frac{wt.\ of\ \ moist\ \ gluten\ (g)}{wt.\ of\ \ sample\ (g) * (100 - M)} * 100$$

where M is the moisture content of flour as %.

5. Also note its color, toughness and elasticity.
6. Then dry it at 100°C to constant weight, weigh it and express it as a percentage of the flour.

$$\%\ \ Dry\ \ gluten\ (in\ dry\ matter) = \frac{wt.\ of\ \ dry\ \ gluten\ (g)}{wt.\ of\ \ sample\ (g) * (100 - M)} * 100$$

where M is the moisture content of flour as %.

4.1.3.3. Acidity of Wheat Flour

a) Water Extract Method:

1. Shake 18 g of flour with 200 ml of CO_2-free water in a conical flask.
2. Place in a water bath at 40°C for 1 h with the flask loosely stoppered.
3. Filter and titrate 100 ml of clear filtrate with 0.05 M NaOH (Section 5.3) solution by using phenolphthalein (Section 5.1.1.) as an indicator.
4. The acidity of the water extract increases during storage and is calculated as lactic acid or KH_2PO_4

1 ml 0.05 M NaOH = 0.0068 g KH_2PO_4

$$\%\ Acidity\ (KH_2PO_4\ in\ dry\ matter) = \frac{1.36 * V\ (ml)}{wt.\ of\ \ sample\ (g)\ (100 - M)}$$

where M is the moisture content of flour as %.

1 ml 0.05 M NaOH = 0.0045 g lactic acid

$$\%\ Acidity\ (lactic\ acid\ in\ dry\ matter) = \frac{0.9 * V\ (ml)}{wt.\ of\ \ sample\ (g)\ (100 - M)}$$

where M is the moisture content of flour as %.

b) Alcohol Extract Method:

1. Extract 10 g of the flour with 100 ml of 96 % neutral alcohol by shaking vigorously for 5 min.
2. Then filtrate the mixture until 50 ml filtrate is collected.
3. Titrate the 50 ml of filtrate with 0.1 N NaOH (Section 5.3) until the solution is a faint pink color using 3 drops of phenolphthalein indicator.
4. Acidity (sulphuric acid) is generally expressed as ml of alkali required per 100 g flour.
5. Then use the following equation to express the acidity in terms of sulphuric acid.

$$\%\ Acidity\ (sulphuric\ acid\ in\ dry\ matter) = \frac{9.8 * V\ (ml) * N}{wt.\ of\ \ sample\ (g)\ (100 - M)}$$

where M is the moisture content of flour as %, V is the ml of NaOH used in the titration and N is the normality of NaOH.

4.1.3.4. Detection of Soy Flour in Wheat Flour (Not applicable to cooked cereal products)

Procedure:

1. Mix approximately. 0.5 g sample with 5 ml of 2 % urea solution in a test tube.
2. Partly immerse a strip of red litmus paper in the liquid.
3. Stopper the test tube and heat in a water bath held at 40°C for 1 h. If soy flour is present in more than traces, the litmus paper will turn to blue from evolution of NH_3 liberated from the urea by urease present in soy flour, urease has not been found in wheat flour.

4.1.3.5. Moisture Content of Wheat Flour

Procedure:

1. Weigh out accurately 5 g of sample into a pre-dried nickel or steel dish spreading the sample as thinly as possible over the base of the dish.
2. Put the dish and contents in an oven maintained at 105°C, and dry for 2 h.
3. Remove, cool in a desicator and weigh.
4. Return the dish to the oven and redry for a further 30 min, remove, cool and weigh.
5. Continue drying until a constant weight has been reached.
6. Calculate the moisture content from the weight loss of the sample.

4.1.3.6. Protein Determination

Protein in flour is estimated by the Kjeldahl procedure. The protein is then calculated from the factor N * 5.7. Strong bread flours should contain 11-12 % of protein, but those suitable for cake making should be weak flours containing less than 10 % of protein. Protein in flour can also be estimated rapidly by using absorptiometric methods.

Procedure (Determination of Nitrogen by Kjeldahl Method):

1. The sample of flour is first prepared as described in the sampling section (2.2.).
2. Before the determination can be carried out it is necessary to obtain a fat free sample by carrying out a soxhlet extraction on about 10 g of sample.
3. Obtain two clean and dry Kjeldahl digestion flasks and stoppers. Label the digestion flasks with sample number.

4. Accurately weigh about 2 g sample and transfer to about a 500 ml Pyrex Kjeldahl flask.
5. Add catalyst and 25 ml concentrated sulphuric acid (Section 5.4).
6. Place a glass bulb stopper in the neck of the flask. Then with the flask in a slightly inclined position.
7. Heat the contents gently over a low Bunsen flame in the fume cupboard using the racks provided.
8. Carry out the experiment in duplicate.
9. Decomposition of the sample is complete when a clear liquid is obtained. Continue to heat the flask strongly for 1 hour after the liquid has been become clear.
10. Allow the digest to cool, then wash it into an 800 ml distilling flask (use a funnel).
11. Add distilled water until volume in the flask is approximately 400 ml.
12. Then add 10 drops of phenolphthalein indicator (Section 5.1.1.) and about 1 g antibumping granules, (use a funnel to add these) and 1 ml of antifoam.
    Connect up the distillation apparatus (Figure 4.1) , greasing the joints well, with the condenser delivery tube dipping into 100 ml of 4 % boric acid solution (Section 5.11.) plus a few drops of screened methylene red indicator (Section 5.1.6.).
13. Note that the outlet of the delivery tube must be completely submerged in the boric acid.
14. Add 50 % sodium hydroxide (Section 5.3.) via the dropping funnel (approximately 80 ml will be required), until the test solution becomes alkaline.
15. Replace the plug in the funnel and seal with a few ml of water.
16. Rotate the scaffolding gently to ensure mixing.
17. Heat the distillation flask at constant rate until a minimum of 250 ml of distillate has been collected.
18. Wash down the delivery tube into boric acid solution, remembering to remove the Bunsen burner after lifting the tube out of boric acid solution to prevent suck back.

Figure 4.1. Kjeldahl Distillation Apparatus

19. Remove the conical flask.
20. Titrate the solution with ammonia contained in the boric acid solution with 0.5 N (0.25 M) sulphuric acid.
21. Calculate the results as % nitrogen in the sample.-

    1 ml of 0.25 M sulphuric acid = 0.0070 g nitrogen

So;

$$\%\ Nitrogen = \frac{Volume\ of\ acid\ used\ (ml) * 0.007}{wt.\ of\ \ sample\ (g)} * 100$$

Note: Correct sample weight used to original sample weight before defatting

4.1.3.7. Sieving

1. Take 100 g flour which is prepared as mentioned in the sampling

procedures (2.2.).

2. Place first 149 micron mesh size sieve and then place 210 micron mesh size sieve on the top of 149 micron mesh size sieve on an electrical siever.
3. Place the prepared flour sample into 210 micron mesh size sieve.
4. Screen diameter of the sieve must be 200-300 mm.
5. Sieve the flour for 5 minutes at a speed of 220 rpm (revolution per minute).
6. Weigh the flour coarser than the sieve No 70 (210 micron mesh size) and finer than the sieve No 100 (149 micron mesh size).
7. Take the average of the replicates.

4.1.3.8. pH of Wheat Flour

The pH of flour usually falls within the range of 6.0-6.8. Bleaching of flour with chlorine gas causes a fall in the pH value.

Procedure:

1. Shake 10 or 20 g of flour with 100 ml of water and allow to stand for at least 30 min.
2. Filtrate and determine pH of the filtrate with a pH-meter.

4.1.3.9. Determination of Crude Fiber in Wheat Flour

Reagents:

Bellucchi solution: 90 ml of diluted nitric acid is taken and made up to 1 liter with 80 % acetic acid.

Procedure:

1. Take 5 ±0.1 g sample of flour.
2. Place into 100 ml volumetric flask and add 50 ml Bellucchi solution.
3. Connect the condenser to the volumetric flask.
4. Heat the solution in the boiled water bath until the flour is dissolved.
5. Remove the volumetric flask from the water bath and place it onto Bunsen burner with a light flame.
6. Shake it frequently and waited for 25 min.

7. At the same time, dry a dish (platinum or porcelain) and ashless filter paper at 103-105 °C until a constant weight is reached.
8. Record the weight of dish as W_1 and filter paper as W_2.
9. Filtrate the hot solution by using the weighed ashless filter paper.
10. Wash the solid on the filter paper with 5 ml Bellucchi solution , then boiled water, after that wash it two times with 5 ml of ethanol and two times with 10 ml of petroleum ether.
11. Place the filter paper into weighed dish, and dry them at 103-105 °C until constant weight is reached.
12. Weigh and record the weight as W_3.
13. Ash another dish at 500-550 until constant weighed.
14. Place the dried ashless paper and crude fiber into weighed dish, and ash the dish at 500-550 °C for two hours.
15. Weigh the dish again record the weight as W_4.

Calculation:

$$\%\ CrudeFiber = \frac{(W_3 - (W_4 + W_2))}{W} * 100$$

where W is weight of sample.

% Crude fiber in dry material = (Crude fiber*100)/(100 – M)

where M is the moisture content of flour as %.

4.2. ANALYSIS OF BISCUITS

Biscuit can be made from hard dough which possess a gluten network (e.g. cream crackes), hard sweet dough which are similar, but contain sugar (e.g. semi-sweet biscuits) and soft dough which have no elasticity (e.g. shortbread). The dough pieces are usually baked in a travelling oven, which consists essentially of three sections, the humidity and temperature of each being carefully controlled. In the first section the dough structure is raised by either CO_2 (chemical or yeast fermentation) or by steam. The next section causes the setting of the dough structure either by coagulation of the protein network where present, or by the

early stages of drying out of the dough. The last section of the oven is used to dry out the formed biscuit to the required low moisture content, whilst caramelisation and Maillard-type reactions develop the crust color and flavor. The dough is rolled to thin sheet, cut into appropriate shapes and quickly baked at a high temperature.

The principal ingredients of the biscuit are flour, salt, fat, sugars and flavoring agents. The handling properties of the dough, as well as the eating qualities of the biscuit, depend on the relative proportions of these materials in the recipe. Water is an important ingredient at the dough stage, but most of it removed during the baking, the final product having a moisture content of 1-3 %.

A different type of biscuit products are wafers, which are made from a fluid batter of flour and water, together with small amounts of the other ingredients, such as sodium bicarbonate.

The energy value of the sweet biscuit may be twice as high as that of bread because of their low water content and the extra sugar and fat they contain.

4.2.1. Composition of Biscuits

The common basic ingredients of the biscuits are flour, fat, sugar and syrups, aerating chemicals and milk or water.

Table 4.4. Composition of various biscuits (Pearson, 1976)

Type	Moisture	Fat	Protein (Nx5.7)	Sugars	Ash	Salt
	%	%	%	%	%	%
Cream crackers	4.3	16.3	9.6	Trace	--	--
Digestive	4.5	20.5	9.6	16.4	--	--
Plain	5.2	13.2	7.4	15.8	--	--
Sweet	0.7	30.7	5.5	25.0	--	--
Wafer	4.5	12.5	10.7	2.3	--	--
Shortcake	2.2	23.5	5.1	20.0	1.0	0.4
Shortbread	2.6	26.0	4.0	20.0	1.0	0.5

4.2.2. Standards of Biscuit (TS 2383)

Physical properties of biscuit should be; creaky, appropriate flavor and color for its variety, no bad flavor and color, no foreign matter present in it, etc.

Table 4.5. General properties of biscuit

Component	Limits in weight
Moisture content;	
for biscuit	max. 6 %
for cream and marmalade added	no limit
Fat (extracted) as oleic acid,	max. 1.5 %
Ash in salt free dry material;	
for salty biscuit	max. 1.5 %
for sweet biscuit	max. 1.0 %
for enriched biscuit	max. 1.0 %
for biscuit (only)	max. 3.0 %
Ash, insoluble in HCl in dry material	max. 0.2 %
Sugar in sweet biscuit, as sucrose in dry material	min. 1.5 %
Salt for salty biscuit in dry material	max. 8 %

4.2.3. Analysis of Biscuits

Analysis of biscuit should include examination of water (dry at 100°C), oil, sugar, ash, salt and protein (nitrogen).

The following methods are especially adapted for the analysis of biscuit:

4.2.3.1. Moisture Content Determination

Moisture content determination is made as mentioned in the analysis of flour (Section 4.1.3.5.).

4.2.3.2. Determination of Ash

Ash content of biscuit is determined as described in the analysis of flour (Section 4.1.3.1.).

4.2.3.3. Protein Determination

Protein content of biscuit is determined by using the Kjeldahl method as described in the analysis of flour (Section 4.1.3.6.).

4.2.3.4. Determination of Oil in Biscuits

1. Weigh 2 g minced sample into a Majonnier extraction tube, add 2 ml alcohol and 10 ml dilute HCl (25 parts conc. HCl:11 parts water by volume) (Section 5.2.).

2. Maintain at 65 °C for 30 min.
3. Cool, add 10 ml alcohol and shake with 25 ml diethyl ether.
4. Then add 25 ml light petroleum ether, shake again.
5. Allow to separate, pour the upper layer into a weighed flask.
6. Repeat the extractions at least twice more.
7. Remove the solvent and dry at 100°C.
8. Cool and weigh flask.
9. Record the oil in biscuit as percentage.

4.2.3.5. Determination of Sugars

Reagents:
Carrez I solution: 150 g potassium ferrocyanide ($K_4Fe(CN)_6.3H_2O$) is dissolved and made up to 1 liter with water.
Carrez II solution: 300 g zinc acetate ($Zn(CH_3COO)_2.H_2O$) and dissolved and made up in water to 1 liter.

Procedure:
1. Blend 5 g minced sample mechanically with 150 ml water, transfer to a 250 ml volumetric flask with more water and add carrez I and carrez II clearing agents before making up to the mark.
2. Shake, filter and heat at 70 °C to invert 50 ml filtrate in a 100 ml volumetric flask.
3. Determine the sugars before and after the inversion as invert sugars and sucrose respectively by using the Lane and Eynon's method.

Lane and Eynon's Method:

The non-stochiometric volumetric method of Lane and Eynon is often the most convenient method for determining reducing sugars. The volume of sugar solution required to reduce completely 10 or 25 ml mixed Fehling's solution is determined using methylene blue as the redox indicator for assessing the end point. A minute excess of reducing sugar solution is necessary for the reduction of the indicator itself (Pearson, 1976).

Procedure:

Preparation of Sample:

1. Boil 20 g of sample gently with water and make up to 100 ml at room temperature.
2. Clarify and neutralise the solution with lead and calcium salts (these salts should be removed by potassium oxalate).
3. Filter.
4. Invert 10 ml of the filtrate with hydrochloric acid, make up to 200 ml.
5. Fill the burette with this solution.

Preliminary Titration

1. Pipette 10 or 25 ml of mixed Fehling's solution (Section 5.12.) into 300 ml conical flask.
2. Add 15 ml of the solution from the burette.
3. Boil the liquid on an asbestos-covered gauze.
4. Add further quantities of sugar solution (1 ml at a time) at 10 -15 second intervals to the boiling liquids until the blue color is nearly discharged.
5. Then add 3 - 5 drops of aqueous methylene blue solution (1%) (Section 5.1.2.) and continue titration the boiling liquid until the indicator is completely discolorized.

Accurate Titration

1. Repeat the titration, adding before heating, almost sugar solution required to effect reduction of copper.
2. Boil the liquid gently for two minutes, add 3 - 5 drops of methylene blue and complete the titration within a total boiling time of 3 minutes.
3. At the end point all blue color should be discharged and liquid should be orange-red color.
4. The proportion of reducing sugar, equivalent to 10 or 25 ml Fehling's solution is given in Tables 4.6 and 4.7.
5. From Tables 4.6 and 4.7 find total reducing sugar (total reducing sugar required) corresponding to titer.

Calculate as follows:

$$mg\ \ Reducing\ \ Sugar\ \ in\ 100\ ml = \frac{Total\ Reducing\ \ Sugar\ \ Required}{Titer\ (ml)} * 100$$

Determination of Sucrose in Solution and in Mixtures of Sugars

Sucrose can be determined in a solution containing no other sugar by inverting a portion containing about 0.5 g sucrose in a 100 ml volumetric flask with acid and neutralising. After titrating against Fehling's solution the amount of invert sugar produced can be obtained by reference to the Tables (Tables 4.6 and 4.7). Then:

$$\% \, Sucrose = \% \, Invert \, Sugar * 0.95$$

For a mixture containing both sucrose and reducing sugars, The Lane and Eynon titration is carried out before and after the inversion and the both results are calculated as the percentage of invert sugars in the original material. The sucrose figure is then obtained by multiplying the difference (as invert sugar) by 0.95.

Table 4.6. Total reducing sugar required for complete reduction of 10 ml Fehling's Solution to be used in conjunction with Lane-Eynon general volumetric method

Titer	Invert sugar, No sucrose	g Sucrose/100 ml Invert sugar				Glucose	Fructose	Maltose		Lactose	
		1	5	10	25			Anhydrous $C_{12}H_{22}O_{11}.H_2O$		Anhydrous	$C_{12}H_{22}O_{11}.H_2O$
Required for Reduction of 10 ml Fehling's Solution											
15	50.5	49.9	47.6	46.1	43.4	49.1	52.2	77.2	81.3	64.9	68.3
16	50.6	50.0	47.6	46.1	43.4	49.2	52.3	77.1	81.2	64.8	68.2
17	50.7	50.1	47.6	46.1	43.4	49.3	52.3	77.0	81.1	64.8	68.2
18	50.8	50.1	47.6	46.1	43.3	49.3	52.4	77.0	81.0	64.7	68.1
19	50.8	50.2	47.6	46.1	43.3	49.4	52.5	76.9	80.9	64.7	68.1
20	50.9	50.2	47.6	46.1	43.2	49.5	52.5	76.8	80.8	64.6	68.0
21	51.0	50.2	47.6	46.1	43.2	49.5	52.6	76.7	80.7	64.6	68.0
22	51.0	50.3	47.6	46.1	43.1	49.6	52.7	76.6	80.6	64.6	68.0
23	51.1	50.3	47.6	46.1	43.0	49.7	52.7	76.5	80.5	64.5	67.9
24	51.2	50.3	47.6	46.1	42.9	49.8	52.8	76.4	80.4	64.5	67.9
25	51.2	50.4	47.6	46.0	42.8	49.8	52.8	76.4	80.4	64.5	67.9
26	51.3	50.4	47.6	46.0	42.8	49.9	52.9	76.3	80.3	64.5	67.9
27	51.4	50.4	47.6	46.0	42.7	49.9	52.9	76.2	80.2	64.4	67.8
28	51.4	50.5	47.7	46.0	42.7	50.0	53.0	76.1	80.1	64.4	67.8
29	51.5	50.5	47.7	46.0	42.6	50.0	53.1	76.0	80.0	64.4	67.8
30	51.5	50.5	47.7	46.0	42.5	50.1	53.2	76.0	80.0	64.4	67.8
31	51.6	50.6	47.7	45.9	42.5	50.2	53.2	75.9	79.9	64.4	67.8

32	51.6	50.6	47.7	45.9	42.4	50.2	53.3	75.9	79.9	64.4	67.8
33	51.7	50.6	47.7	45.9	42.3	50.3	53.3	75.8	79.8	64.4	67.8
34	51.7	50.6	47.7	45.8	42.2	50.3	53.4	75.8	79.8	64.4	67.9
35	51.8	50.7	47.7	45.8	42.2	50.4	53.4	75.7	79.7	64.5	67.9
36	51.8	50.7	47.7	45.8	42.1	50.4	53.5	75.6	79.6	64.5	67.9
37	51.9	50.7	47.7	45.7	42.0	50.5	53.5	75.6	79.6	64.5	67.9
38	51.9	50.7	47.7	45.7	42.0	50.5	53.6	75.5	79.5	64.5	67.9
39	52.0	50.8	47.7	45.7	41.9	50.6	53.6	75.5	79.5	64.5	67.9
40	52.0	50.8	47.7	45.6	41.8	50.6	53.6	75.4	79.4	64.5	67.9
41	52.1	50.8	47.7	45.6	41.8	50.7	53.7	75.4	79.4	64.6	68.0
42	52.1	50.8	47.7	45.6	41.7	50.7	53.7	75.3	79.3	64.6	68.0
43	52.2	50.8	47.7	45.5	41.6	50.8	53.8	75.3	79.3	64.6	68.0
44	52.2	50.9	47.7	45.5	41.5	50.8	53.8	75.2	79.2	64.6	68.0
45	52.3	50.9	47.7	45.4	41.4	50.9	53.9	75.2	79.2	64.7	68.1
46	52.3	50.9	47.7	45.4	41.4	50.9	53.9	75.1	79.1	64.7	68.1
47	52.4	50.9	47.7	45.3	41.3	51.0	53.9	75.1	79.1	64.8	68.2
48	52.4	50.9	47.7	45.3	41.2	51.0	54.0	75.1	79.1	64.8	68.2
49	52.5	51.0	47.7	45.2	41.1	51.0	54.0	75.0	79.0	64.8	68.2
50	52.5	51.0	47.7	45.2	41.0	51.1	54.0	75.0	79.0	64.9	68.3

Table 4.7. Total reducing sugar required for complete reduction of 25 ml Fehling's Solution to be used in conjunction with Lane-Eynon general volumetric method

Titer	Invert sugar, No sucrose	1 g Sucrose/100 ml Invert sugar	Glucose	Fructose	Maltose		Lactose	
					Anhydrous	$C_{12}H_{22}O_{11}.H_2O$	Anhydrous	$C_{12}H_{22}O_{11}.H_2O$
Required for Reduction of 25 ml Fehling's Solution								
15	123.6	122.6	120.2	127.4	197.8	208.2	163.9	172.5
16	123.6	122.7	120.2	127.4	197.4	207.8	163.5	172.1
17	123.6	122.7	120.2	127.5	197.0	207.4	163.1	171.7
18	123.7	122.7	120.2	127.5	196.7	207.1	162.8	171.4
19	123.7	122.8	120.3	127.6	196.5	206.8	162.5	171.1
20	123.8	122.8	120.3	127.6	196.2	206.5	162.3	170.9
21	123.8	122.8	120.3	127.7	195.8	206.1	162.0	170.6
22	123.9	122.9	120.4	127.7	195.5	205.8	161.8	170.4
23	123.9	122.9	120.4	127.8	195.1	205.4	161.6	170.2
24	124.0	122.9	120.5	127.8	194.8	205.1	161.5	170.0
25	124.0	123.0	120.5	127.9	194.5	204.8	161.4	169.9
26	124.1	123.0	120.6	127.9	194.2	204.4	161.2	169.7
27	124.1	123.0	120.6	128.0	193.9	204.1	161.0	169.5
28	124.2	123.1	120.7	128.0	193.6	203.8	160.8	169.3
29	124.2	123.1	120.7	128.1	193.3	203.5	160.7	169.2
30	124.3	123.1	120.8	128.1	193.0	203.2	160.6	169.0
31	124.3	123.2	120.8	128.1	192.8	202.9	160.5	168.9

32	124.4	123.2	120.8	128.2	192.5	202.6	160.4	168.8
33	124.4	123.2	120.9	128.2	192.2	202.3	160.2	168.6
34	124.5	123.3	120.9	128.3	191.9	202.0	160.1	168.5
35	124.5	123.3	121.0	128.3	191.7	201.8	160.0	168.4
36	124.6	123.3	121.0	128.4	191.4	201.5	159.8	168.2
37	124.6	123.4	121.1	128.4	191.2	201.2	159.7	168.1
38	124.7	123.4	121.2	128.5	191.0	201.0	159.6	168.0
39	124.7	123.4	121.2	128.5	190.8	200.8	159.5	167.9
40	124.8	123.4	121.2	128.6	190.5	200.5	159.4	167.8
41	124.8	123.5	121.3	128.6	190.3	200.3	159.3	167.7
42	124.9	123.5	121.4	128.6	190.1	200.1	159.2	167.6
43	124.9	123.5	121.4	128.7	189.8	199.8	159.2	167.6
44	125.0	123.6	121.5	128.7	189.6	199.6	159.1	167.5
45	125.0	123.6	121.5	128.8	189.4	199.4	159.0	167.4
46	125.1	123.6	121.6	128.8	189.2	199.2	159.0	167.4
47	125.1	123.7	121.6	128.9	189.0	199.0	158.9	167.3
48	125.2	123.7	121.7	128.9	188.9	198.9	158.8	167.2
49	125.2	123.7	121.7	129.0	188.8	198.7	158.8	167.2
50	125.3	123.8	121.8	129.0	188.7	198.6	158.7	167.1

4.3. ANALYSIS OF MACARONI

Pasta is wheat-based products that are formed from a dough but are not leavened. The processes by which they are formed are quite different, as are the types of flours used. The formulation is generally very simple, often only flour and water for pasta.

Pasta, paste or alimentary pastes are terms that describe a large number of products. The most common types are macaroni (US standards state that it must be tube-shaped, hollow, and more than 0.11 in. but not more than 0.27 in. in diameter) and spaghetti (cord-shaped, not tubular, and more than 0.06 in. but less than 0.11 in. in diameter, according to US standards).

It is generally believed that the ideal raw material for pasta is durum semolina. The uncooked pasta should be mechanically strong so that it will retain its size and shape during packaging and shipment. It should also be uniformly yellow. Consumer acceptance has been strongly linked to a uniform, translucent, yellow color. When cooked in boiling water, the product should maintain its shape with no splitting or falling apart. Also, after it is cooked, the pasta should give a

firm bite (the so-called “al dente”) and the surface should not be sticky. The cooking water should be free of starch. Finally, the pasta should be resistant to overcooking.

The durum wheat differ from the common wheat. They are tetraploids and the common wheat are hexaploids. Durums are mostly spring wheat, although winter durums are known. They are usually amber in color; actually, they are white wheat with a translucent endosperm that gives them an amber color. The durum wheat are high in carotenoid pigments; these are what give the pasta its yellow color. Because of the relationship between the yellow color and consumer acceptance, the amount of pigmentation has been used as a selection tool for good-quality durum.

The wheat can be milled to give good yields of semolina, which is purified middling from the durum wheat. Durum wheat is so hard it is difficult to reduce to a flour fines. When it is reduced to flour, the percentage of damaged starch is several times higher than that found with common wheat. Durum flour generally gives excellent products except that they are not as resistant to overcooking as are products made with semolina (Hoseney, 1986).

4.3.1. Standards of Spaghetti and Macaroni (TS 1620)

Physically, macaroni should be free from foreign matters and appropriate for its flavor and color.

Table 4.8. Chemical properties of macaroni

Component	Limits
Protein in dry material	10.5 -13.5 %
Ash in dry material	max. 1.0 %
Ash insoluble in 10 % HCl	max. 0.15 %
Moisture	max. 13 %
Acidity, in 93 % ethanol	max. 0.05 %

All the macaroni should be cooked in distilled water for max. 20 min., and dissolved portion in water should not be exceed 10 % (dry basis).

4.3.2. Analysis

4.3.2.1. Determination of Moisture Content

Vacuum oven procedure carried out in this analysis method.

1. Accurately weigh 2 g well mixed sample in covered dish previously dried at 98 –100 °C, cooled and weighed.
2. Loosen cover (do not remove) and heat at 98 –100 °C to constant weight in partial vacuum having pressure equivalent to ≤ 25 mm Hg (3.3 kPa).
3. Immediately tighten cover on dish, transfer to desicator, and weigh.
4. Report flour residue as total solids and loss in weight as moisture (indirect method).

4.3.2.2. Determination of Ash Content

Ash content can be determined as in section (4.1.3.1.).

4.3.2.3. Determination of Protein

Determination of protein carried out as in section (4.1.3.6.) using 1 g prepared sample. % protein = % N * 5.7 for wheat, % N * 6.25 for all other grains.

4.3.2.4. Determination of pH

1. Weigh 10.0 g sample into clean, dry erlenmeyer and add 100 ml recently boiled water at 25 °C.
2. Shake until particles are evenly suspended and mixture is free of lumps.
3. Digest 30 min. more, decant supernate into the H-ion vessel.
4. Determine pH using electrode.
5. Standardise pH meter by buffer solutions of pH 4.01 and 9.18 both at 25 °C before the analysis.

4.3.2.5. Determination of Crude Fiber

1. Extract 2 g of ground material with ether or petroleum ether (initial boiling point temperature 35–38 °C; dry-flask end point, 52–60°).
2. If fat is < 1 % extraction may be omitted.

3. Transfer to 600 ml beaker, avoiding fiber contamination from paper or brush.
4. Add 1.5–2 g dry weight prepared ceramic fiber, 200 ml boiling 1.25 % H_2SO_4 (Section 5.4.), and 1 drop antifoam (excess antifoam give high result; use only if necessary to control foaming.).
5. Bumping chips or granular may also be added.
6. Place beaker on digestion apparatus with preadjusted hot plate and boil exactly 30 min., rotating beaker periodically to keep the solids from adhering to sides.
7. Remove beaker, and filter using buchner funnel.
8. Filter contents of beaker through the buchner (precoated with ceramic fiber if extremely fine materials are being analysed), rinse beaker with 50–75 ml boiling water, and wash through buchner.
9. Repeat with three 50 ml portions of water and suck dry.
10. Remove material and residue by snapping bottom of buchner against top while covering stem with thumb or forefinger and replace in beaker.
11. Add 200 ml boiling 1.25 % NaOH and boil exactly 30 min.
12. Remove beaker, and filter as above.
13. Wash with 25 ml boiling 1.25 % H_2SO_4, three 50 ml portions of water and 25 ml alcohol.
14. Remove residue; transfer to ashing dish.
15. Dry material and residue 2 hour at 130 ±2 °C.
16. Cool in desicator and weigh.
17. Ignite 30 min. at 600 ±15 °C, and cool in desicator and then weigh.

$$\%\ Crude\ Fiber\ in\ ground\ sample = C = \frac{(loss\ in\ weight\ on\ \ ignition\ (g) - loss\ in\ weight\ of\ \ ceramic\ fiber\ blank\ (g))}{weight\ \ of\ \ sample\ (g)} * 100$$

$$\%\ Crude\ Fiber\ on\ desired\ moisture\ basis = \frac{C * (100 - \%\ moisture\ \ desired)}{(100 - \%\ moisture\ \ in\ \ ground\ \ sample)}$$

4.4. ANALYSIS OF FATS AND OILS

The examination of oils for identity, purity, and freshness can involve a very extensive series of physical and chemical tests. These are density, color, refractive index, and slip point. The latter also describes qualitative tests for identification of various oil values (those for iodine value, saponification value, etc.). Some rancidity tests, and examination for metals, which may promote taints or other forms of deterioration.

4.4.1. Standards of Fats and Oils

4.4.1.1. Margarine -Vegetable Origin (TS 2812)

Table 4.9. Chemical properties of margarine-vegetable origin

Chemical Properties	Limits (max.)
Peroxide (meq./1000 g sample)	5
Metallic Contamination :	
Iron, Fe, (mg/kg)	1.5
Copper, Cu, (mg/kg)	0.1
Lead, Pb, (mg/kg)	0.1
Arsenic, (mg/kg)	0.1

Table 4.10. Physical properties of margarine-vegetable origin

Physical Properties	For Breakfast	For Cooking	For Industry Type I (as ingredients)	For IndustryType II (as ingredients)
Slip point (°C)	36	36	45	45

% Fat (w/w) min.	82	99	99	82
% Acidity (oleic acid) max. (w/w)	1.5	0.2	0.2	1.5
% Moisture content, max. (w/w)	16	---	---	16

For salty margarine allowable salt content (NaCl) is maximum 2.0 % (w/w), for margarine with no salt addition is 0.2 % (w/w) and for cooking and Industry Type I margarine is 1 % (w/w).

4.4.1.2. Standards for Oils (TS 341, TS 886, TS 887, TS 888)

Table 4.11. Turkish standards for oils

	Specific gravity	Refractive index	% Volatile matter (max.)	Saponification value	Iodine value	% Free fatty acid (max.)	Peroxide (max.)
Olive oil (TS 341)	0.912-0.918	1.468-1.470	0.2	184-196	78-88	0.3	20
Sunflowerseed oil (TS 886)	0.918-0.923	1.467-1.469	0.2	188-194	110-143	0.3	10
Cottonseed oil (TS 887)	0.918-0.926	1.458-1.466	0.2	189-198	99-119	0.3	10
Corn oil (TS 888)	0.917-0.925	1.465-1.468	0.2	187-195	103-128	0.3	10

4.4.2. Analysis

4.4.2.1. Determination of Saponification Value of Oil

Definition:

The saponification value denotes the weight of KOH in mg required to saponify 1 g of the oil or fat.

Principle of Method:

The oil is saponified by heating it with excess alcoholic caustic alkali. The amount of alkali consumed is estimated by back-titration with HCl (Section 5.2.). The saponification value is inversely proportional to the mean of the molecular weights of the fatty acids in the glycerides present in the oil or fat. As many oils give similar values the saponification value is less valuable than the iodine value

for identification of an unknown oil.

Procedure:

1. Weigh out accurately about 2 g of the oil or melted fat into flask (A) and blank (B).
2. Add exactly 25 ml of 0.5 N alcoholic KOH solution (Section 5.9.).
3. Attach the reflux condenser and immerse the flask in boiling water for 60 min.
4. Swirl the flask frequently during the heating
5. After the refluxing, add 0.5 ml of 1 % phenolphthalein (Section 5.1.1.) and titrate very carefully, whilst still hot, with 0.5 N HCl (Section 5.2.).
6. Retain A if the unsaponifiable matter is also to be determined. If the volumes of 0.5 N HCl used are A ml (for sample) and B ml (for blank).
7. Then;

$$Saponification\ \ Value = \frac{(B-A)}{wt.of\ \ sample(g)} * 28.05$$

4.4.2.2. Determination of Free Fatty Acids

Principle of Method:

The oil is dissolved in neutral solvent and the acidity is titrated with standard alkali. The value obtained represents the extent to which the glycerides in the oil have been decomposed by lipase. the free fatty acids (FFA) are usually calculated as oleic acid.

Procedure:

1. Mix 20 ml of alcohol (95 %) with 20 ml of diethyl ether, add 1 ml of phenolphthalein indicator (1 % in alcohol) (Section 5.1.1.).
2. Neutralise the mixture by adding 0.1 N NaOH (Section 5.3.) from a burette.
3. Weigh out 5 g of sample into a baker-flask (200-400 ml cap.).
4. Then add the solvent to the oil, swirl and titrate with 0.1 N NaOH, shaking constantly, until a pink color persists for 30 sec.

5. If two layers are separate, repeat the titration using a smaller amount of samples. If V is the ml of 0.1 N NaOH used and the weight of sample taken (g).
6. Then;

$$\%\ \ Free\ Fatty\ Acids\ = \frac{V * 28.2 * N}{wt.of\ sample(g)}$$

where N is the normality of NaOH.

Maximum limits of edibility vary according to the oil, but a critical limit of 1 % can be taken as a general guide. During storage, the FFA of oils and fats usually increases steadily. The rate is, however, inhibited as the temperature is lowered.

4.4.2.3. Determination of The Iodine Value of Oil by Hanus Method

The iodine value is the quantity of iodine absorbed per g of oil. It is an index of the unsaturation of the oil.

Reagent:

Hanus solution: Take 6.5 g iodine and add glacial acetic acid which cover the iodine. Add 4 g (1.3 ml) bromine into this solution. Make up to 500 ml with glacial acetic acid in volumetric flask. Shake vigorously until the iodine is just dissolved.

Procedure:

1. Weigh 0.5 g sample into a 250 ml flask.
2. Add 10 ml CCl_4 and 25 ml Hanus solution.
3. Carry out a blank at the same time. Let stand it 1 h in a dark place.
4. Add 20 ml of 10 % KI solution, shake though and add 100 ml of distilled water.
5. After 10 min., titrate the solution with 0.1 N $Na_2S_2O_3$ solution (Section 5.6.) until the color is changed from brown to yellow.
6. At this point, starch solution is added and titration is continued until the blue-starch iodine color disappears.
7. Calculate the iodine value by using the following equation:

$$Iodine\ Number = \frac{1.269*(B - A)}{wt.\ of\ \ sample\ (g)}$$

where A: ml of 0.1 N $Na_2S_2O_3$ solution used for the sample,

B: ml of 0.1 N $Na_2S_2O_3$ solution used for the blank.

4.4.2.4. Determination of Peroxide Value (PV)

Principle of Method:

During the storage of oils and fats, oxygen is adsorbed at the unsaturated bonds, which react like those in peroxides. At a certain level, volatile products are formed that have a deleterious effect on the taste and odour, known as oxidative rancidity.

In the usual methods, the sample is dissolved in an acetic acid-chloroform mixture and potassium iodide is added. Peroxide oxygen liberates iodine from the KI and is titrated with $Na_2S_2O_3$.

Procedure:

1. Weigh 5 g of sample into a 250 ml glass-stoppered erlenmeyer flask.
2. If your sample is margarine, melt it at about 40°C avoiding excessive heating.
3. And then add 30 ml of acetic acid-chloroform solution (3 parts acetic acid + 2 parts chloroform by volume).
4. Swirl the flask until the sample is dissolved in the solution.
5. Add 0.5 ml of saturated potassium iodide (Section 5.8.) using measuring pipette.
6. Allow the solution to stand with occasional shaking for exactly 1 minute
7. Then add 30 ml of distilled water.
8. Titrate with 0.01 N sodium thiosulphate (Section 5.6.) adding it gradually and with constant and vigorous shaking
9. Continue the titration until the yellow color has almost disappeared.
10. Add ca. 0.5 ml of starch (1 %) indicator (Section 5.13.) solution.
11. Continue the titration, shaking the flask vigorously near the end point to liberate all the iodine from the chloroform layer.

12. Add the thiosulphate dropwise until the blue color has just disappeared.
13. Conduct a blank determination of the reagents daily.

$$PV\ (meq.\ of\ \ peroxide\ \ per\ 100\ g\ of\ \ sample) = \frac{(S-B)*N*1000}{wt.\ of\ \ sample\ (g)}$$

where B : ml of 0.1 N $Na_2S_2O_3$ solution used for the sample,

S : ml of 0.1 N $Na_2S_2O_3$ solution used for the blank.

4.4.2.5. Melting Point Determination

The melting point of a substance is defined as the temperature at which the liquid and solid phases exist in equilibrium with one another without a change in temperature.

A crystal consists of an orderly arrangement of molecules held together by gravitational and electrostatic forces. When the crystal is heated, the kinetic energy of the molecules is increased, with the result that the molecules vibrate at an ever increasing frequency and amplitude. Finally at a certain temperature, the forces tending to make the molecules fly apart, exceed those holding them together and the crystals collapses, that is it melts.

The melting point of an impure substance is usually lower than that of the pure substance and melting point. range is almost always wider. Pure substances have rather sharp melting point.

Procedure:

1. Prepare the melting point apparatus (Figure 4.2.),
2. The bulb of the thermometer should be at the same level as the bottom of the capillary tube, and they should be just below the side-arm.
3. Fill the substance in to a closed-end capillary tube. The easiest way to fill the capillary tube to place a bit of the powdered sample on a watch glass and tap the open end of the capillary in to the solid a few times.
4. The solid should be tightly packed in the tube, and this is accomplished by dropping the capillary though a piece of glass tubing approximately 60 cm

long (closed end of the capillary facing downwards).

5. The size of the sample should be such as to fill the capillary to a height of 0.5 cm.

Figure 4.2. Apparatus used for capillary tube melting point determination.

6. Add sample into the Thiele Tube just above the top of the side arm. Melt the fats thoroughly at first, then let it cool to 10 °C.

4.4.2.6. Color

The color of oils is usually compared in a LOVIBOND TINTOMETER using a 1 in. or 5.25 in. cell. Alternatively the optical density can be measured, using a spectrophotometer, against carbontetrachloride in a suitable cell (0.5-5 cm) at the vawelength of maximum absorbance.

Fats should be melted and an oil or fat that is cloudy should be filtered at a temperature not greater than 60 °C. During matching, the sample should be at

room temperature (for fats) or not more than 10 °C above the melting point.

4.4.2.7. Refractive Index

For determination of the refractive index the following temperatures are employed: 20 °C for oils; 40 °C for solid fats and mixture of fatty acids liquid at that temperature; 65 °C for solid fats, etc., not liquid at 40 °C (using sodium light of vawelength 589.3 nm).

Procedure:

1. Put one drop of the sample (at a proper temperature) on a refractometer prism.
2. Read the refractive index.

During the determination of refractive index, it is important to be careful on the points given in figure 4.3 (Harwood and Moody, 1989) .

Figure 4.3. Appearance of the field of view using an Abbe refractometer: a) before compensation for dispersion effects; b) after compensation for dispersion effects; c) insufficient sample on prism surfaces; d) using an instrument with poor quality optical surfaces.

4.5. ANALYSIS OF DAIRY PRODUCTS

Prior to taking portions for each determination, the milk should be thoroughly mixed by continuous slow inversions of the sample bottle or by slowly pouring it into a beaker and back into another beaker and repeating the process many times. In the routine examination of milk it is convenient to take the lactometer reading and, after determining the fat by the Gerber method, the total solids and hence the non-fatty solids content can be calculated. The development of sourness can be assessed by determining the acidity of the milk. The assessment of the degree of pasteurisation from the results of the phosphatase test is also a common routine procedure for milks which are sold as "Pasteurised". Other determinations which may be required are those of protein, lactose, ash, chloride and citric acid and examination for dirt, added dyes, preservatives, detergent and antibiotics. The methylene blue test and the bacteriological examination must be carried out on samples taken under aseptic precautions.

4.5.1. Composition of Milk

Table 4.12. Compositions of cow, goat and sheep milk

Source	Water (%)	Fat (%)	Protein (%)	Lactose (%)	% Total acidity*	Density	Ash (%)
Cow	87.6	3.8	3.3	4.7	7.6	1.033	0.6
Goat	87.0	4.5	3.3	4.6	8.2	1.035	0.6
Sheep	81.6	7.5	5.6	4.4	10.0	1.038	0.9

* SH° (soxhlet henkel degree)

4.5.2. Composition of Cheese

Table 4.13. Composition of the some of the cheese

Type of Cheese	Water %	Oil %	Total Nitrogen %	Soluble (NaCl) %	Insoluble %
Camembert	53.8	22	17.1	3.2	1.2
Gruyere	35.7	28	28.9	0.4	3.1
Roquefort	36.9	29.5	20.5	5.1	1.9
Emmentaler(hard)	33	30.5	30.4	4.2	4.2
Neufchatel (soft)	59.2	18.2	21.3	2.4	2.4
Edirne	55	27	14	3.5	
Tulum	37	27	27	5	
Bursa Cheddar	31	30	34	4.5	

4.5.3. Standards for Milk, Yoghurt and Cheese (TS 591, 1018 and 1330)

4.5.3.1. TS for Milk (TS 1018)

Table 4.14. Turkish standards for milk

	Cow milk	
Component	Lowest	Highest
Total Acidity as Lactic acid (%)	0.135	0.202
Density	1.028	1.039
Fat Content (%)	3.0	---
Solid Non-Fat Content %	8.5	---
Lactose (%)	3.5	5.5
Protein (%)	2.8	5.0
Ash (%)	0.6	0.9
Dirt (mg/100 ml)	--	6

4.5.3.2. TS for Yoghurt (TS 1330)

Table 4.15. Turkish standards of yoghurt

Type	% Fat (min.)	% Total dry matter (min.)
High fat	3.8	15.0
Full fat	3.0	12.0
Half fat	1.5	10.5
Fat-free	--	9.0

4.5.3.3. TS for Cheese (TS 591)

Physical properties: dimensions of cheese should be 7-11 cm of each side and should be in a rectangular or cubic form. Chemical properties are given in Table 4.16.

Table 4.16. Chemical Properties of Cheese

Chemical properties	Limits
Acidity, Lactic acid,	max. 3 % (v/v)
pH	min. 4.5
Moisture	max. 60 % (v/v)
Salt	max. 10 % of the total solid
Copper, Cu, (mg/kg)	max. 1
Tin, Sn , (mg/kg)	max. 250
Mercury, Hg, , (mg/kg)	max. 0.03
Lead, Pb, (mg/kg)	max. 0.3

4.5.4. Analysis

4.5.4.1. Determination of Ash in Milk

Ash is determined by heating the dried residue of milk at a temperature not exceeding 500°C so that the chlorides are not volatilized.

Chloride in Ash:

1. Pipette and weigh 10 ml milk into a 250 ml flask.
2. Then add by pipette 10 ml 0.05 M silver nitrate (Section 5.7.).
3. Add 10 ml conc. nitric acid and a few anti-bump granules and boil gently for a few min. The liquid should then be pale yellow.
4. Cool, add 60 ml water and 1 ml ammonium ferric sulphate ($Fe_2(SO_4)_3.(NH_4)_2.SO_4.24H_2O$) solution as indicator
5. And titrate the excess silver nitrate with the potassium thiocyanate solution (Section 5.5.).
6. Perform a blank determination by repeating the procedure with 10 ml water instead of milk.

   1 ml of 0.050 M potassium thiocyanate = 0.001773 g chloride.

4.5.4.2. Analysis of Lactose in Milk

The reducing sugar contents of milk samples can be determined by using Dinitrosalicylic acid (DNS) method. The DNS reagent can be prepared as follows; 2.65 g of Dinitrosalicylic acid and 4.95 g of NaOH are dissolved and mixed in 354 ml of distilled water. To this mixture 76.5 g of sodium-potassium tartarate, 1.9 ml of phenol and 2.075 g sodium-meta-bisulfite are added. In order to prevent the effect of proteins in Milk on spectrophotometric measurement, ca. 10 ml of milk is mixed with 3-5 drops of 1 M Lactic acid solution to precipitate the proteins of milk and then it is centrifuged at 4500 rpm for 5 min.

Procedure:

1. Mix 1 ml of centrifuged sample is mixed with 3 ml of DNS reagent in a test tube.
2. The test tube is placed into a boiling water bath for 5 min.
3. Then, 16 ml of distilled water is added and mixed thoroughly.
4. The colored solution obtained after this process is placed into a

spectrophotometer cell and the absorbance is measured at 575 nm.

5. A standard curve is obtained by preparing glucose (a reducing six carbon sugar) solutions in distilled water ranging in concentrations of 0.1-7 g/L.
6. The above procedure is applied for glucose solutions and corresponding absorbances are obtained.

4.5.4.3. Determination of Total Solids in Milk

Procedure:

1. Weigh out 3-4 g of mixed milk into a dish and place the dish on a boiling water bath for 30 min.
2. Then transfer the dish to a well-ventilated oven at 100°C.
3. Dry for 2 1/2 hour, cool the dish for 30 min. in a desicator and weigh.
4. Calculate the weight of residue in the dish as a percentage of the sample.

4.5.4.4. Determination of Protein in Milk

The rapid determination of protein in the milk can be made by formal titration method. The protein in Milk can also be determined on 10 g milk by the macro Kjeldahl method using the factor N*6.38. Formal titration method depends on the fact that when formaldehyde is added to neutralised milk, free acid(which can be titrated by alkali) is produced in proportion to the amount of protein present. The protein content is then obtained by multiplying the titration by an empirical factor, which depends on the ratio of casein to albumin and also the particular technique employed in which interference due to calcium is prevented by addition of oxalate give reasonably accurate results.

Procedure:

1. To 10 ml of milk add 0.5 ml of 0.5 % phenolphthalein indicator and 0.4 ml of neutral saturated potassium oxalate ($(COOK)_2.H_2O$ or $C_2K_2O_4.H_2O$).
2. Mix and allow to stand for a few min. and neutralise with 0.1 M NaOH to the standard pink color.
3. Add exactly 2 ml. of formalin.
4. Mix and allow to stand for a few minute and titrate the new acidity produced

with 0.1 M NaOH (Section 5.3.) to the same pink color (a).

5. Titrate separately 2 ml. of formalin and 10 ml. of water with the same alkali (b) as blank.
6. Then the protein content of the milk is

$$\% \ Protein = 1.7 * (a - b)$$

If the oxalate is omitted, the first titration gives the acidity and a higher formal factor is usually appropriate;

$$\% \ Protein = 1.95 * (a - b)$$

4.5.4.5. Determination of Fat in Milk

a) Werner-Schmid Method: The gravimetric **Werner- Schmid** method can be carried out in the extraction tubes used for the Gottlieb method.

Procedure:

1. Weigh out 10 g of the milk into the tube.
2. Add 10 ml of concentrated HCI and immerse in boiling water until all the casein is dissolved.
3. At this stage the mixture should be brown (or violet) in color and the fat will be seen to collect on the surface.
4. Cool the tube in running water.
5. Extract the fat by shaking with 30 ml of diethyl ether and blow the extract, after allowing the layers to separate, into a weighed flask.
6. Repeat the extraction three-times more and distil off the solvent. Then dry the fat at 100°C, cool and weigh.
7. If any non-fatty matters appears to be present, dissolve the fat in light petroleum, reweigh and correct the result accordingly.

b) Gerber Method: Rapid volumetric methods are often used for routine purposes for determining fat in Milk. This method is performed in butyrometer tubes which are closed with special rubber stoppers.

Procedure:

1. Pipette the following liquids into a milk butyrometer, ensuring that they do not mixed with one another: 10 ml of sulphuric acid (density 1.812-1.818 at 20 °C ≡ 90 % w/w approximately), 10.94 ml of mixed milk and 1 ml of fat-free amyl alcohol (density 0.809-0.813 at 20 °C).
2. Close the tubes with stopper.
3. Mix the contents thoroughly and immediately centrifuge at 1100 rpm for 4 min.
4. Transfer the tube (stopper downwards) to a water bath at 65 °C far at least 3 min.
5. Read off the percentage fat directly from the scale (extending from the bottom of the upper meniscus to the flat bottom of the fat column).
6. Readings to an accuracy of 0.05 % are usually adequate for routine purposes.
7. As it is difficult to separate the small fat globules in homogenised (e.g. sterilised) milk, it is advisable to re-centrifuge after warming in the 65 °C bath until the reading reaches a maximum.

4.5.4.6. Specific Gravity

The specific gravity of milk varies according to the proportions of fat (sp. gr. 0.93), non-fatty solids, NFS, (sp. gr. 1.614) and water (sp. gr. 1.0). The density of milk can be conveniently measured by means of the lactometer which is a special hydrometer calibrated over the range 1.025-1.035 (25 ° - 35 ° as lactometer degrees). For determination, the milk should be reasonably fresh and be thoroughly but gently mixed, avoiding incorporation of air. The temperature of the sample being measured must always be taken.

If the percentage of fat is determined by Gerber method, the total solid (T) figure can be calculated from the modified **Richmond's formula**

$$T = 0.25 * D + 1.22 * F + 0.72$$

where D is the density hydrometer reading (lactometer degrees) at 20 °C and F is the fat in percentage.

$$NFS = T - F$$

4.5.4.7. Dirt in Milk

The limits of dirt in milk in American standards is 2 parts of moist dirt per 100,000 is a proper maximum amount.

Procedure:

1. Add formalin to the milk and allow it to stand in a covered sedimentation vessel connected to a standardised centrifuge tube, also fill with the milk, for 72 hours.
2. Stopper the lower end of the vessel by a glass rod with a rubber cork.
3. Remove the centrifuge tube and spun at 200 rpm for 5 min.
4. After pour off the milk the separated dirt is washed by filling the tube with water.
5. Centrifuge again for 3 min.
6. Wash it similarly with 1 M ammonia solution and 1 M hydrochloric acid (Section 5.2.).
7. Read off the volume of the moist dirt in the calibrated tube.
8. A macroscopic determination of the dirt can be made before the ammonia wash, and the part removed returned to the tube before the final measurement.

4.5.4.8. Total Acidity in Milk

The acidity value can be expressed in different manners. These are:

1. Degree of Soxhlet Henkel (SH°) : Amount of N/4 NaOH required to neutralise 100 ml of milk. Or use 25 ml of milk then multiply the amount of alkali consumed by 4. Generally, SH° of milk is about 6-7.
2. Degree of Dornic (D°): Amount of 0.1 N NaOH required to neutralise 10 ml milk multiplied by 9. Generally the acidity of milk is 15-16 D° (1 SH° ≡ 2.25D°).
3. % Lactic Acid.

Procedure:

1. Take 20 g milk into a flask and dilute with twice its volume of CO_2-free water.
2. By using (1% in alcohol) phenolphthalein (Section 5.1.1.) as indicator.
3. Titrate the sample with 0.1 N NaOH (Section 5.3.) to the first persistent pink color.
4. Calculate the percent acidity as lactic acid.

1 ml of 0.1 N NaOH = 0.0090 g lactic acid

4.5.4.9. Turbidity Test for Sterilised Milk

When milk is heated at 80°C or above, all the albumin becomes denatured and if solutions of inorganic salts or acids are added the albumin separates with the casein.

Procedure:

1. Weigh out 4.0 g of ammonium sulphate into a 50 ml conical flask.
2. Add 20.0 ml of milk sample and shake the mixture for 1 min. to dissolve the ammonium sulphate.
3. Allow the solution to stand 5 min., then filter it.
4. When at least 5 ml of clear filtrate have been collected, place the tube in boiling water for 5 min.
5. Then cool it in cold water and examine the solution for turbidity.
6. A sterilised milk that has been satisfactorily heat treated gives no turbidity. UHT milk gives a faint turbidity and raw pasteurised milk give a white precipitate.

4.5.4.10. Determination of Fat in Cheese

The fat content of cheese can be determined by Werner- Schmid method also Gerber method can be applied.

Procedure for Determination of Fat in Cheese by Werner-Schmid Method:

1. Weigh an amount of cheese (approximately 1-2 g) in to 100 ml beaker.
2. Macerate the sample by using the rod thoroughly with a few drops of 0.88

ammonia.

3. Then add dilute hydrochloric acid (7 volume concentrated HCl + 3 volume water).
4. Cover the beaker with a clock glass and heat carefully while stirring over a small flame or in a boiling-brine bath.
5. When all particles have dissolved, cool slightly, add 10 ml of alcohol, mix, then cool and transfer the liquid down the rod into an extraction tube or separator rinsing in with several small volumes of ether (total 25 ml).
6. Shake the tube vigorously for 1 min.
7. Then add 25 ml light petroleum, shake again and transfer the separated extract into weighed flask.
8. Repeat the extraction 3 more times, combining the extracts and finally remove the solvent and weigh the residual fat.

Gerber method:

Same method as mentioned in the milk section can be applied for the determination of the fat content in cheese (Section 4.3.4.5).

4.5.4.11. Determination of Water in Cheese

Oven method can be applied for determining the water content in cheese.

Procedure:

1. Weigh out 3 g sample into dried and weighed dish.
2. Add water to assist with the spreading of the sample by means of the rod before drying on a boiling water bath.
3. Put into oven at 100 °C for overnight.
4. Weigh the dish and calculate the weight loss and determine the water content of cheese.

4.5.4.12. Determination of Salt in Cheese

Procedure:

1. Weigh 2 g of sample into 200-500 ml conical flask.
2. Add 25 ml of 0.05 M silver nitrate (Section 5.7.) solution and 10 ml of water.
3. Disperse the cheese by swirling and warming to 80 °C.

4. Add 10 ml of concentrated nitric acid and boil gently until the precipitate is granular (about 10 min.).
5. To the hot solution add 0.5 g urea, mix, then cool and add 1 ml nitrobenzene and 50 ml of water.
6. Titrate the unused silver nitrate with 0.05 M potassium thiocyanate (Section 5.5.) using ferric alum (Section 5.1.4.) as indicator.

$$1 \text{ ml } 0.05 \text{ M } AgNO_3 \equiv 0.002925 \text{ g NaCl}$$

4.6. ANALYSIS OF TOMATO PUREE

Tomato puree is made by concentration the strained liquid pulp of good quality tomatoes. The raw tomato (5% total solids) is converted to "double concentrate" (28-32 % total solids) and "triple concentrate" (36-40 % total solids). During manufacture it is important to know the degree of concentration of pulp i.e. the dry or total solids content; an idea of the condition of the tomato being pulped, as reflected in the Howard Mould count; the color of the product, and also the acidity and sugar content. Physical tests such as determination of consistency, number of black and brown specks (points), color, flavor, taste can be applied during and/or after manufacture.

4.6.1. Composition of Tomato

Table 4.17. Composition of tomato

Component	Composition (%)
Water	93.6
Nitrogenous material	1.2
Protein	0.8
Oil	0.05
Glucose+Fructose	3.3-3.5
Cellulose	0.6
Acidity, citric acid	0.35-0.4
Crude Fibre	0.35
Non-nitrogenous material	4.1
Ash	0.6

4.6.2. Standards for Tomato Paste (TS 1466)

Turkish standards for tomato paste are given in Table 4.18.

Table 4.18. Turkish standards for tomato paste

Component	Limits
Physical Properties:	
Filling Percentage	min. 90 %
Black Point	max. 2 / g
Hunter a/b	min. 1.8
Foreign Material	none
Chemical Properties:	
Reducing Sugar	min. 40 % (w/w)
Acidity	max. 10 % of total solid
Insoluble Ash in 10 %HCl	max. 0.3 % (w/w)
Salt (w/w) for salty	10 % of total solid
Soluble solid (w/w) for puree	min. 11 %
for double concentrated	min. 28 %
for triple concentrated	min. 36 %
Metallic Contamination:	
Arsenic, As	max. 0.1 mg/kg
Lead, Pb	max. 3 mg/kg
Zinc, Zn	max. 15 mg/kg

Iron, Fe	max. 30 mg/kg
Copper, Cu	max. 15 mg/kg
Tin, Sn	max. 150 mg/kg

4.6.3. Analysis

4.6.3.1. Testing for Black Points

A very simple, but effective test to determine the homogeneity of the puree, consists of placing 1 g of tomato paste between two pieces of glass. The glasses are pressed together and paste made to squash out into a thin film which can be viewed through the glasses. According to TS, black points should not be more than 2 # in 1 g.

4.6.3.2. Determination of Sugar Content of Tomato Paste

Sugar content of tomato paste is determined by the Lane and Eynon's method as mentioned in section 4.2.3.5.

4.6.3.3. Determination of Color

This can be assessed by means of the **Lovibond Tintometer.** Red should be predominant over yellow. An excess of blue may indicate scorching

4.6.3.4. Determination of Total Soluble Solids In Tomato Paste

a) By oven drying:

1. Place 3 g tomato paste into a dry and weighed dish.
2. Spread evenly over the bottom surface.
3. Dry the dish content in a vacuum oven at 70°C until constant weight is attained.

b) By refractometer:

It is very rapid and accurate for production control purpose.

i) Direct measurement:

Undiluted tomato paste is put on a refractometer prism. Approximately total solids can be obtained by reference to the tables.

ii) Measurement after dilution:

1. One part by weight of the tomato paste is diluted with three parts of distilled water and mixed well.
2. After filtration the refractive index is read by one drop of filtrate.
3. The actual solids of the sample can be calculated by multiplying the dilution factor i.e.4.

4.6.3.5. Determination of Acidity

The acid in tomatoes is generally considered to be almost entirely citric, and free acids are almost always determined as citric monohydrate.

Procedure:

1. A 1:3 dilution, by weight, of the tomato paste, in which the solid content is known, is filtered.
2. The first few drops may carry though some suspended matter, but this may be returned to the filter. So that the filtrate for test is as clear as possible.
3. 10 ml of the filtrate is mixed with 50 ml of water and using 1 ml of 1 % phenolphthalein (Section 5.1.1.) and 0.1 N NaOH (Section 5.3.) the titration is made until a pink color persists at least 30 sec.

1 ml of 0.1 N NaOH = 0.0064 g citric acid.

4.6.3.6. Determination of Salt

Procedure:

1. Weigh out 10 g of puree into a 250 ml beaker.
2. Add about 90 ml of water, with the rod, warm the mixture slightly and transfer it carefully to a 500 ml volumetric flask.
3. Cool, make-up to 500 ml, mix and filter (A). To 50 ml of A (=1 g of sample) add 5 % potassium chromate solution (Section 5.1.5.) and a piece of marble.

4. And titrate with 0.05 N silver nitrate solution (Section 5.7.).

1 ml of 0.05 N silver nitrate = 0.00292 g of NaCl.

It is permitted to add salt (max. 10 % of dry solids) and spices.

4.6.3.7. Determination of Lycopene

The red color of tomato is due to the pigment Lycopene ($C_{40}H_{56}$). Tomatoes contain other carotenoid pigments besides Lycopene, but in fully ripe fruit, the latter predominates. Unripe green and yellow fruits, however, contain no Lycopene, but mainly chlorophyll and other carotenoid pigments. Therefore, estimation of Lycopene is a good index of the quality of fruit used in the manufacture of tomato products.

Principle:

Lycopene has absorption maximum at 473 nm and 503 nm. The molecular extinction coefficient for all trans-lycopene at 473 nm is 18.6×10^4. A rapid method for the estimation of Lycopene in tomato products is based on the measurement of absorption of the petroleum ether extract of the total carotenoids at 503 nm. The errors involved in the method is very small (2 - 5 %), since the carotene has a comparatively negligible absorbance, while Lycopene has a large absorbance at 503 nm.

Extraction:

1. Weigh 5-10 g of the paste.
2. Extract repeatedly with acetone in a blender until the residue is colorless.
3. Transfer the acetone extract to a separation funnel containing 10 ml petroleum ether and mix gently.
4. Take up the carotenoid pigment into the petroleum ether by diluting the acetone (lower phase) with water or water containing 5% Na_2SO_4.
5. Transfer the lower phase to another separating funnel and the petroleum ether extract containing the carotenoid pigments to an amber colored bottle.
6. Repeat the extraction of the acetone phase similarly with petroleum ether

until it is colorless.

7. Discard the acetone phase.
8. To the petroleum ether extract, add a small quantity of anhydrous Na_2SO_4, transfer to a 50 ml volumetric flask and dilute to mark with petroleum ether.
9. Dilute an aliquot (2 ml) to 50 ml with petroleum ether.
10. Measure the color in a 1 cm cell at 503 nm. in a spectrophotometer using petroleum ether as blank.

Calculation:

Calculate the Lycopene content of the sample as given below using the optical density (OD) relationship;

(OD) in 1.0 cm cell at 503 nm = 3.1206 micro g of Lycopene per ml.

$$mg\ of\ \ Lycopene\ per\ 100\ g\ sample = \frac{3.12 * OD\ of\ \ sample * Vol.made\ up\ (ml) * DF}{wt.\ of\ \ sample\ (g)} * 100$$

where DF is the dilution factor.

4.7. ANALYSIS OF CARBONATED NONALCOHOLIC BEVERAGES

The products obtained from fruits and vegetables are canned, frozen, concentrated, dried, powdered fruits, and juices prepared from citrus fruits (orange, lemon, lime, etc.). Sometimes these juices are carbonated. A large number of methods are available for checking the quality of these products e.g. specific gravity, refractive index, total solids, alcohol, total and volatile acidity, pH, sugars, ash, moisture, chloride, pectin, enzymatic activity, vitamin C, benzoic acid, sorbic acid, color measurement, CO_2 content, pulp content, microbial examinations, etc.

4.7.1. Standards for Carbonated Nonalcoholic Beverages (TS 4080)

Table 4.19. Turkish standards for carbonated nonalcoholic beverages

Components	Natural	Cola	Tonic	Synthetic
Flavor and Odor	Appropriate for fruit	Suitable for cola		
Chemical Properties				
Soluble Solid min.	9	9	7	8

max.	12	12	10	13
Total Sugar, sucrose, min. %	7.0	7.0	6.0	8.0
Total acidity, citric acid, max. %	0.5	0.3	0.5	0.5
Carbondioxide (w/v) min. %	0.3	0.4	0.4	0.4
max. %	0.6	0.9	---	0.9
Phosphoric acid, (PO_4) max., g/l	---	0.6	0.6	---
Synthetic Sweeteners, g/l	----	---	---	---
Total Preservatives, g/l	0.7	0.7	0.7	0.7
Caffeine max. g/l	---	0.15	---	---
Formol Index (0.1 N NaOH is required for 100 ml) min.	1.0	---	1.0	0.4

4.7.2. Analysis

4.7.2.1. Titratable Acidity

Procedure:

1. Dilute a known amount (in grams) of sample with recently boiled distilled water to 100 ml.
2. Then titrate with 0.1 N NaOH (Section 5.3.) using a few drops of 1 % phenolphthalein solution as indicator (Section 5.1.1.).
3. Note the titrate value.
4. Calculate the result as per cent citric acid.

1 ml 0.1 N NaOH = 0.0064 g citric acid.

4.7.2.2. Formol Index

In order to distinguish natural fruit juices from others, which are prepared from synthetic volatile oils, formal index can be determined. The principle of this method is to titrate amino acids formally which found in natural fruit juices. After neutralisation of free acids and adding neutral formalin, some amount of alkaline is used during titration. Natural fruit juices consumes some amount of alkali (e.g. 8- 50 ml) but synthetic ones consume less amount or nothing

Procedure:

1. Titrate the 25 ml of fruit juice with 0.1 N NaOH (Section 5.3.) until the pH of the solution is 8.50.
2. Then add 10-15 ml of formaldehyde solution, stand for 1 min.

3. Continue the titration with 0.1 N NaOH until pH is 8.50.
4. For 100 ml of fruit juices, ml of 0.1 N NaOH consumed (total) gives formal index.

4.7.2.3. Caffeine in Cola Drinks

Caffeine ($C_8H_{10}N_4O_2$) is present in cola nut, the extract of which is present in cola drinks. Cola drinks usually contain 60-190 mg per litre.

Procedure:

1. Make the sample alkaline with ammonium by using red litmus paper or a pH-meter.
2. Extract the solution with chloroform for several times.
3. Then measure the absorbance at 273 nm. (E=530) by using a spectrophotometer.
4. Remember the Beer's Law for calculation (A = Ebc).
5. The method may lead to high results due to interference from other substances extracted.

4.7.2.4. Determination of Sodium Benzoate

Benzoic acid is usually used as a preservative in the form of the calcium, sodium, or potassium salt. Benzoic acid retards the growth of yeast and molds, the effective agent being the undissociation acid.

When the sodium benzoate solution is acidified with excess HCl, water-insoluble benzoic acid is formed which is extracted with chloroform. The chloroform is removed by evaporation and the residue containing benzoic acid is dissolved in alcohol and then titrated with standard NaOH

Procedure:

1. Mix 100-150 g of the sample in approximately 30 ml saturated NaCl solution.
2. Add 15 g of NaCl and make alkaline to litmus paper.
3. Transfer to a 500 ml volumetric flask and dilute to mark with saturated salt solution.

4. Allow to stand at least 2 h, shaking frequently, centrifuge, if necessary, and filter.
5. Pipette a convenient portion (100 ml) of the filtrate into a 500 ml separation funnel.
6. Neutralise to litmus paper with HCl (Section 5.2.) and add 5 ml of HCl in excess.
7. Extract carefully with chloroform using successive portions of 70, 50, 40, and 30 ml.
8. To avoid the formation of emulsion, shake continuously each time using rotatory motion.
9. Chloroform layer usually separates readily after standing for a few minutes. If emulsion forms, break it by stirring the chloroform layer with a glass rod.
10. Transfer the combined chloroform extracts from the separation funnel to a dry conical flask and rinse the funnel three-times with chloroform.
11. Distill very slowly at low temperature to app. one fourth of the original volume, and evaporate to dryness at water bath until only a few drops remain.
12. Dissolve the residue of benzoic acid in 50 ml of alcohol neutral to phenolphthalein; add 12-15 ml of water and add 1 or 2 drops of phenolphthalein indicator and titrate against 0.005 N NaOH.

1 ml of 0.005 N NaOH = 0.0072 g anhydrous benzoate
1 ml of 0.05 N NaOH = 0.0061 g benzoic acid

4.7.2.5. Determination of Sugar

The presence of added sucrose can be detected by determining the sugars before and after inversion by Lane and Eynon's method (Section 4.2.3.5.).

4.8. ANALYSIS OF FRUIT JUICE

Fruit is refreshing to eat and adds color and flavor to the diet. Most fruits consist largely of water, however, and hence their nutrient contents is low. Their main importance is a source of vitamin C and dietary fibre. Most of this comes from citrus fruit and, especially, fruit juices and little from bananas, apples and pears which are the most popular fruits. Some fruits also contain vitamin A and they also make a small contribution to the mineral content of the diet.

When fruit is stored there is a progressive loss of vitamin C and up to 20 per cent of that present in citrus fruit may be lost in one month. There is usually also loss of thiamine but as only small amounts are present initially the loss is not significant. Small amount of carotene is lost.

Canned fruit can be a good source of vitamin C, despite losses of 20-30 per cent which may occur during the canning process. If canned fruits are stored for a long time some loss of vitamin C may occur but less than 15 per cent is usually lost during one year's storage.

Frozen fruit retain most of the vitamin C during the freezing process and subsequent storage. Some losses occur during the preliminary trimming, washing and blanching.

When fruit is dried to produce raisins, sultanas, prunes or dried currants its vitamin C content and about 50 % of its thiamine are destroyed. The carotene

content is little affected.

4.8.1. Composition of Some Fruits

Table 4.20. Composition of some fruits (Cemeroğlu, 1982)

Type of fruit	Protein (g)	Carbohydrate (g)	Water (g)	Vit A (μg)	Thiamine (mg)	Vit C (mg)
Apples	0.3	11.9	84	5	0.04	5
Bananas	1.1	19.2	71	33	0.04	10
Cherries	0.6	11.9	82	20	0.05	5
Grapes	0.6	16.1	79	0	0.04	4
Grapefruit	0.6	5.3	91	0	0.02	40
Oranges	0.8	8.5	86	8	0.10	50
Peaches	0.6	9.1	86	83	0.02	8
Strawberries	0.6	6.2	89	5	0.02	60

4.8.2. Standards for Fruit Juice (TS 1535 and TS 1537)

Table 4.21. Turkish standards for Orange juice (TS 1535)

Chemical Properties	Limits
Soluble Solid Matter (refractometric), %w/w	min. 10.00
Total acidity (citric acid), % w/v	max. 2.00
Lactic acid, v/v	max. 0.06
Volatile acid (acetic acid), % w/v	max. 0.04
Ethanol, % v/w	max. 0.50
Volatile oil	max. 0.30
Hydroxymethly furfural, mg/l	max. 10.00
Formol index	min. 15.00
Proline, mg/l	min. 500.00
Ascorbic acid (Vitamin C), mg/l	min. 200.00
Preservatives	none
Metallic contamination:	
Arsenic, As, mg/l	max. 0.20
Copper, Cu, mg/l	max. 5.00
Zinc, Zn, mg/l	max. 5.00
Iron, Fe, mg/l	max. 15.00
Tin, Sn, mg/l	max. 150.00
Lead, Pb, mg/l	max. 0.30
Precipitatible metal ions, as iron, mg/l	max. 20.00

Table 4.22. Turkish standards of Orange juice concentrates (TS 1537)

Chemical Properties	Limits
Soluble Solid Matter (refractometric), %w/w	min. 60.00
Total acidity (citric acid), % w/w	max. 1.50
Lactic acid, w/w	max. 0.06
Volatile acid (acetic acid), % w/w	max. 0.04
Ethanol, % w/w	max. 0.50

Volatile oil, % v/w	max. 0.30
Hydroxymethly furfural, mg/l	max. 10.00
Formol index (required for 100 ml 0.1 N NaOH)	min. 10.00
Proline, mg/l	min. 0.50
Ascorbic acid (Vitamin C), mg/l	min. 200.00
Preservatives	none
Metallic contamination:	
Arsenic, As, mg/l	max. 0.20
Copper, Cu, mg/l	max. 5.00
Zinc, Zn, mg/l	max. 5.00
Iron, Fe, mg/l	max. 15.00
Tin, Sn, mg/l	max. 150.00
Lead, Pb, mg/l	max. 0.30

4.8.3. Analysis

4.8.3.1. Titratable Acidity

Acidity can be found as in the nonalcoholic beverages (Section 4.7.2.1.). The flavor of fruit juice is related to the ratio of soluble solids to the total acidity called the "maturity ratio" which increases as the fruit ripens.

4.8.3.2. Formol Index

Formal index can be found as in the nonalcoholic beverages (Section 4.7.2.2.)

4.8.3.3. Determination of Sodium Benzoate

Sodium benzoate concentration in the fruit juice can be determined as in the non-alcoholic beverages (Section 4.7.2.4.)

4.8.3.4. Determination of Ascorbic Acid (Vitamin C)

1. The dye (indophenol) solution supplied contains 0.05 g 2:6 dichlorophenol indophenol dissolved in water, and has been diluted to 100 ml and filtered.
2. Place this in a 10 ml micro-burette.
3. To standardise, weigh approximately 0.05 g (accurately) of pure ascorbic acid into a 250 ml volumetric flask and dissolve in 60 ml of 20 % metphosphoric acid and dilute with water to exactly 250 ml.

4. Then pipette 10 ml of this solution into small flask and titrate with indophenol solution until a faint pink color persists for fifteen seconds.
5. Express the concentration as mg ascorbic acid equivalent to 1 ml of the dye solution.
6. The dye solution keeps for a few weeks if stored in the refrigerator, but it should be standardised before use against a freshly prepared solution of ascorbic acid.

$$Ascorbic\ acid\ (g/ml\ of\ dye\ ) = \frac{wt\ of\ \ ascorbic\ acid\,(g) * 10}{Vol.\ of\ \ indophenol\ \ solution(ml) * 250}$$

Vitamin C Determination in Fresh Lemon Juice

1. Use a lemon juice supplied. This has been squeezed and filtered.
2. Pipette 25 ml into a 50 ml volumetric flask, add 12 ml of 20 % metaphosphoric acid, dilute to 50 ml with water.
3. Pipette 10 ml of this solution into a small flask and titrate with indophenol solution.
4. Calculate Vitamin C as mg per 100 ml of juice.

$$Vitamin\ C\ (mg/100\ ml) = Vol.indophenol\,(ml) * equivalent\ \ ascorbic\,acid\,to\,1ml * \frac{100}{5}$$

Vitamin C Determination in Commercial Lemon Concentrates

1. Pipette 10 ml of the juice into a 50 ml graduate flask, add 12 ml of 20 % metaphosphoric acid as stabilising agent and make up to the mark with water.
2. Pipette 10 ml of this solution into a small flask, add 2.5 ml of acetone and titrate with indophenol solution until a faint pink color persists for fifteen seconds.
3. Calculate Vitamin C content in the sample as mg per 100 ml.

$$Vitamin\ C\,(mg/100\ ml) = Vol.indophenol * equivalent\ \ ascorbic\,acid\,to\,1ml * 50$$

4.8.3.5. Determination of Hydroxymethyl furfural (HMF)

Hydroxymethyl furfural (HMF) is a cyclic aldehyde formed by dehydration when hexoses are heated. HMF reacts like other aldehydes with barbituric acid and p–toluidine forming a red-colored complex. The intensity of the red color is dependent upon the amount of HMF and it can thus be made the basis of a quantitative colorimetric determination. The reaction proceeds to a color-maximum that can be defined by observing the extinction. HMF reacts as an aldehyde with any sulphurous acid that may be present and in such a case it cannot be estimated without pre-treatment When sulphurous acid present the HMF is set free under weakly alkaline conditions and the sulphurous acid oxidised by iodine-solution (IFFJP, 1964).

Reagents:

Barbituric acid solution: 500 mg barbituric acid dried at 105 °C is dissolved in distilled water in graduated flask by gently in a water bath and made up to 100 ml. The solution is suitable for 1 week.

p-Toluidine solution: 10 g p-toluidine (melting point. 45 °C, analytical grade) is dissolved in 50 ml isopropanol (2-propanol) and treated with 10 ml glacial acetic acid; it is then made up with isopropanol in a 100 ml graduated flask. Sufficient for about 20 determinations. The solution is to be made up freshly each day.

Carrez I solution: Prepare the carrez I solution as mentioned in section 4.2.3.5.

Carrez II solution: Prepare the Carrez II solution as mentioned in section 4.2.3.5.

Determination procedure for HMF:

1. Take 25 ml of fruit juice or corresponding amount of concentrate
2. Make weakly alkaline with pure $NaHCO_3$.
3. If very vigorous foaming occurs add a few drops of isopropyl alcohol.
4. Check the pH value of 7-8 by litmus paper.
5. Then add 4 ml of 0.5 % starch solution (Section 5.13.)
6. Titrate the solution by drop-wise addition of 0.1 N iodine-solution (Section 5.8.) sufficient to give a blue color of starch-iodine that persists for 15-20

second.

7. Add 1 ml each of Carrez solution I and Carrez solution II.
8. Make up to 50 ml with distilled water in a 50 ml graduated flask and filter.
9. Pipette 2 ml of filtrate into each of 2 small flasks with ground stoppers (designated s; sample and b; blank).
10. Immediately before the measurement add 5 ml p-toluidine solution and 1 ml distilled water into flask b, mix the solutions, transfer to cuvette.
11. Mix and measure the extinction value (blank value) in photometer at 550 nm. after 3 min..
12. Add 5 ml p-toluidine solution and instead of water 1 ml barbituric acid solution into flask s.
13. Mix and measure the extinction value (sample value) at a wavelength of 550 nm. after 3 min.
14. Dilute the solution to a proper concentration, if the solution contains more than 50 mg HMF/l.

Calculation:

HMF–Extinction Value = Extinction value of the sample with 1 ml barbituric acid solution – Blank value.

Standard Graph for HMF:

The amount of HMF corresponding to the extinction of the sample is taken from a standard graph that is prepared from an HMF solution of known concentration in a similar manner. The graph is linear up to a concentration of 50 mg HMF/l.

Procedure:

1. Prepare 5-HMF solution by dissolving 125 mg HMF in 100 ml water.
2. Prepare standard graph with standard HMF solutions: 0, 5, 10, 15, 20, 25, 30, 40, 50, 70, 80 and 100 ppm.
3. Apply same procedure for all samples as in the determination procedure.
4. Plot standard curve for HMF by using the obtained absorbance values of the standard HMF solution versus ppm HMF.
5. Determine the slope of the standard curve as E/(ppm HMF). Calculate concentration of HMF as:

HMF mg/l = (1/slope of calibration curve)* E

where E is the extinction value of the sample.

HMF can be defined for fruit juices in mg per liter, for concentrates in mg per kg.

Remarks:

1. An elevated content of HMF indicates that heating of the fruit juice or concentrate has occurred.
2. If a spectrophotometer is available, the HMF can be determined in the UV-range near 284 nm as in the manner used for honey, after complete extraction with ether.
3. Absolutely pure HMF should be used for the preparation of standard curve. The necessary solutions must always be freshly prepared. Commercial HMF can e.g. be distilled in a bulb-tube (high vacuum, Bp. 155 °C at 0.01 Torr) and kept under nitrogen.
4. Deep red fruit juices (black currant etc.) or deep brown apple juice concentrates should be given active carbon treatment for complete decolorization instead of Carrez-finning.
5. High quality juices in general contain only a few mg HMF/l or none.

4.8.3.6. Determination of Sugars

The presence of added sucrose can be determined before and after the inversion by Lane and Eynon's method (Section 4.2.3.5.).

4.8.3.7. Determination of Ash and Ash Alkalinity

The ash is obtained by igniting the sample at 550 °C after a preliminary evaporation. The alkalinity of the ash is then determined by boiling with a measured excess of 0.1 N HCl (Section 5.2.), cooling and back titration with 0.1 N NaOH (Section 5.3.) using a methyl orange (Section 5.1.3.) as indicator.

$$1 \text{ ml } 0.1 \text{ N NaOH} \equiv 0.0069 \text{ g } K_2CO_3$$

4.8.3.8. Determination of Degree of Concentration

Degree of concentration is usually assessed industrially from the refractive index (or specific gravity). The following figures are extracted:

Table 4.23. Refractive index values of some concentrated fruits juices

Degree of concentration	Grapefruit RI (at 20 °C)	Lemon RI (at 20 °C)	Orange RI (at 20 °C)
3 x	1.3756-1.3790	1.3681-1.3710	1.3816-1.3860
4 x	1.3926-1.3970	1.3796-1.3820	1.3996-1.4040
5 x	1.4086-1.4120	1.3911-1.3940	1.4181-1.4230
6 x	1.4236-1.4270	1.4041-1.4070	1.4381-1.4430
7 x	1.4391-1.4430	1.4149-1.4175	1.4566-1.4610

4.9. ANALYSIS OF JAMS, MARMALADES AND HONEY

The basic principle of jam and marmalade manufacture is the boiling together of fruit (suitable prepared), sugars (sucrose syrup, invert syrup, glucose syrup) and water. During the boiling the proportion of solid matter in the mixture increases, a proportion of the sucrose is converted to invert sugar and a gel is produced on cooling.

When the process is carried out under atmospheric conditions, whereby the boiling point reaches is about 106 °C, the sugar tends to caramelise excessively and the flavor, etc., may be impaired.

Honey is the saccharine product gathered by bees from the nectar of flowers. The color of honey varies from almost colorless to nearly black according to the flower from which the nectar is taken. Storage at cool temperatures causes honey granulate, i.e. the sugar crystallises.

The main constituents of the honey are dextrose, fructose, sucrose, dextrin, mineral matter, protein and waxes. Pollen is invariable present in comb honey, but the absent in products which have been finely strained.

Most of the natural honey has negative optical rotation, but sucrose and glucose syrup both make the rotation more positive.

4.9.1. Composition of Jam, Marmalade and Honey

4.9.1.1. Composition of Honey

Table 4.24. Composition of honey

Chemical Properties	Composition
Water	17-20 %
Reducing Sugar	70-80 % (32-49 % Fructose) (22-44 % Glucose)
Sucrose	1.9 %
Nitrogenous material	0.3 %
Organic acid	0.1-0.4 %

Lactone	0.2-2.0 mg/100 g
Ash	0.1-0.35 %
Dextrin	1.5 %
Chlorine (Cl^-)	150 mg/kg
Sulphate (SO_4^{2-})	200 mg/kg

pH of the honey is around 3.3-4.6.

4.9.1.2. Composition of Jams

Table 4.25. Composition of some selected jams

Type of Jams	Insoluble matter (%)	Soluble solids (%)	Acidity (as malic acid) (%)	Invert sugar (%)	Sucrose (%)	Ash (%)	Glucose (%)
Apple	1.39	52.25	0.42	20.84	29.11	0.15	---
Apricot	1.90	80.66	1.87	52.53	21.38	0.57	---
Cherry	7.42	69.66	1.0	43.00	21.25	0.45	---
Marmalade	3.80	70.40	0.25	35.70	19.30	0.63	12.82
Strawberry	2.37	73.45	1.22	36.60	32.82	0.41	---

4.9.2. Standards for Sour Cherry Jam (TS 3958):

Soluble solid content should be (min.) 68 %, pH of the jam should be 2.8-3.4 and coloring matter should not be used in the manufacture.

Table 4.26. Turkish standards for sour cherry jam

Component	for first quality Jam	for second quality Jam
5-Hydroxymethyl furfural (5-HMF)	max. 25 (mg /kg)	max. 50 (mg /kg)
Preservatives	---	max. 1 (g/kg)
Fruit percentage (w/w) %	min. 45	min. 33
Amount of Seed (#/100 g)	max. 1	max. 2

Metallic contamination:

Copper, Cu, (mg/kg) : max. 5.0
Zinc, Zn, (mg/kg) : max. 5.0
Iron, Fe, (mg/kg) : max. 15
Tin, Sn, (mg/kg) : max. 250
Lead, Pb, (mg/kg) : max. 0.3

4.9.3. Analysis of Marmalade and Jams

The routine analysis of jams and marmalade should include the determinations of soluble solids, reducing sugars and total sugar, pH, HMF, fruit content and seed count.

4.9.3.1. Soluble Solids

The soluble solid content can be found by the refractometer at 20 °C (Section 4.4.2.7.).

4.9.3.2. Acidity and pH

1. Determine acidity (Section 4.7.2.1.) by titration 10 g of sample with 0.1 N NaOH (Section 5.3.) using phenolphthalein as indicator (Section 5.1.1.).
2. Calculate the total acidity as percentage of citric acid.
3. pH value is determined by pH-meter.

4.9.3.3. Determination of HMF

HMF value is determined as mentioned in the section of analysis of fruit juice (Section 4.8.3.5.).

4.9.3.4. Fruit Content

The minima for fruit jam and marmalade are prescribed in the Preservers Order. The fruit content can be assessed from;

$$Fruit\ (\%) = \frac{Percentage\ of\ \ A\ in\ the\ jam}{Average\ Percentage\ of\ \ A\ in\ the\ fruit}$$

where A is any suitable constituent, e.g. Insoluble solids, non-sugar solids, seed count, potassium, phosphorous.

Procedure:

1. Take 500 g of sample and add 500 ml of distilled water.
2. Wait 5 minute and sieve the mixture from 1.0 mm mesh size sieve.
3. Then wait again 5 minute.
4. Weigh the matter top of the sieve.

$$Fruit\ (\%) = \frac{M\,(g) * F}{M_i\ (g)}$$

where M_i is the weight of sample (g), M is the weight of sample top of the sieve, F is 1.5 for cherry, strawberry and apricot and 0.9 for quince.

4.9.3.5. Seed Count

1. Heat 50 g of sample in a porcelain basin with a little water on a water bath.
2. When the insoluble matter has sufficiently disintegrated, count the seeds.
3. Calculate the number of seeds present in 100 g of jam or marmalade.
4. The seed count is of assistance for mixed jams, where one fruit contains seeds and the other does not.
5. The individual seed should also be examined under the lens to confirm that they are genuine.
6. Set of genuine seeds should be kept for comparison purposes.

4.9.3.6. Determination of Sugar

The individual sugars present can be determined on the cleared solution, both before and after the inversion by either a volumetric, polarimetric or a gravimetric method. **Reducing sugars** alone are best determined by **Lane and Eynon's** method (Section 4.2.3.5.) by titration 10 ml of Fehling's solution with a 1.5 % solution of jam using a methylene blue as indicator.

4.9.4. Analysis of Honey

Methods of analysis are also include determination of reducing sugar, apparent sucrose (Lane and Eynon's method), moisture (by refractometer), insoluble solids, ash (600 °C), acidity and hydroxymethly furfural.

4.9.4.1. Determination of Total Solids and Water

The total solids can be estimated by drying in a vacuum oven at 70 °C. Alternatively the figure can be obtained from the specific gravity of a 20 % w/v solution.

$$Total\ solid\ in\ solution\ (\%) = \frac{sp.\,gr.\,of\ \ 20\,\%\ solution - 1}{0.00386}$$

Most genuine honeys contain 13-23 % water. Figures relating to the refractive index of honey with water content at 20 °C are given in Table 4.27.

Table 4.27. Figures related to the refractive index of honey with water content

Refractive Index (RI)	Water (%)
1.5044	13.0
1.5018	14.0
1.4992	15.0
1.4966	16.0
1.4940	17.0
1.4915	18.0
1.4890	19.0
1.4865	20.0
1.4840	21.0
1.4815	22.0
1.4790	23.0
1.4765	24.0
1.4740	25.0
1.4714	26.0

Temperature correction = - 0.00023 per degree.

4.9.4.2. Determination of Ash

Ignite the sample at 600 °C. The ash of genuine honey seldom exceeds 0.35 %. Only a minute trace of sulphate should be present in pure honey.

4.9.4.3. Determination of Total Nitrogen

Nitrogen content of honey can be determined by Kjeldahl method as mentioned in the section of analysis of flour (Section 4.1.3.6.).

4.9.4.4. Determination of HMF in Honey

Determination of HMF carried out as mentioned in the fruit juice section (4.8.3.5.).

4.9.4.5. Determination of Sugars

The principal sugars present in honey are fructose and dextrose together with a little sucrose and little dextrin.

Procedure:

1. Dilute 2 g to 250 ml and estimate the total reducing sugars by Lane and Eynon's method (4.2.3.5.).
2. Estimation of the dextrose:
    a) Add excess 0.1 M iodine (at least twice the iodine is used) to 25 ml of honey solution and 100 ml equal parts of 0.2 M sodium bicarbonate and carbonate solution.
    b) Allow to stand in the dark for 2 hours.
    c) Acidify with 12 ml dilute sulphuric acid (25 %) and titrate with 0.1 M thiosulphate.
    d) Carry out a blank at the same time.
    e) The difference between the two titrations represents the dextrose

    1 ml 0.1 M iodine ≡ 0.009005 g dextrose
3. Fructose = Total reducing sugars - Dextrose

Sucrose content can be determined before and after inversion by the Lane and Eynon's method.

4.9.4.6. Determination of Free acid and pH

1. Dilute 10 g with 75 ml water.
2. Titrate with 0.1 M sodium hydroxide (Section 5.3.) using phenolphthalein (Section 5.1.1.).
3. Express the result as percentage of formic acid or as milliequivalents of acid per kg of sample (= titrate x 10).
4. The pH value can be determined on a 10 % solution.

4.10. ANALYSIS OF CHOCOLATE

The essential ingredients of chocolate are cocoa, cocoa butter and sugar. The cocoa and cocoa butter are both obtained from the cocoa beans which grow in pods on cacao trees.

In the manufacture of cocoa and chocolate, the beans are first roasted in revolving drums and are then broken into small pieces by passing through special rollers. The husk is removed, leaving behind the small pieces of roasted bean.

Chocolate is made by mixing cocoa-mass with sugar, cocoa-butter and, for milk chocolate, dried milk or condensed milk. The mixing is carried out in melanguers, in which massive rollers rotate in contact with a heated plate. The mixture is then passes to a refining machine where it pinched between rollers revolving at different speeds. To complete the process the molten chocolate is then conhed for a period of up to 24 hours.

Conversion of liquid chocolate to a familiar solid chocolate bar is not simply a matter of pouring the chocolate into moulds and allowing it to cool. The fats present in the cocoa butter can solidify in six different forms, or polymorphs, with different melting point. One of them melts at 33.8 °C and when only this form is present in the solid chocolate it will be smooth and glossy and will easily melt in the mouth. To produce as much of this polymorph as possible chocolate is subjected to a special heat treatment process called Tempering. The molten chocolate is cooled until it just begins to solidify and then it is reheated to just below the melting point of the desirable polymorph. The chocolate is then stirred at this temperature so that a high proportion of the fat will solidify in the preferred polymorphic form when the chocolate is finally moulded or used for coating.

Chocolate is a nutritious food and a small bar (100 g) of milk chocolate provides about 9 g of protein and 220 mg calcium, approximately one-eight and one-half respectively of the RDA of the these nutrient for a moderate active man.

4.10.1. Composition of Chocolate

Ranges of the composition of the some type of chocolate is given in the following Table.

Table 4.28. Composition of some chocolates

	Plain chocolate	Milk chocolate	Nut chocolate

	%	%	%
Moisture	0.8-2.3	0.8-1.8	1.1
Total ash	1.0-2.5	1.8-2.4	1.5
Water soluble ash	0.7-1.1	--	--
Fat	30-40	30-40	36.6
Sucrose	37-55	35-47	45.2
Lactose	--	7-11	6.9
Starch	2-7	--	--
Total nitrogen	0.6-1.3	0.8-1.5	1.12
Theobromine	0.4-0.7	0.2-0.5	--
Crude fibre	0.8-1.5	0.4-1.0	--
Values on the fat:			
Reichert value	--	4.3,5.6	4.8
Polenske value	--	1.2,1.1	1.3
Kirschner value	--	4.0,5.3	4.2

4.10.2. Standards of Chocolate (TS 7800)

Table 4.29. Turkish standards for Chocolate

Component	Limits		
	for milk type	for bitter type	for white type
Moisture	max. 1.5 %	max. 1.5 %	max. 1.5 %
Metallic contamination			
Arsenic, As, mg/kg	max. 0.5	max. 1.0	max. 0.5
Copper, Cu, mg/kg	max. 15.0	max. 30.0	max. 15.0
Lead, Pb, mg/kg	max. 1.0	max. 2.0	max. 1.0
Cacao oil	min. 20 %	min. 24 %	min. 20 %
Fat free cacao	min. 5 %	min. 16.0 %	—
Fat free milk powder	min. 10.0 %	—	min. 10.0 %
Total sugar, sucrose	max. 60 %	max. 60 %	max. 60 %
Starch	max. 2.0 %	max. 4.0 %	—
Crude fiber	max. 2.5 %	max. 6.0 %	—
Ash	max. 2.5 %	max. 2.0 %	max. 2.5 %
Ash in alkaline cacao	max. 4.0 %	max. 6.0 %	—

4.10.3. Analysis

The quality of chocolate can be assessed by determining the following: moisture (by drying at 100 °C), fat (by continuous extraction using a petroleum ether), sugars (by Lane and Eynon's method), nitrogen (by Kjeldahl method), ash, water-soluble ash, crude fibre and trace metals (e.g. As, Pb and Cu). In addition, the oil values should be determined on the fat.

4.10.3.1. Determination of Moisture Content

Moisture content can be determined by drying at 100 °C.

4.10.3.2. Determination of Sucrose (Polarimetric Method)

Polarimetric method can be carried out for determination of sucrose in chocolate products.

1. Transfer 26 g prepared sample (Section 2.9.) into 250 ml centrifuge bottle.
2. Add 100 ml petroleum ether, shake 5 min., and centrifuge.
3. Decant clear solvent carefully and repeat extraction with petroleum ether.
4. Place bottle containing defatted residue in warm place until petroleum ether is expelled.
5. Add 100 ml water and shake until most of the chocolate is detached from sides and bottom of bottle.
6. Loosen stopper and carefully immerse bottle 15 min. in water bath kept at 85–90 °C, shaking occasionally to remove all chocolate from the sides of bottle.
7. Remove from bath, cool, and add basic $Pb(OAc)_2$ solution (sp.gr. 1.25) to complete precipitation (5 ml is usually enough).
8. Add water to make total of 110 ml added liquid.
9. Mix thoroughly, centrifuge, and decant supernate through small filter.
10. Precipitate excess Pb with powder dry $K_2C_2O_4$ and filter.
11. Pipette 50 ml portion Pb-free filtrate into 100 ml volumetric flask and add 20 ml of water.
12. Add, little by little, while rotating flask, 10 ml HCl (sp. gr. 1.029 at 20/4 °C or 24.85° Brix at 20 °C) (Section 5.2.).
13. Heat water bath and adjust heater to keep bath at 60 °C.
14. Place flask in water bath, agitate continuously 3 min., and leave flask in bath exactly 7 min. longer.
15. Place flask at once in water at 20 °C.
16. When contents cool to 35 °C, dilute almost to mark.
17. Leave flask in bath at 20 °C at least 20 min. longer and finally dilute to mark.
18. Mix well and polarise solution in 200 mm tube provided with lateral branch and water jacket, keeping temperature at 20 °C.

19. This reading must be multiplied by 2 to obtain invert reading.
20. If it is necessary to work at temperature other than 20 °C, which is permissible within narrow limits, volume must be completed and both direct and invert polarisations must be made at exactly same temperature.
21. Calculation of % sucrose, S, by following formula

$$S = \frac{100 * (P - l)}{132.56 - 0.0794 * (13 - m) - 0.53 * (t - 20)}$$

where *P* is direct reading, normal solution; *l* is invert reading, normal solution; *t* is temperature at which readings are made; and *m* is total solids from original sample in 100 ml inverted solution. To obtain *m* for liquids determine total solids in original solution as % by weight, and multiply this figure by weight original sample in 100 ml invert solution.

4.10.3.3. Determination of Fat Content

Applicable to cacao products with or without milk ingredients to products prepared by cooking with sugar and water, and drying.

1. Accurately 3–4 g prepared sample liquor, 4–5 g cacao, 4–5 g sweet chocolate, or 9–10 g milk chocolate into 300–500 ml beaker.
2. Add slowly, while stirring, 45 ml boiling water to give homogeneous suspension.
3. Add 55 ml 8 N HCl (2+1) (Section 5.2.) and few defatted SiC chips or other antibumping agents, stir.
4. Cover with watch glass, bring slowly to boil, and boil gently 15 min.
5. Rinse watch glass with 100 ml water.
6. Filter digest through 15 cm S&S 589 medium fluted paper, or equivalent, rinsing beaker 3 times with water.
7. Continue washing until last portion of filtrate is Cl free as determined by addition of 0.1 N $AgNO_3$ (Section 5.7.).
8. Transfer wet paper and sample to defatted extraction thimble and dry 6-18 hours in small beaker at 100 °C.
9. Place glass wool plug over paper.

10. Add few defatted antibumping chips to 250 ml erlenmeyer and dry 1 hour at 100 °C.
11. Cool to room temperature in desicator and weigh.
12. Place thimble containing dried sample in soxhlet apparatus (Figure 4.4).

Figure 4.4. Soxhlet apparatus for the extraction of solids.

13. Rinse digestion beaker, drying beaker, and watch glass with three 50 ml portions petroleum ether, and washings to thimble.
14. Reflux digested sample 4 hour, adjusting heat so that extractor siphons ≥ 30 times.
15. Remove flask and evaporate solvent on steam bath.

16. Dry flask at 100–101 °C to constant weight (1.5–2 hour), cool in desicator to room temperature and weigh.
17. Constant weight is attained when successive 1 hour drying periods show additional loss of < 0.05 % fat.

$$\% \ Fat = \frac{wt.\ of\ \ fat\ (g)}{wt.\ of\ \ sample\ (g)} * 100$$

4.10.3.4. Determination of Protein

Determination of protein in chocolate products carried out by Kjeldahl method as in section (4.1.3.6.).

4.10.3.5. Determination of Ash

Determination of ash content in chocolate products can be followed as in section (4.1.3.1.).

4.10.3.6. Determination of Crude Fiber

Determination of crude fiber can be done as in section (4.1.3.9.).

4.11. ANALYSIS OF MEAT AND MEAT PRODUCTS

In most society, it is suitable to subject meat to some form of heat treatment prior to consumption and it is readily apparent to even the most native consumer that the quality of the cooked products varies markedly with the type of the heat treatment. Flavor, color and the texture are all important attributes of cooked-meat quality but it is well established that the texture (tenderness) is of paramount

importance and this quality factor usually dictates the method of cooking

Although texture, flavor and color are the main factors involved in selecting the mode of domestic cooking, for several commercial processes a heating regime may be used primarily to control bacterial growth. For example, a relatively mild treatment may be employed to pasteurise fresh or cured meat or a more intense heat treatment may be applied to sterilise canned products. These treatments will also cook the meat and so affect its quality.

Fresh sausage meat normally consists of a mixture of various cuts of meat, usually beef, wheat rusk, seasoning (salt, spices, or spice extracts), water and sulphite preservative. Some products contain flavor enhancers, polyphosphates and other emulsifiers, vitamin C, coloring matter and various protein supplements derived from soya milk and possibly cereals and peanut.

4.11.1. Composition of Meat

Typical values for the concentration of the different components to be found in the skeletal muscle (lean) are given in Table 4.30.; the actual composition for a given muscle will vary with the breed, age, sex, and diet of the animal as well as its anatomical location.

Table 4.30. Composition of meat

Component	Composition (%)
Water (range 65-80%)	75.0
Protein (range 16-22%)	18.5
Lipids (Variable range 1.5-13.0 %)	3.0
Non-Protein nitrogenous substances	1.5
Carbohydrates and non-nitrogenous substances (range 0.5-1.5 %)	1.0
Ash	1.0

4.11.2. Composition of Chicken and turkey

Edible meat varies from 34.4 to 39.1 % for roasted turkey, of which approximately 60 % is light and 40 % is dark. The yield of edible meat may be as low as 28.4 % from chicken wings and as high as 62.3% from breast and thigh. Edible yield of poultry is influenced by age, size, strain, processing techniques, phosphate treatment and cooking methods. The composition of chicken and turkey are given in Table 4.31.

Table 4.31. Composition of turkey and chicken

Species and description	Moisture (%)		Protein (%)		Fat (%)	
	Raw	Cooked	Raw	Cooked	Raw	Cooked
Chicken:						
Fryers	75.7	53.3	18.6	30.7	4.9	11.8
Roasted	63.0	53.5	18.2	25.2	17.9	20.2
Hens and cocks	56.9	45.9	17.4	24.0	24.8	29.5
Mean	65.2	50.9	18.1	26.6	15.9	20.5
turkey*:*						
All classes	64.2	55.4	20.1	27.0	14.7	16.4

4.11.3. Composition of Sausage

Table 4.32. Composition of beef sausage

Components	Composition		
	Minimum	Maximum	Mean
Water (%)	40.6	52.5	48.2
Fat (%)	16.6	33.2	24.9
Protein (%) Nx6.25	7.5	12.7	9.4
Ash (%)	2.2	2.9	2.5
Carbohydrate (%)	7.8	18.1	13.2
Defatted meat (%)	25.0	57.9	37.3
Total meat (%)	51.8	75.9	61.5
Salt	1.4	2.0	1.7
Sulphur dioxide (mg/kg)	45.0	450	233

4.11.4. Standards of Sausage (TS 980) and Turkish Soudjuck (TS 1070)

Table 4.32. Common Turkish standards for sausage

Components	Limits
Moisture (mass)	max. 65 %
Salt (mass)	max. 3 %
Total Animal Fats (mass)	min. 15 %
Potassium or Sodium Nitrate	max. 500 mg /kg
Potassium or sodium Nitrite	max. 200 mg/kg
Starch	max. 5 %
Color and Flavor	Appropriate

Bad Flavor Test	Negative
pH	max. 6.30
Coloring matter	No

Table 4.33. Turkish standards for Turkish Soudjuck

Chemical Properties	Limits
Moisture (mass)	max. 40 %
Salt (mass)	max. 5 %
Coloring matter	None
pH	max. 5.8
	min. 5.4
Fat (mass)	
for first quality	max. 30 %
for second quality	max. 40 %
for third quality	max. 50 %
Protein (mass) Nx6.25	
for first quality	min. 22 %
for second quality	min. 20 %
for third quality	min. 20 %

4.11.5. Analysis of Turkish Soudjuck

4.11.5.1. Determination of Fat

1. Extractable Fat:
   a) Extract the fat from the dried residue with light petroleum ether or n-Hexane in a continuous extraction apparatus.
   b) After approximately 90 minutes of extraction, remove and regrind the material in order to effect complete extraction.

2. Total Fat:
   c) Boil 3 5 g with 50 ml 4 M HCl (Section 5.2.), dilute and extract with petroleum ether or n-Hexane.

4.11.5.2. Determination of Moisture

1. Weigh 5 g sample and dry at 100 - 103 °C.
2. Determine the moisture content.

4.11.5.3. Determination of Protein

Kjeldahl method is applied to determine the protein content (Section 4.1.3.6.).

4.11.5.4. Determination of Salt

1. Take 25 g sample with 240 ml cold water.
2. Shake the sample and filter.
3. Then titrate 10 ml of the filtrate with 0.1 M silver nitrate solution (Section 5.7.) using potassium chromate (Section 5.1.5.) as an indicator.

4.11.5.5. Determination of pH

Procedure:

1. Take minimum 200 g of sample. pH analysis should be done as soon as possible.

a) For Homogenizable sample:

2. Mince the sample twice.
3. Homogenise the minced sample
4. Take a portion of homogenised sample which should be cover the electrode.
5. Place the electrode into the sample.
6. Read the pH.
7. Wash the electrode first with diethyl ether and than with ethanol.

b) For Non-Homogenizable sample:

2. If the sample is hard drill it at different points.
3. Immerse the electrode into the drilled point.
4. Read the pH value of each point.
5. Calculate the arithmetic mean of the pH values read for different points.
6. Wash the electrode with the same was as in the Homogenizable section.

4.11.5.6. Off-Odor TEST

This method based on the detection of ammonia in the sample. Positive result means that the odor of sample is bad.

Reagent:

Nessler reagents (16 g potassium iodine, 24 g mercury iodine, 75 g potassium hydroxide are dissolved in 560 g water).

Procedure:

1. Put a small piece of sample into petri dish.
2. Pour the Nessler reagent on the sample.
3. If sample is in bad odor, color of the sample turns to dark orange-brown color.

4.11.6. Analysis of Sausage

4.11.6.1. Determination of Moisture

Moisture content can be determined as in section (4.11.5.2.).

4.11.6.2. Determination of Total Animal Protein

Total protein can be determined as in section (4.1.3.6.).

4.11.6.3 Determination of Salt

Salt content of the sausage can be determined as in section (4.11.5.4.).

4.11.6.4. Determination of pH

Determination of pH carried out as mentioned in the section of Turkish Sudjuck (4.11.5.5.)

4.11.6.5. Determination of Potassium or Sodium Nitrate

In view of possible rapid changes, determinations of nitrate and nitrite should be commenced as soon after sampling as possible. Nitrite can be determined on the 10 % extract. If cereal is present a clearing agent should preferably be employed as mentioned in the method for nitrate below.

Regents:

1. Zinc rods: length about 15 cm and diameter 5-7 mm.
2. Cadmium sulphate solution: Dissolve 37 g cadmium sulphate (3 $CdSO_4$ .H_2O) in water and dilute to 1000 ml.
3. Activated Charcoal
4. Borax solution: Dissolve 50 g borax in a litre of warm water and allow to cool.
5. Coupling reagent: Freshly prepare a 0.5 % solution of N-(1-Napthhyl)-ethylene-diamine hydrochloride in water.
6. Sulphanilamide solution (0.5 %): Dissolve 1.25 g sulphanilamide in 250 ml hydrochloric acid (1+1).
7. Standard nitrite solution: Dissolve 0.150 g of sodium nitrite in water and dilute to one litre (1 ml = 100 μg NO_2^-). Dilute to produce solutions containing 10 and 1 μg NO_2^- per ml.
8. Ammonia buffer solution: Dilute 20 ml concentrated hydrochloric acid with 500 ml water, carefully add 50 ml 0.88 ammonia, dilute to one liter with water, mix and check the pH is 9.6-9.7.

Procedure:

Preparation of cadmium reducing column:

1. Place 5 zinc rods in sufficient cold cadmium sulphate solution in a beaker.
2. Remove the spongy metallic deposit from the zinc rods about every hour by swirling them in the solution or rubbing them against each other.
3. After about 7 hours decant the solution and wash the deposit with a litre of water, ensuring that the cadmium is always covered with a layer of liquid.
4. Transfer the cadmium deposit with approximately 0.1 M HCl and blend in a mechanical mixer for 10 second and return to the beaker.
5. Occasionally stir up the deposit with a glass rod and after allowing to stand overnight under 0.1 M acid, stir once more to remove all bubbles of gas from the metal.
6. Decant the liquid and wash the cadmium slurry twice with a litre of water.
7. Fit a glass wool plug to the bottom of the glass column (Figure 4.5).
8. Then wash the cadmium occasionally during filling, ensuring that the metal

is always covered with liquid.

9. Inclusions of the gas can be eliminated with a knitting needle.
10. The liquid should flow out at a rate not exceeding 3 ml per minute or there is danger of incomplete reduction.

Preparation of Extract of Sample:

1. To a 250 ml wide necked conical flask add 10 g sample (sausage), 100 ml hot water (80 °C), 5 ml borax solution and 0.5 g activated charcoal and heat, with repeated agitation, for 15 min. on a boiling water bath.
2. Allow to cool and after not less than an hour add 2 ml of each of the freshly prepared zinc ferrocyanide clearing reagents (mixture of zinc acetate solution, 21.9 g zinc acetate Zn $(C_2H_3O_2)_2.2H_2O$ and 3 ml glacial acetic acid in water dilute to 100 ml, and potassium ferrocyanide solution, 10.6 % aqueous solution), swirling after each addition.
3. Add 5 ml more borax solution and transfer to a 200 ml volumetric flask. After allowing to stand for 30 min. make up to the mark with water, mix and filter
4. though a No. 44 filter paper.

Figure 4.5. Apparatus for nitrate reduction (Dimensions in mm).

Determination of nitrite

1. Pipette a suitable volume of sample filtrate (containing less than 100 μg NO_2^-) into a 50 ml volumetric flask, dilute to approximately 40 ml add 5 ml sulphanilamide solution and allow to stand for 3 min.
2. Then add 2 ml coupling reagent, make up to the mark with water and mix.
3. After 20 min. measure the optical density at 540 nm in a 1 cm cell against a solution produced by mixing 40 ml water with 5 ml sulphanilamide solution and 2 ml coupling reagent.
4. Treat suitable volumes of the dilute nitrite solutions in 50 ml volumetric flask as described for the sample extract.
5. Construct the graphs covering the ranges 0-20 μg NO_2^- and 20-100 μg NO_2^- in the final solution.
6. Convert the sample reading to μg NO_2^- and express as mg/kg $NaNO_2$ in the sample.

Alternative method for determination of the nitrite:

1. Pipette a suitable volume of the sample add to drop of the nitrite indictor reagent (reagent I:1.5 g sulphanilic acid is dissolved in 300 ml o 15 % acetic acid, reagent II: boil a 0.5 g α-nafhtphalamine in 60 ml of water, and filter the hot solution onto 300 ml 15 % acetic acid).
2. Allow to stand 5 min.
3. Light pink color indicates 200 ppm of nitrite, after 5 min. pink color indicates 160-200 ppm of nitrite.
4. Compare the color of the samples having the indicator with the standards of known concentrations of sodium nitrite.

Determination of Nitrate

1. Pipette 20 ml of the sample filtrate into a 50 ml beaker, add 5 ml ammonia buffer, mix and transfer to the reservoir of the freshly prepared cadmium reducing column.
2. Collect the column elute, washing the column with water until 100 ml has been collected.
3. Pipette a suitable volume of elute into a 50 ml volumetric flask and continue as for the determination of nitrite.
4. Convert the difference between the nitrite content before and after reduction to μg $NaNO_2$ and express the result as mg/kg $NaNO_3$ in the sample.
5. Allowances must be made for blanks due to the reagents (up to 20 mg/kg $NaNO_3$) and the sample extract constituents which produce colors on diazotisation. (Approximately 10 mg/kg).

4.11.6.6. Off-Odor Test

Determination of bad flavor is done as in section (4.11.5.6.).