

Cow ghee — Specification

PUBLIC REVIEW DRAFT

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Cow ghee — Specification

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Foreword

This Kenya Standard was prepared by the Milk and Milk Products Technical Committee under the guidance of the Standards Projects Committee, and it is in accordance with the procedures of the Kenya Bureau of Standards

Animal ghee is a coarse, granular anhydrous milk fat product obtained from churning dairy cream to separate the butter and then heating until the water is driven off.

Since this product can easily be adulterated with animal body fat or vegetable fats, it is therefore necessary that specifications be set to regulate the quality of animal ghee offered for consumption.

In the preparation of this standard, reference was made to the following documents:

Codex Stan 280

The Pearsons' Book of Food Analysis – 9th Edition.

KS 326 Specification for edible fats and oils Parts 1-6 (*Second Revision*).

The assistance derived from these documents is hereby acknowledged.

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1 Scope

- 1.1 This Kenya standard specifies the requirements and methods of sampling and test for cow ghee, Suitable for human consumption.
- 1.2 This standard shall only apply to the ghee obtained from cow milk fat.

2 Normative references

The following referenced documents are indispensable for the application of this standard:

AOAC 942.17, *Arsenic in foods molybdenum blue method*

KS EAS 38 *Labelling of prepackaged foods*

CAC/RCP 57, *Code of hygiene practice for milk and milk products*

KS 1552 *Code of hygienic practice for production, handling and distribution of milk and milk products*

KS ISO 2446:2008, *Milk — Determination of fat content (Routine method)*

KS ISO 663 *Animal and vegetable fats and oils — Determination of insoluble impurities*

KS ISO 5555:2001; *Animal and vegetable fats and oils -- Sampling*

KS ISO 3960 *Animal and vegetable fats and oils — Determination of peroxide value*

KS ISO 3961 *Animal and vegetable fats and oils — Determination of iodine value*

KS ISO 8294 *Animal and vegetable fats and oils — Determination of copper, iron and nickel contents*

KS ISO 12193 *Animal and vegetable fats and oils — Determination of lead content*

AOAC 980.21, *organochlorine and organophosphorous pesticide residues in milk and milk products*

KS ISO 662:2016; *Animal and vegetable fats and oils -- Determination of moisture and volatile matter content*

KS ISO 6579:2002 *Microbiology of food and animal feeding stuffs - Horizontal method for the detection of Salmonella spp*

KS ISO 6785, *Milk and milk products -- Detection of Salmonella spp*

KS ISO 14501, *Milk and milk powder — Determination of aflatoxin M content — Clean-up by immunoaffinity chromatography and determination by high-performance liquid chromatography*

KS ISO 4833-1, *Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 1: Colony count at 30 degrees C by the pour plate technique*

KS ISO 6611, *Milk and milk products — Enumeration of colony-forming units of yeasts and/or moulds — Colony-count technique at 25 degrees C*

KS ISO 6888-1:1999; Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) -- Part 1: Technique using Baird-Parker agar medium

KS ISO 4832:2006; Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coliforms - Colony-count technique

KS ISO 660:2009; *Animal and vegetable fats and oils -- Determination of acid value and acidity*

Weights and Measures Act, Cap. 513

3 Terms and definitions

For the purposes of this standard, the following terms and definitions apply.

Cow ghee

Product exclusively obtained from cow milk, cream or butter, by means of processes which result in almost total removal of water and non-fat solids, with an especially developed flavour and physical structure

4 Quality requirements

4.1 General requirements

4.1.1 Colour

The colour of cow ghee when in liquid form shall be transparent amber and while in solid form, cow ghee shall be golden in colour.

4.1.2 Odour and taste

The odour and taste shall be characteristic of cow ghee and free from foreign odour and taste.

4.2 Physico-Chemical requirements

Cow ghee shall comply with the physico-chemical requirements stipulated in Table 1:

Table 1— Physico-Chemical requirements for cow ghee

S/N	Characteristic	Requirement	Test method
i.	Melting point range, °C	28 – 44	EAS 313
ii.	Reichert value	26 – 29	Annex
iii.	Free fatty acid, as oleic acid (%) m/m, max.	0.3	KS ISO 660
iv.	Milk fat % (m/m), min.	99.8	KS ISO 2446
v.	Moisture % (m/m), max.	0.2	KS ISO 662:2016
vi.	Peroxide Value (milli eqvt of Oxygen/ Kg fat),max.	0.6	KS ISO 3960
vii.	Iodine value (Wijs)	26 – 38	KS ISO 3961
viii.	Refractive index at 40 °C; OR BR reading at 40 °C	1.4483 - 1.452 40 - 44	Annex

ix.	Saponification value, (mg KOH/g oil), (min.)	220	Annex
x.	Boudouins Test	Negative	Annex

5 Hygiene

5.1 Cow ghee shall be processed in accordance with the hygienic requirements stipulated in CAC/RCP 1 and CAC/RCP 57.

5.2 Cow ghee shall comply with the maximum limits in Table 2

Table 2 — Microbiological limits

S/N	Micro-organisms	Maximum limits per g	Method of test
i.	Total plate count per gram, max	500	KS ISO 4833
ii.	Coliform count per gram	Absent	KS ISO 4833
iii.	<i>E. Coli</i>	Absent	ISO 11866-1 or ISO 11866-2
iv.	<i>Salmonella spp per 25g</i>	Absent	KS ISO 6785
v.	<i>Shigella per 25g</i>	Absent	KS ISO 4833
vi.	<i>L. monocytogenes per gram</i>	Absent	ISO 10560
vii.	<i>Yeast and Moulds per gram</i>	Less than 10	KS ISO 6611
viii.	<i>Staphylococcus aureus per 25g</i>	Less than 10/g	KS ISO 6888
ix.	<i>Bacillus cereus</i>	Absent	KS ISO 7932
x.	<i>Clostridium botulinum.</i>	Absent	

6 Contaminants

6.1 Heavy metals, detergents and insoluble impurities

Cow ghee shall not contain any substances harmful to human health and when tested in accordance with the test methods given in Table 3, the contaminant limits shall not exceed the levels specified in the same table

Table 3 — Contaminant limits for cow ghee

SL No.	Contaminant	Maximum limit	Test method
i)	Matter volatile at 105 °C, % (m/m), max.	0.2	KS ISO 662:2016
ii)	Insoluble impurities, % (m/m)	0.05	KS ISO 663
iii)	Detergent residues/ Soap content	absent	KS EAS 318
iv)	Iron (Fe), mg/kg	0.2	KS ISO 8294
v)	Copper (Cu), mg/kg	0.05	KS ISO 8294
vi)	Lead (Pb), mg/kg	0.1	KS ISO 12193
vii)	Arsenic (As), mg/kg	0.1	AOAC 942.17,

6.2 Pesticide and veterinary drug residues

The milk and milk products used in the manufacture of Cow ghee shall comply with the maximum residue limits for veterinary drug residues and pesticides established for milk by the Codex Alimentarius Commission in CODEX STAN 193, Codex general standard for contaminants and toxins in foods

6.3 Aflatoxin M1

Cow ghee shall not contain more than 0.05 µg/kg when tested in accordance to KS ISO 14501:2007/ AOAC 980.21, Aflatoxin M1 in milk and cheese- thin layer chromatographic method

7 Packaging

Cow ghee shall be packaged in airtight food grade containers, which shall be sealed to prevent contamination.

8 Labelling

In addition to EAS 38, the following specific provisions shall apply:

- a) the name of the product;
- b) name, address and physical location of the manufacturer/processor;
- c) net contents in g or kg;
- d) manufacturing date;
- e) expiry date;
- f) country of origin;
- g) conditions of storage;
- h) batch/lot number.

9 Fill of the container

The fill of the container shall be in accordance with the relevant regulations on weights and measures

10 Sampling

Sampling for the purpose of testing shall be done in accordance with ISO 5555:2001; Animal and vegetable fats and oils -- Sampling

Annex A (Methods of test)

1.0 Determination of the Refractive Index

1.1 Definition:

The ratio of velocity of light in vacuum to the velocity of light in the oil or fat: more generally, it expresses the ratio between the sine of angle of incidence to the sine of angle of refraction when a ray of light known wave length (usually 589.3nm, the mean of D lines of Sodium) passes from air into the oil or fat.

Refractive index varies with the temperature wave length.

1.1.2 Principle:

Measurement of the refractive index of sample is done by means of a suitable refractometer

1.1.3 Apparatus: Refractometer- Abbe or Butyro Refractometer

By Abbe's Refractometer: - Open double prism with the help of the screw head and place a drop of oil on the prism. Close prisms firmly by tightening screw heads.

Circulate water through the instrument. Let instrument stand for few minutes before taking reading so that the temperature of test sample and instrument are the same. Clean prism between readings by wiping off oil with cotton pad moistened with ethyl alcohol / toluene or petroleum ether and let dry.

By Butyro-refractometer: - Place 1-2b drops of sample on the lower prism. Close prisms and adjust mirror until it gives sharpest reading. If reading is indistinct after running constant temperature water through instrument for sometime, test sample is unevenly distributed on prism surfaces. As refractive index is greatly affected by temperature, use care to keep temperature constant

The temperature of the refractometer should be controlled to within $\pm 0.1^{\circ}\text{C}$ and for this purpose it should be provided with a thermostatically controlled water bath and a motor driven pump to circulate water through the instrument

When butyro-refractometer is used its reading can be converted to refractive index with the help of table

1.1.4 Light Source

If the refractometer is equipped with a compensator, a tungsten lamp or day light may be used. Otherwise a monochromatic light such as sodium vapour lamp (589.3 nm) may be used.

1.1.5 Procedure

Melt the sample if it is not already liquid and filter through a filter paper to remove impurities and traces of moisture. Make sure sample is completely dry.

Circulate stream of water through the instrument. Adjust the temperature of the refractometer to the desired temperature. Ensure that the prisms are clean and dry

Place a few drops of the sample on the prism. Close the prisms and allow standing for 1-2 min. Adjust the instrument and lighting to obtain the most distinct reading possible and determine the refractive index or butyro-refractometer number as the case may be.

5.1.6 Temperature correction- Determine refractive index at the specified temperature. If the temperature correction is necessary use the following formula:

$$R = R_1 + KI (T_1 - T_0)$$

Where,

R = Reading of the refractometer reduced to the specified temperature $T^{\circ}\text{C}$

R_1 = Reading at $T_1^{\circ}\text{C}$

5.1.7 Significance

Refractive index of oils increases with increase in a saturation and also chain length of fatty acids

(Ref: - A.O.A.C 17th edn, 2000, official method 921.08- Index of refraction of oils and fats / I.S.I Handbook of food analysis (Part XIII)- 1984, Page 70.) Table for conversion of BR readings to refractive index

2.0 Determination of Saponification Value

7.1 Definition:

The saponification value is the number of mg of potassium hydroxide required to saponify 1 gram of oil/fat

The oil sample is saponified by refluxing with a known excess of alcoholic potassium hydroxide solution. The alkali required for saponification is determined by titration of the excess potassium hydroxide with standard hydrochloric acid

2.2 Analytical importance:

The saponification value is an index of mean molecular weight of the fatty acids of glycerides comprising a fat. Lower the saponification value, larger the molecular weight of fatty acids in the glycerides and vice-versa.

2.3 Apparatus:

- 250 ml capacity conical flask with ground glass joints.
- 1 m long air condenser, or reflux condenser (65 cm minimum in length) to fit the flask (a).
- Hot water bath or electric hot plate fitted with thermos

2.4 Reagents:

(i) Alcoholic potassium hydroxide solution- Reflux 1.2 litre alcohol 30 minutes with 10 gm KOH and 6 gm granulated Aluminium or Al foil. Distill and collect 1 litre after discarding first 50 ml.

Dissolve 40 g of potassium hydroxide in this 1 litre alcohol keeping temperature below 15°C while dissolving alkali. Allow to stand overnight, decant the clear liquid and keep in a bottle closed tightly with a cork or rubber stopper. \

- ii) Phenolphthalein indicator solution- Dissolve 1.0 g of phenolphthalein in 100 ml rectified spirit
- iii) Standard hydrochloric acid: approximately 0.5 N

2.5 Procedure:

Melt the sample if it is not already liquid and filter through a filter to remove any impurities and the last traces of moisture. Make sure that the sample is completely dry. Mix the sample thoroughly and weigh about 1.5 to 2.0 g of dry sample into a 250 ml Erlenmeyer flask. Pipette 25 ml of the alcoholic potassium hydroxide solution into the flask. Conduct a blank determination along with the sample. Connect the sample flasks and the blank flask with air condensers, keep on the water bath, boil gently but steadily until saponification is complete, as indicated by absence of any oily matter and appearance of clear solution. Clarity may be achieved within one hour of boiling. After the flask and condenser have cooled somewhat wash down the inside of the condenser with about 10 ml of hot ethyl alcohol neutral to phenolphthalein. Titrate the excess potassium hydroxide with 0.5N hydrochloric acid, using about 1.0 ml phenolphthalein indicator.

2.6 Calculation:

$$\text{Saponification Value} = \frac{56.1 (B-S)N}{W}$$

Where,

B = Volume in ml of standard hydrochloric acid required for the blank.

S = Volume in ml of standard hydrochloric acid required for the sample

N = Normality of the standard hydrochloric acid and

W = Weight in gm of the oil/ fat taken for the test.

Note: - when titrating oils and fats which give dark coloured soap solution, the observation of the end point of titration may be facilitated either (a) by using thymolphthalein or alkali blue or b) by shaking 1 ml of 0.11 (w/v) solution of methylene blue in water to each 100 ml of indicator solution before the titration.

(Ref: - A.O.A.C 17th edn, 2000, Official method 920.160 Saponification number of oils and fats / IUP AC 2. 202 / I.S.I Handbook of Food Analysis (Part XIII) 1984, page 78)

3.0 Determination of Reichert-Meissl Value

Butter is distinguished from other fats by the presence of glyceryl esters of relatively low molecular weight fatty acids, especially butyric but also caproic, capric, caprylic, lauric and myristic acids. These acids are wholly or partially steam volatile and water soluble. The Reichert value reflects the amount of butyric and caproic acids present and Polenske chiefly caprylic, capric and lauric acid with some contribution from myristic and even palmitic acid.

3.1 Definition:

The Reichert-Meissl value is the number of millilitres of 0.1N aqueous sodium hydroxide solution required to neutralise steam volatile water soluble fatty acids distilled from 5g of an oil/fat under the

prescribed conditions. It is a measure of water soluble steam volatile butyric and caproic acids present in oil or fat.

3.2 Principle:

The material is saponified by heating glycerol sodium hydroxide solution and then split by treatment with dilute sulfuric acid. The volatile acids are immediately steam distilled. The soluble volatile acid in the distillate are filtered out and estimated by titration with standard sodium hydroxide solution.

3.3 Analytical Importance

These determinations have been used principally for analysis of butter and margarines. Butter fat contains mainly butyric acid glycerides. Butyric acid is volatile and soluble in water.

No other fat contains butyric acid glycerides, and therefore, the Reichert-Meissl value of the butter fat is higher than that for any other fat. Coconut oil and palm kernel oil contain appreciable quantities of capric and lauric acid glycerides. These fatty acids are steam volatile but not soluble in water, and hence give high Polenske value.

3.4 Apparatus:

- a. An all-glass distillation assembly conforming to specifications given in AOCs Official Methods Cd 5-40 or Methods of Analysis, AOAC- 17th Edn,' Figure 925.41 chapter 41 page 14 or distillation apparatus as shown in the diagram below
- b. 25 ml beaker
- c. 100 ml graduated cylinder
- d. 100 ml pipette
- e. Graduated burette
- f. Asbestos board with a hole about 65mm dia for supporting the flask over the burner. During distillation the flask shall fit snugly into the hole of the board to prevent the flame from impinging on the surface of the flask above the hole.
- g. Bunsen burner sufficiently large to allow completion of distillation in the prescribed time

3.5 Reichert- Meissl Distillation Apparatus

10.6 Reagents

- a). Glycerine:

b. concentrated sodium hydroxide solution: 50 % (w /w) Dissolve sodium hydroxide in equal wt of water and store solution a bottle. Use clear solution free from deposit

c.) Pumice stone grains

d.) Dilute sulfuric acid solution: Approximately 1.0 N

e.) Sodium hydroxide solution:

- 0.1 N solution in water, accurately standardized

3.6 Apparatus

f.) Phenolphthalein indicator:

Dissolve 0.1 g of phenolphthalein in 100 ml of ethyl alcohol

g.) Ethyl alcohol:

90% by volume and neutral to phenolphthalein

3.7 Procedure

Weigh accurately 5 ± 0.1 g of filtered oil or fat into a clean, dry 300ml distilling flask. Add 20 ml of glycerin and 2ml of concentrated sodium hydroxide solution, and heat while swirling over a flame until completely saponified, as shown by the mixture becoming perfectly clear. Cool the contents slightly and add 90 ml of boiling distilled water, which has been vigorously boiled for about 15min. after thorough mixing the solution, should remain clear. If the solution is not clear (indicating incomplete saponification) or is darker than light yellow (indicating over-heating), report the saponification with a fresh sample of the oil or fat. If the sample is old, the solution may sometimes be dark and not clear

Add about 0.6-0.7 gm of pumice stone grains, and 50 ml of dilute sulfuric acid solution. Immediately connect the flask to the distillation apparatus. Place the flask on asbestos board so that it fits snugly into the aperture. This will prevent the flame from impinging on the surface of the flask above the level of the liquid and avoid super heating. Heat very gently until the liberated fatty acids melt and separate. Then set the flame so that 110 ml of distillate shall be collected within 19 to 21 min. The beginning of the distillation is to be taken as the moment when the first drop of the distillate falls from the condenser in the receiving flask.

Keep the water in the condenser flowing at a sufficient speed to maintain the temperature of the outgoing water from the condenser between 15 and 20 °C. Collect the distillate in a graduated flask.

When the distillate exactly reaches the 110 ml mark on the flask, remove the flame and quickly replace the flask by a 25 ml measuring cylinder. Stopper the graduated flask and without mixing it in a water bath maintained at 15 Oc for 10 min so that the 110 ml graduation mark is 1 cm below the water level in the bath. Swirl round the contents of the flask from time to time. Remove the graduated flask from the cold water bath, dry the outside and mix the content gently by inverting the flask 4 to 5 times without shaking. Avoid wetting the stopper with the insoluble acids. Filter the liquid through a dry, 9 cm whatman filter paper. Reject the first

2-3 ml of the filtrate and collect the rest in a dry flask. The filtrate should be clear. Pipette 100 ml of the filtrate and add 5 drops of the phenolphthalein solution, and titrate against standard 0.1N sodium reagents.

Run a Blank Test without the fat, but using the same quantities of the reagents

Calculation

Reichert-Meissl value = $(A-B) \times N \times 11$

Where,

A = Volume of standard sodium hydroxide solution required for the test;

B = Volume in ml in standard sodium hydroxide solution required for the blank; and

N = Normality of standard sodium hydroxide solution.

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