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DRAFT TANZANIA STANDARD

Macaroni, spaghetti, vermicelli and noodles – Specification

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TANZANIA BUREAU OF STANDARDS

0 Foreword

Macaroni, spaghetti and vermicelli and noodles belong to a class of food products generally known as pasta products.

In Tanzania the production of pasta products is of recent origin. This Tanzania Standard, therefore, has been developed in order to encourage the growth of the industry in the country, and at the same time ensure safe and good quality products.

The principal raw materials for making pasta products are semolina, wheat flour and water. The minor ingredients which may be added are milk powder, vegetables, spices and eggs. Besides, they may also be enriched with vitamins and minerals.

In the preparation of this Tanzania Standard assistance was derived from:-

a) IS: 1485 – 2015 *Macaroni, Spaghetti and Vermicelli and Egg noodles- Specification*; published by the Bureau of Indian Standards.

b) SLS 420:1989 *Specification for pasta products*; published by Sri Lanka Standards Institution.

In reporting the results of a test or analysis made in accordance with provision of this Tanzania Standard, if the final value observed or calculated is to be rounded off, it shall be done in accordance with TZS 4 (See clause 2)

1.0 SCOPE

This Tanzania Standard prescribes the requirements, methods of sampling and test for macaroni, spaghetti, vermicelli and noodles intended for human consumption.

2.0 REFERENCES

The following referenced standards are indispensable for the application of this Tanzania Standard. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced standard (including any amendments) applies:

TZS 109 - Code of hygiene for food processing units - General.

TZS 59 - Water - Distilled quality - Specification

TZS 4 - Rounding off numerical values.

TZS 538/ EAS 38 – Packaging and labeling of food

TZS 439 – Wheat flour – Specification

TZS 132/ EAS 35 – Fortified food grade salt - Specification.

TZS 111 Bakery and biscuits manufacturing units – Code of hygiene

TZS 1335 Animal and vegetable fats and oils – Determination of copper, iron and nickel content – graphite furnace atomic absorption

TZS 268 General atomic absorption spectrophotometric method for determination of lead in food stuffs.

TZS 76 Methods for determination of arsenic content.

TZS 118 Microbiology of food and animal feeding stuffs – Horizontal method for enumeration of micro-organisms – Colony count technique at 30°C

TZS 122 Microbiology of food and feeding stuffs – Horizontal method for the detection of salmonella ssp

TZS 125 Microbiology of food and animal feeding stuffs – Horizontal method for enumeration of coagulase – Positive staphylococcus aureus and other species

TZS 131 Microbiology – General guidance for enumeration of yeast and moulds – Colony count technique at 25°C

3.0 TERMS AND DEFINITIONS

For the purpose of this Tanzania standard the following terms and definitions shall apply:-

3.1 macaroni

Is a variety of dry pasta traditionally shaped into narrow tubes produced in various shapes and sizes, made with durum wheat.

3.2 spaghetti

Is long, thin, cylindrical, solid pasta, made of milled wheat and water.

3.3 vermicelli

Is a traditional type of pasta round in section similar to spaghetti.

3.4 egg noodles

It is a narrow strip of pasta dough made with eggs.

3.5 noodles

4.0 Requirements

4.1 General requirements

4.1.1 Form and dimensions

The forms and dimensions of macaroni, spaghetti, vermicelli and egg noodles shall be as given below:-

4.1.1.1 Macaroni

They may be of two types, namely long macaroni or cut macaroni.

a) Long macaroni – shall be in the form of tubular rods, smooth or corrugated. These shall have the outer diameter ranging between 3mm and 5 mm and of wall thickness of about 1 mm.

b) Cut-macaroni - shall be obtained by extrusion and may be in the form of tubular elbows, shells, alphabets, numerals, stars, wheels, rings, rice, melon seeds etc.

4.1.1.2 Spaghetti - shall be in the form of solid rods of diameter 1 mm.

4.1.1.3 Vermicelli - shall be in the form of solid rods or of slightly curved or bend product of diameter between 0.5 mm and 1.25 mm.

4.1.1.4 Egg noodles – The material shall be in the form of ribbons, and shall have not less than 5.5 percent by mass of egg solids or egg yolk solids when tested by the method given in Annex H.

4.1.2 Other physical characteristics

Macaroni, spaghetti, vermicelli and noodles shall be of good characteristic colours, flavour and odour and shall be free from rancidity, bitterness, mustiness or any other undesirable taste or odour. It shall also be free from impurities, any foreign matter, cracks, flaws, mould, insect infestation or other spoilage. The material shall retain its shape and show no signs of disintegration and shall swell appreciably when plunged into vigorously – boiling water and boiled for 10 minutes.

NOTE - In case of instant or quick cooking noodles, the material shall be boiled for the time declared in the cooking instructions. The material shall be smooth to the touch (not stick together) and shall not contain any added colouring matter.

4.1.3 Enrichment

Subject to the agreement between the purchaser and the supplier, the product may be fortified with one or more of the following:

- a) Minerals – calcium, phosphorus and iron.
- b) Vitamins – A, B complex and D.

4.2 Ingredients

4.2.1 Essential ingredients

The following materials shall be used in the manufacture of macaroni, spaghetti, vermicelli and noodles and shall be of food grade quality and conform to the relevant Tanzania standard specifications, where available and other materials shall be free from foreign matter, insect infestation and objectionable flavours and odours.

The materials are wheat flour, water and Egg (only in the case of egg noodles).

4.2.2 Optional Ingredients

In addition to the essential ingredients specified under 4.2.1, any of the following ingredients may be used in the manufacturer of macaroni, spaghetti, vermicelli and egg noodles:

- a) Milk
- b) Soya flour
- c) Vegetable or vegetable products
- d) Spices
- e) Gluten
- f) Edible oilseeds flour
- g) Edible common salt
- h) Fruit or fruit products (preserved, dehydrated or pulp).

4.3 Specific requirements

4.3.1 The product shall also comply with the requirements given in Table 1.

Table 1 - Requirements for Macaroni, Spaghetti, Vermicelli and Egg noodles

SIN	Characteristics	Requirements	Method of Test (ref. to annex)
1	Moisture, <i>per cent by mass</i> , max	12	A
2	Total ash (on dry <i>basis</i>), <i>per cent by mass</i> max.	0.7	B
3	Acid insoluble ash (on dry <i>basis</i>) <i>per cent by mass</i> max.	0.05	C
4	Total protein (Nx 5.7) (on dry <i>basis</i>), <i>per cent by mass</i> , min	10	D
5	Cooking test: Total solids in gruel, <i>per cent by mass</i> , max	8	E
6	Free acidity (ml of 1N, NaOH solution per 100g of product), max.	4	F

4.4 Food additives

Only the food additives permitted by CODEX STAN 192 in the manufacture of macaroni, spaghetti, vermicelli and egg noodles may be used without exceeding the limits.

5.0 Hygiene

5.1 The macaroni, spaghetti, vermicelli and noodles shall be prepared and packaged in premises built and maintained under hygienic condition in accordance with TZS 109 and TZS 111 (see clause 2)

5.2 Macaroni, spaghetti, vermicelli and noodles shall be free from pathogenic organisms and shall comply with microbiological limits in Table 2.

Table 2 - Microbiological limits for macaroni, spaghetti, vermicelli and noodles

Types of micro-organism	Limits	Test methods
Yeast and mould count (cfu per g, max).	10 ²	TZS 131
<i>E.coli</i> (MPN per 1g)	absent	TZS 731
Salmonella in 25g	absent	TZS 122

6.0 CONTAMINANTS

The macaroni, spaghetti, vermicelli and noodles shall comply with the heavy metal contaminants limits stipulated in Table 3

Table 3. Requirements for Heavy metal in macaroni, spaghetti, vermicelli and noodles

S/N	Contaminant	Requirements (max.limits. mg/kg)	Test method
1	Lead (Pb)	0.5	TZS 268
2	Copper(Cu)	2.0	TZS 1335
3	Arsenic(Ar)	1.0	TZS 76

7.0 Sampling and method of test

7.1 Sampling

Representative samples of the product (macaroni, spaghetti, vermicelli and egg noodles) shall be drawn as prescribed in annex G.

7.2 Test methods

Test shall be carried out as prescribed under 4.3.1, 6.2 and 7.0 and appropriate appendices specified in Tables 1, 2 and 3.

7.3 Criteria for conformity

Criteria for conformity - The criteria ascertaining conformity to the requirements specified in this Tanzania Standard shall be as given in annex G.

8.0 Packaging, marking and labelling

8.1 Packaging

The product shall be packed in suitable cardboard cartons with a lining of moisture proof material or in suitable plastic film or moisture – proof paper bags, sealed to prevent entrance of moisture. Tinline containers may also be used .The size of the container is subject to agreement between the purchaser and the supplier.

8.2 Marking and labelling

Labelling of macaroni, spaghetti, vermicelli and egg noodles shall be done in accordance with the requirements of the TZS 538(EAS 38) and the following particular shall be clearly and indelibly marked on each pack of macaroni, spaghetti, vermicelli and egg noodles on the outer side of the wrapper; printing ink if any, shall be non-toxic and non-transferable:

- a) Name of the product;
- b) Type of the product
- c) If enriched with vitamins; (i) the word 'vitaminized' placed in brackets, below (a); and (ii) the details of enrichment and quantities added;
- d) If spices have been added, the word 'spiced' to be given on the label
- e) If egg has been added, it shall be declared on the label;
- f) All ingredients shall be declared on the label in descending order of proportions;
- g) Name and address of the manufacture;
- h) Batch or code number;
- i) Net mass;
- j) Date of manufacture;
- k) Expiry date;
- l) Cooking instructions; and

8.2.2 The language on the label shall be "Kiswahili" or Kiswahili and English. A second language may be used depending on the designated market.

8.3 Each pack may also be marked with the TBS Certification mark.

NOTE – The use of TBS Certification mark is governed by the provisions of the standards Act, 2009. Details of conditions under which a licence for use of the TBS Certification mark may be granted to manufacturers, may be obtained from TBS.

ANNEX A - DETERMINATION OF MOISTURE CONTENT

A.1 PROCEDURE

A.1.1 Preparation of sample - Grind in a pestle and mortar about 30 g of the material so that at least 90 per cent passes through 425-micron sieve. Transfer this prepared sample to a well-stoppered glass bottle for use as indicated in A-1.2 and 0-3.1.

A-1.2 Weigh accurately about 5 g of the prepared sample in a suitable moisture dish, made of porcelain, silica or platinum, previously dried in an air-oven and weighed. Place the dish in the air-oven maintained at $105^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for five hours. Cool the dish in a desiccator and weigh the dish with the lid on. Heat again at $105^{\circ}\text{C} \pm 2^{\circ}\text{C}$ in the air-oven for 30 minutes. Cool the dish in the desiccator and weigh. Repeat this process of heating for 30 minutes, cooling and weighing till the difference in mass between two successive weighings is less than one milligram. Note the lowest mass.

NOTE - Preserve the dish containing this dried material for the determination of total ash (see B.1).

A.2 CALCULATION

$$\text{Moisture, per cent by mass} = \frac{100 (m_1 - m)}{(m_2 - m)}$$

Where

m_1 = mass in g of the dish with the material before drying

m = mass in g of the empty dish, and

m_2 = mass in g of the dish with the material after drying

ANNEX B
DETERMINATION OF TOTAL ASH

B-1 PROCEDURE

Ignite the dried material (see A.1.2) in the dish with the flame of a suitable burner for about one hour. Complete the ignition by keeping in a muffle furnace at $600^{\circ}\text{C} \pm 20^{\circ}\text{C}$ until grey ash results. Cool in a desiccator and weigh. Repeat the process of heating for 30 minutes, cooling and weighing till the difference in mass between two successive weighing is less than one milligram. Note the lowest mass.

NOTE - Preserve the dish containing the ash for the determination of acid insoluble ash (see C.2).

B.2 CALCULATION

Total ash (on dry basis), per cent by mass =
$$\frac{100 (m_2 - m)}{m_1 - m}$$

Where

m_2 = mass in g of the dish with the ash,
 m = mass in g of the empty dish, and
 m_1 = mass in g of the dish with the dried material (m_2 in A.2)

ANNEX C
DETERMINATION OF ACID INSOLUBLE ASH

C.1 REAGENTS

C.1.1 Dilute Hydrochloric Acid - approximately 5 N, prepared from concentrated hydrochloric acid.

C.2 PROCEDURE

To the ash contained in the dish (see B.1) add 25 ml of dilute hydrochloric acid, cover with a watch-glass and heat on a water-bath for 10 minute. Allow to cool and filter the contents of the dish through Whatman filter paper No. 42 or its equivalent. Wash the filter paper with water until the washings are free from the acid and return it to the dish. Keep it in an air-oven maintained at $105^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for about three hours.

Ignite in a muffle furnace at $600^{\circ}\text{C} \pm 20^{\circ}\text{C}$ for one hour. Cool the dish in a desiccator and weigh. Heat again at $600^{\circ}\text{C} \pm 20^{\circ}\text{C}$ in the muffle furnace for 30 minutes. Cool the dish in the desiccator and weigh. Repeat the process of heating for 30 minutes, cooling and weighing till the difference in mass between two successive weighing is less than one milligram. Note the lowest mass.

C.3 CALCULATION

C.3.1 Acid insoluble ash (on dry basis) per cent by mass =

$$\frac{100(m_2-m)}{m_1-m}$$

Where

m_2 = Mass in g of the dish with the acid insoluble ash,

m = Mass in g of the empty dish, and

m_1 = mass in g of the dish with the dried material

ANNEX D
DETERMINATION OF TOTAL PROTEIN

D.1 APPARATUS

D -1.1 Kjeldahl apparatus

D-1.2 Kjeldahl flask - of capacity 500 ml.

D -2 REAGENTS

D -2.1 anhydrous sodium sulphates

D -2 .2 Copper sulphate

D -2.3 Concentrated sulphuric acid - sp. gr. 1.84

D -2.4 Sodium hydroxide solution - Dissolve about 225 g of sodium hydroxide in 500 ml of water.

D -2 .5 Standard sulphuric acid - 0.1 M.

D -2 .6 Methyl red indicator solution - dissolve one gram of methyl red in 200 ml of rectified spirit (95 per cent by volume)

D-2.7 Standard sodium hydroxide solution - 0.1 M.

D.3 PROCEDURE

D.3.1 Transfer carefully about one gram of the prepared sample, accurately weighed, into the Kjeldahl flask, taking precaution to see that particles of the material do not stick on the neck of the flask. Add about 10g of anhydrous sodium sulphate, about 0.5 g of copper sulphate and 25 ml concentrated sulphuric acid.

Place the flask in an inclined position. Heat below the boiling point of the acid until frothing ceases. Increase heat until acid boils vigorously and digest for 30 minutes after the mixture becomes clear and pale green or colourless. Cool the contents of the flask. Transfer quantitatively to the round bottom flask, with water, the total quantity of water used being about 200 ml. Add with shaking a few pieces of pumice stones to prevent bumping. Add about 30 ml of the sodium hydroxide solution (enough to make the solution alkaline) carefully through the side of the flask so that it does not mix at once with the acid solution but forms a layer below the acid layer.

Assemble the apparatus taking care that the tube extends below the surface of the standard sulphuric acid contained in the beaker. Mix the contents of the flask by shake until all ammonia has passed over into the standard sulphuric acid. Shut off the burner and immediately detach the flask from the condenser. Rinse the condenser thoroughly with water into the beaker. Wash the dip tube carefully so that all traces of the condensate are transferred to the beaker. When all the washing has drained into the beaker add two or three drops of methyl red indicator solution and titrate with the standard sodium hydroxide solution.

D.3.2 Carry out a blank determination using all reagents in the same quantities but without the material to be tested.

D.4 CALCULATION

$$\text{Total protein (on dry basis), per cent by mass} = \frac{798 (A - B) N}{M1 (100 - M)}$$

Where

B = volume in ml of the standard sodium hydroxide solution used to neutralize the acid in the blank determination,

A = volume in ml of the standard sodium hydroxide solution used to neutralize the excess of acid in the test with the material,

N = normality of the standard sodium hydroxide solution,

M1 = mass in g of the prepared material taken for the test,
and

M = moisture, percent by mass of the material.

ANNEX E DETERMINATION OF TOTAL SOLIDS IN GRUEL

E -1 APPARATUS

Lipless beaker - tall-form, of capacity 500 ml.

E-2 PROCEDURE

Take 250 ml of water in the lipless beaker and heat over hot-plate or any suitable burner till the water boils. Introduce 25 g of the material (previously broken into about 10 mm lengths in case of long goods) and stir thoroughly with a glass rod. Cook for 10 minutes with occasional stirring. At the end of 10 minutes allow the material to drain for five minutes. Measure the volume of gruel collected. Pipette out 20 ml of the gruel; after stirring well to give an even distribution of the solids content, into a tared petri dish and evaporate to dryness on a water-bath. Transfer the petri dish to a hot air-oven maintained at $105 \pm 2^\circ\text{C}$ and dry to constant mass.

E - 3 CALCULATION

Total solids in gruel, percent by mass
$$\frac{(m_2 - m_1)V}{5}$$

Where

m_2 = mass in g of petri dish with total solids,
 m_1 = mass in g of empty petri dish, and
 V = volume of gruel in ml.

ANNEX F DETERMINATION OF ACIDITY

F-1 REAGENTS

F-1.1 Sodium Hydroxide – 0.1N solution.

F-1.2 Phenolphthalein Indicator Solution

F-2 PROCEDURE

F-2.1 Grind 10grams of the product and add 100 millilitres of distilled water (start the test from within an hour from the grinding). Leave for one hour, stirring 3 times for two minutes each time, at approximately equal intervals. Titrate with 0.1N solution of NaOH using phenolphthalein as an indicator.

F-3 CALCULATION

F-3.1 Free acidity (ml of 1N NaOH) =
$$\frac{10,000 \times V \times N}{M}$$

Where: V = volume, in ml, of standard sodium hydroxide solution used in the titration;
 N = normality of sodium hydroxide solution; and
 M = mass, in g, of the sample taken.

ANNEX G

SAMPLING

G-1 GENERAL REQUIREMENTS FOR SAMPLING

In drawing, preparing, storing and handling samples, the following precautions shall be observed.

G.1.1 Samples shall be taken in a protected place not exposed to damp air, dust or soot.

G.1.2 The sampling instrument shall be clean and dry when used.

G.1.3 Precautions shall be taken to protect the samples, the material being sampled, the sampling instrument and the containers for samples from adventitious contamination.

G.1.4 The samples shall be placed in clean, odourless and dry containers. The sample containers shall be of such a size that they are almost completely filled by the sample.

G.1.5 Each container shall be sealed air-tight after filling and marked with full details of sampling, batch or code number, name of the manufacturer and other important particulars of the consignment.

G.1.6 Samples shall be stored in such a manner that the temperature of the material does not vary unduly from the normal temperature.

G.1.7 Sampling shall be done by a person agreed to between the purchaser and the vendor.

G.2 SCALE OF SAMPLING

G.2.1 Lot - All the packages in a single consignment belonging to the same batch of manufacture shall constitute a lot.

Samples shall be tested from each lot for ascertaining conformity of the material to the requirements of the specification.

G.2.2 The number of containers to be tested from a lot shall depend on the size of the lot and shall be in accordance with table 2.

TABLE 2 - Number of containers to be selected for sampling

Lot size (number of packages in the lot), N	Sample size (number of packages to be selected) n
Up to 50	3
51 to 150	5
151 to 500	8
501 to 3000	13
Above 3000	20

G.2.3 The packages to be selected for sampling shall be chosen at random from the lot. In order to ensure randomness of selection, random number tables shall be used.

G.3 TEST FOR CONFORMITY

G.3.1 Each of the selected packages shall be opened and the contents examined separately for appearance and dimension (see 4.1.1).

G.3.2 The contents of the packages shall then be mixed to obtain a composite sample. The sample shall be tested for chemical requirements (see 4.3.1).

G.4 CRITERIA FOR CONFORMITY

G.4.1 The lot shall be considered satisfactory in respect of the requirements of G.3.1 if each individual sample satisfies all the requirements.

G.4.2 The lot shall be considered satisfactory in respect of the requirements of G.3.2 if the test results on the composite sample satisfy the corresponding requirements.

G.4.3 The lot shall be declared to be in conformity with all the requirements of this specification, if it has been found satisfactory in accordance with G.4.1 and G.4.2.

ANNEX H

(Clause 3.1.1.4)

DETERMINATION OF EGG SOLIDS IN EGG NOODLES

H-O PRINCIPLE

Egg solid content of egg noodles is estimated closely from cholesterol content of unsaponifiable matter. When analyzing egg-free products or material containing less than 0.23 percent unsaponifiable matter, add 10 mg cholesterol before analyzing and correct results accordingly. The added Cholesterol should have melting point not less than 147°, and its purity should be checked by submitting 20 mg to the determination as given below.

H.1 APPARATUS

H.1.1 Cold Baths

Prepare one bath with crushed ice and one with salt-ice mixture.

H.1.2. Mohr Pipettes.

One graduated to 0.01 ml and one to 0.1 ml.

H.1.3 Filtration Bell Jar

Size to accommodate 300-ml Erlenmeyer flask connected to vacuum source by 2-way stopcock

H-1.4 Filtering Device

For filtering at 0° C, prepare filter tube of Knorr type. Tube should be about 20 mm internal diameter, body about 11cm long, and stem about 10cm long, provided with usual nickel or Monel metal disk. Insert this tube through bottom of container of such size that crushed ice can be packed

around body of tube to height of 7-8 cm. Fit tube into 500-ml suction flask. In tube prepare mat of asbestos 6-8 mm thick, packed fairly tight, and cover with layer of sand about 12 mm deep. Digest asbestos with acid and alkali. Purify sand by passing through 60-mesh sieve, then treat with warm hydrochloric acid until extract is practically colour less. Wash, dry, and ignite sand.

H-1.5 Separating Funnels

Separating funnels, one 250-ml and one 500-ml. Wash free of grease. Funnels must be ether-tight, with stopcock lubricated only with water.

H-1.6 Sintered Glass Filter- 30-50 ml capacity.

H-2 REAGENTS

H-2.1 Bromine Solution

Weigh to 0.1g a narrow-mouthed, glass- stoppered 25-ml flask containing 5ml carbon tetrachloride. Add 4-5 g bromine, weigh again and dilute with carbon tetra chloride to calculated final concentration of 0.22 ± 0.02 g bromine per ml. (This reagent should not be more than 2 days old.) Store under refrigeration.

H-2.2 Acetic Acid Solution

Pipette or burette 200 ml glacial acetic acid into 250-ml glass-stoppered volumetric flask; dilute to mark with water and mix cautiously,

H-2.3 Sodium Hypochlorite Solution

Dissolve 88g reagent grade sodium hydroxide in 200 ml water in a wide-mouthed 3-litre flask. Add about 1500 g crushed ice and pass in chlorine until 71 g is absorbed. Dilute to 2 litre and then store in dark bottles under refrigeration. Solution should be alkaline to phenolphthalein. Before using, check concentration of available chlorine as follows: Pipette 5ml solution into 100 ml water containing 2 g potassium iodide. Add 5ml hydrochloric acid (1: 1) and titrate with 0.1 N $\text{Na}_2\text{S}_2\text{O}_3$ Solution should be equivalent in available chlorine to 0.95 - 1.05 N NaOCl.

H-2.4 Sodium Formate Solution

Dissolve 50 g reagent grade sodium formate and dilute 100 ml.

H-2.5 Hydrochloric Acid

Approximately 6 N. Mix 500 ml concentrated hydrochloric acid with water and dilute to 1 litre.

H-2.6 Potassium Iodide Solution

20 percent. Dissolve 20 g potassium iodide and dilute to 100 ml. This solution must be colorless when acidified with hydrochloric acid.

H-2.7 Soluble Starch Solution - 1 percent.

H-2.8 Sodium Thiosulphate Solution - 0.02 N.

H-2.9 Sodium Sulphate Anhydrous

Powdered to pass through 60-mesh sieve.

H-2.10 Potassium Hydroxide Solution

a) 100 percent - Dissolve 10 g potassium hydroxide in 10ml water

b) 1 percent - Dissolve 10 g potassium hydroxide in water and make up to 1 litre.

H-2.11 Ammonium Molybdate Solution

Dissolve 5 g ammonium molybdate and make to 100 ml.

H-2.12 Ether

a) Anhydrous, reagent grade

b) Dry and peroxide free - (check for presence of peroxides, just prior to use). Rinse glass-stoppered cylinder with ether, place 10ml ether in rinsed cylinder, and add 1ml freshly prepared 10 percent solution of cadmium potassium iodide. Let stand protected from light for one hour, shaking occasionally, no color should develop in either liquid layer. To dry ether, shake with excess of anhydrous calcium chloride and filter.

H-3 PROCEDURE

H-3.1 Determination of Unsaponifiable Matter

Weigh 10-g sample into 500-ml Erlenmeyer flask and add, with shaking, 30 ml hydrochloric acid (1: 1). Heat on a steam bath for 30 minutes with occasional shaking. Cool under cold-water tap and add carefully with shaking, 30 g, potassium hydroxide pellets. Add pellets at such rate that liquid may boil, but not so fast as to cause splattering. While flask is still hot, place on steam bath, cover with small watch-glass and heat for 3 hours with occasional swirling. Cool, add 30 ml of 95 percent alcohol and 500 ml water and mix well. (Samples may be allowed to stand overnight at this point).

Add 100ml ether, swirl mixture vigorously for one minute, and transfer to 500-ml separating funnel, washing flask with 50-ml and 25-ml portions of ether. Wash flask with 50-ml dilute potassium hydroxide solution. Pour washings slowly into funnel while gently swirling liquid and continue swirling for 10 to 15 seconds.

Let liquid separate (about 10 minutes) and slowly draw off soap solution into 250-ml separating funnel, but do not draw off any emulsion or insoluble matter at interface. If emulsion forms and does not break to give sharp interface within 10 minutes, pour 5 ml alcohol into funnel and let stand until emulsion breaks. Rinse down sides of 500-ml funnel with 10 ml dilute potassium solution and draw this off into smaller funnel. Add 50 ml ether to smaller funnel and shake vigorously:

After liquids have separated, discard lower layer. Add ether layer to solution in larger funnel and rinse smaller funnel with 10 ml ether. Wash ether solution as before with 100 ml dilute potassium hydroxide solution, still retaining any insoluble matter or emulsion in funnel. In the same manner wash ether solution with two additional 100-ml portions of dilute potassium hydroxide solution, testing portions of last washings for soap by acidifying with dilute hydrochloric acid (1: 4). Acidified washings should be clear or only faintly turbid. If necessary, repeat washings with dilute potassium hydroxide solution until acidified washings are clear.

Wash ether solution by swirling with successive portions: 50 ml water, 50 ml water containing 0.5 ml 0.1 N hydrochloric acid, and two more 50-ml portions water. Draw off as much water as possible without loss of ether solution.

Filter ether solution into dry 500-ml suction flask through 50-g layer of sodium sulphate on sintered glass filter, using no suction for first several millilitres and then gentle suction for remainder.

Rinse separate funnel and filter with successive 10-, 5-, 5-, and 5-ml portions dry ether. Rinse stem of funnel, add glass beads to flask, and evaporate on steam bath to about 20-ml volume (current of air directed across neck of flask will increase rate of evaporation). Heat 50-ml glass-stoppered Erlenmeyer flasks at 100 - 105°C for 1 hour, cool in air for 30 minutes, and weigh. Transfer ether solution in suction flask quantitatively to weighed Erlenmeyer flask. Evaporate ether and dry flask at 100°C. Weigh flask again and determine unsaponifiable matter by difference.

Carry out blank determination using all reagents in above method without sample, and deduct this value from weight of unsaponifiable matter. Calculate unsaponifiable matter.

H-3.2 Determination of Egg Solids

Wash down sides of flask containing unsaponifiable matter with 2.0 ml anhydrous ether delivered from Mohr pipette. Pack flask in ice bath up to neck and let stand for at least 10 minutes. From another Mohr pipette add 0.20 ml cold bromine solution, swirl, stopper replace in ice bath for 10 minutes.

During bromination, cool acetic acid solution in ice-salt bath until temperature is -5°C and in same bath cool a 25-ml cylinder. Measure, 15 ml acetic acid solution into cold cylinder and add to reaction flask. Swirl reaction mixture for 3 minutes while holding in ice bath, and let stand in ice bath for 10 minutes. With slight suction pour mixture down stirring rod into cold filter tube, leaving rod in tube. Wash down sides of flask with 5 ml cold acetic acid solution and replace in ice bath. When liquid in funnel recedes below surface of sand, add acetic acid from reaction flask. Again repeat washing with 5 ml cold acetic acid solution and suck free of excess liquid. Wash flask and tube with ice water, filling filter tube about five times. Drain flask and apply suction to filter to remove all excess water, discarding filtrate and washings.

Remove crushed ice from around filter tube and transfer tube to top of filtration bell jar. Place 300-ml Erlenmeyer flask under filter tube so that stem projects well into the neck of the flask. Wash filter with 10 ml alcohol, 10-, 5-, and 5-ml portions of ether, and finally with 10 ml alcohol, stirring sand gently with each portion of solvent, and let mixture stand for about 1 minute before applying suction. Wash stem of filter with few ml ether, remove 300-ml flask, and to filtrate add 1 ml potassium hydroxide solution. Mix and wash down sides of flask with 5 ml ether. Evaporate on steam bath, using stream of clean air to remove all vapors.

Add 40 ml hot water to residue, mix and neutralize with 6 N hydrochloric acid, using 1 drop methyl red indicator. Add 10 g NaCl, 3 g $\text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$, and 20 ml N_2OCL solution. Bring to vigorous boil, remove from heat, and at once, with care, add 5 ml sodium formate solution. Cool and dilute to about 150 ml. Add 5 ml potassium iodide solution, 1-2 drops ammonium molybdate solution and 25 ml 6 N hydrochloric acid. Immediately titrate rapidly with thiosulfate, using starch indicator. Correct titre by blank value obtained on reagents, starting with addition of potassium hydroxide to alcohol ether solution.

H-4 CALCULATIONS

$$\text{H-4.1 Cholesterol} = \frac{0.55}{(\text{mg})} + \frac{0.688}{(\text{ml } 0.02 \text{ N Na}_2\text{S}_2\text{O}_3)}$$

H-4.2 Percent sterol calculated as cholesterol in sample

$$(\text{Moisture-free basis}) = \frac{\text{mg cholesterol}}{(100 - \text{percent moisture})} = C$$

H-4.3 Percent commercial egg-yolk solids

$$(\text{Moisture-free basis}) = \frac{(C - 0.024)100}{(2.88 - 0.024)} = 35(C - 0.024)$$

H-4.4 Percent commercial whole-egg solids

$$(\text{Moisture-free basis}) = \frac{(C - 0.024)100}{(2.11 - 0.024)} = 48(C - 0.024)$$

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