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Pasteurized milk — Specification

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Introduction

This African Standard seeks to lay down requirements for the acceptable production of pasteurized milk in order to ensure quality, consumer safety and promote fair trade practices.

In elaborating this Standard, references were made to inputs from Manufacturers, National and International standards which are hereby duly acknowledged.

Pasteurized milk — Specification

Scope

This African Standard specifies requirements, sampling, and test methods of pasteurized milk of (cow milk (*Bos spp*.); goat (*Capra spp*.); sheep (*Ovis spp*.); camel (*Camelus dromedarius*) for direct consumption or further processing.

Normative references

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

ARS 53, General Principles of Food Hygiene – Code of practice

ARS 56, Pre-packaged Foods – Labelling

ARS 1034, Dairy industry — Glossary of terms

ARS 1036, Code of Hygienic Practice for Milk and Milk Products

CXS 193, General Standard for Contaminants and Toxins in Food and Feed

CXS 206, General Standard for the Use of Dairy Terms

CXS 212, Codex Standard for Sugars

CXS 346, *General Standard for the labelling of non-retail containers of foods*

IDF 21B, Milk, cream and evaporated milk – Determination of total solids content (Reference method)

ISO 1211, Milk - Determination of fat content - Gravimetric method (Reference method)

ISO 5764, Milk - Determination of freezing point - Thermistor cryoscope method (Reference method)

ISO 8968-1, Milk - Determination of nitrogen content – Part 1: Kjeldahl method (including calculation of crude protein content)

Terms and definitions

For the purpose of this standard the terms and definitions given in ARS 1034 shall apply.

pasteurized milk

milk that has been heat-treated to kill harmful bacteria and extend shelf life

NOTE: The process involves any of the following heat-treatments:

a) Low Temperature Long Time (LTLT): at least 63°C and holding at such temperature continuously for at least 30 minutes;

b) High Temperature Short Time (HTST) (also called batch pasteurisation): at least 72°C and holding at such temperature continuously for at least 15 seconds; or

c) other mild heat—typically below 100°C (212°F)—for a specific duration;

to achieve a negative Phosphatase Test and allow for the total destruction of pathogenic microorganisms and almost all of the common microflora contained in milk while preserving its physico-chemical, organoleptic characteristics and nutritional value as much as possible.

Composition and Requirements

4.1 Raw materials

Raw materials and ingredients used shall comply with relevant standards.

These include:

a) Raw cow milk.

b) Raw sheep milk.

c) Raw goat milk.

d) Raw camel milk.

e) Pasteurized milk as part of rework or reprocessing – based on principles of Good Manufacturing Purposes (GMP).

4.2 General requirements

Pasteurized milk shall:

a) Retain the characteristics of the raw material.

b) Have characteristic texture and colour.

c) Be free from off-flavours and odour.

d) Be free from additives, preservatives, any other unauthorized substances, and

e) Be free from added water (except for reconstituted milk).

4.3 Specific requirements

Pasteurized milk shall meet the compositional requirements given in Table 1 to Table 4 when tested in accordance with the test methods specified therein. Pasteurized milk may be classified as identified in Table 1 and meet the compositional requirements, as shown.

4.4 Permitted ingredients

No ingredients are permitted to be added to pasteurized milk of all species, including milk powders

4.5 Compositional Requirements

Requirements for pasteurized cow milk

Pasteurized cow milk shall be classified as set out in Table 1 and comply with the standards for each type and class.

Table 1 — Compositional requirements for cow milk

Class/ Class designation/ Legal Description	Milk fat content (%)(m/m)	Minimum milk solids non-fat content	Minimum milk protein content calculated on a fat-free basis (%) (m/m)	pH value at 20 - 25°C	Specific Gravity (Density) g/ml
		Calculated on a fat-free basis (%) (m/m)			
High fat milk	More than 4.5	8.5	3	6.5 - 6.85	1.026
Full fat milk/ Whole milk	More than 3.0 - 4.5	8.5	3	6.5 - 6.85	1.026
Medium fat milk Fat-reduced/ Semi-skimmed	More than 1.5 - 3.0	8.5	3	6.5 - 6.85	1.026
Partly skimmed milk/ Low Fat	More than 0.5 - 1.5	8.5	3	6.5 - 6.85	1.026
Fat free milk/ Skimmed	Not more than 0.5	8.5	3	6.5 - 6.85	1.026

Requirements for pasteurized sheep milk

Sheep milk shall be classified as set out in Table 2 and comply with the standards for each type and class.

Table 2 — Classes and compositional standards for sheep milk

Class/ Class designation/ Legal description	Milk fat content (%)(m/m)	Minimum milk solids non-fat content	Minimum milk protein content calculated on a fat-free basis (%)(m/m)	pH value at 20 - 25°C
		Calculated on a fat-free basis (%)(m/m)		
Full fat sheep milk/ Whole sheep milk	More than 3.3	8.5	3	6.5 - 6.85

Requirements for pasteurized goat milk

Goat milk shall be classified as set out in Table 3 and comply with the standards for each type and class.

Table 3 — Classes and compositional standards for goat milk

Class/ Class designation/ Legal description	Milk fat content (%)(m/m)	Minimum milk protein content calculated on a fat-free basis (%)(m/m)
Goat milk/ Whole goat milk	At least 2.9	At least 2.5

Requirements for pasteurized camel milk

Camel milk shall be classified as set out in Table 4 and comply with the standards for each type and class.

Table 4 — Classes and compositional standards for camel milk

Class/ Class designation/ Legal description	Milk fat content (%)(m/m)	Minimum milk solids non-fat content		Minimum milk protein content calculated on a fat-free basis (%)(m/m)	pH value at 20 - 25°C
		Calculated on the total content (%)(m/m)	Calculated on a fat-free basis (%)(m/m)		
Full fat camel milk/ Whole camel milk	More than 3.3	8.3	8.6	3	6.5 - 6.85

4.6 General quality characteristics

The pasteurized milk of different species shall be:

a) free from any impurities, foreign particles and extraneous matter and adulteration.
b) natural in odour (characteristic to the origin species).
c) natural in consistency and homogeneous (characteristic to the origin species).
d) free of objectionable flavour, off-flavours and taints and unacceptable taste such as rancid taste and decomposed milk, not be bitter.
e) have a characteristic creamy white colour (characteristic to the origin specie).
f) not contain added water or other added substances, nor shall any proportion of a natural constituent be removed (by standardisation or any other means).
g) not be mixed with milk of any other livestock other than the specified species neither powdered milk, nor liquid.
h) be processed with validated pasteurization method which should make the phosphatase negative (Phosphatase Test Method A – Annex 1)
i) not coagulate in the clot-on-boiling test, when tested. (Annex, test method B).

5 Food Additives

Food additives may be used and only within the limits specified in accordance with CXS 192.

6 Contaminants

6.1 Heavy metals

The products covered by this African Standard shall comply with those maximum limits for metal contaminants specified in CXS 193.

6.2 Aflatoxins

When tested in accordance with ISO 14501, the level of aflatoxin M_1 shall not exceed 0.50 µg/kg.

6.3 Pesticides residues

Pesticide residue limits shall be in accordance with limits set by the Codex Alimentarius Commission for the product.

6.4 Veterinary drug residues

Veterinary drug residue limits shall be in accordance with limits set by the Codex Alimentarius Commission for the product.

7 Hygiene

The products covered by this African Standard shall be produced, prepared and handled in accordance with the appropriate sections of, ARS 53 and ARS 1036.

Pasteurized milk shall be free from microorganisms and products originating from microorganisms in amounts which may represent a hazard to human health.

Pasteurized milk shall comply with microbiological limits given in Table 5 when tested in accordance with the methods specified therein.

When tested by appropriate methods, pasteurized milk shall conform to the limits specified in Table 5:

Table 5 — Microbiological criteria limits related to pasteurized milk

Food Category	Micro-organisms	Sampling plan		Limits		Test method reference[1]	Stage
		n	c	m	M		
Pasteurized Milk	Total plate count (TVC) *(*Aerobic Plate count*)*	5	1	5.10^4 (*cfu/mL*)	10^5 (*cfu/mL)*	End of the manufacturing process	End of the manufacturing process (PHC)
	Enterobacteriaceae	5	0	10*(cfu/mL)*		ISO 21528-2	End of the manufacturing process (PHC)
	Salmonella	5	0	ND (*in 25 ml*)		ISO 6579-1	On the market during the shelf-life (FSC)
	Listeria monocytogenes	5	0	ND (*in 25 mL*)		ISO 11290-1	After packaging (FSC)
	Escherichia coli	5	0	ND (*in 25 mL*)		ISO 11866-1/2/3	On the market during the shelf-life (FSC)
	Coagulase-positive staphylococci	5	0	ND*) (in 25 mL)*		ISO 6888-1/2	On the market during the shelf-life (FSC)
n = number of units comprising the sample c = number of sample units giving values between m and M m= The level of the microbiological criterion required in the product M= Value or level of microbial limit not to be reached or greater than in any unit of the sample							

NOTE:

1) The most recent edition of the standard shall be used.

2) The criterion shall not apply to products intended for further processing in the food industry

3) The total bacteria count is the number of bacteria in a sample that can grow and form countable colonies on Standard Methods Agar after being held at 32°C (90°F) for 48 hours.

8 Packaging

Pasteurized milk shall be packaged in suitable food grade containers which will safeguard the hygienic, nutritional, technological, and organoleptic qualities of the product during dispatch, transport and use of the product until the end of its shelf life.

All pasteurized milk shall be stored at temperature of 4°C.

9 Labelling

In addition to the provisions of the General Standard for the Labelling of Prepackaged Foods (ARS 56) and the General Standard for the Use of Dairy Terms (CXS 206), the following specific provisions apply:

9.1 Name of the product

The name of the food shall be pasteurized milk; or for clarification, the name of the animal may be included in close proximity to the name of the product.

A word denoting the animal from which the milk has been derived, shall be inserted immediately before or after the designation of the product. Such declarations are not required if the consumer would not be misled by their omission.

Any claims such as "organic," "fortified," or "lactose-free" must comply with respective regulatory requirements.

9.2 Declaration of milk fat content

The milk fat content shall be declared in a manner found acceptable in the country of sale to the final consumer, either (i) as a percentage by mass, (ii) as a percentage of fat in dry matter, or (iii) in grams per serving as quantified in the label provided that the number of servings is stated.

10 Methods of sampling

For checking the compliance with this standard, the methods of sampling contained in ISO 707, shall be used.

11 Methods of Analysis

For checking the compliance with this standard, the methods of analysis contained in ISO 1211, ISO 8968-1, IDF 21B and ISO 5764 shall be used.

Annex A
(normative)

Testing method reference

A: Alkaline Phosphatase Test Method

The phosphatase test is a crucial method used to determine the adequacy of pasteurization in milk. Pasteurization is intended to destroy pathogenic microorganisms and deactivate enzymes that may spoil the milk. Alkaline phosphatase, an enzyme naturally present in raw milk, is deactivated by proper pasteurization. The presence of active alkaline phosphatase in pasteurized milk indicates that the milk may not have been heated sufficiently.

Principle:
The negative phosphatase test is based on the principle that alkaline phosphatase, which is deactivated during proper pasteurization, should not be detectable in pasteurized milk. A colorimetric reaction is used to determine the presence of this enzyme. If the enzyme is still active, it will catalyze a reaction that results in a color change, indicating inadequate pasteurization.

Materials and Reagents:

a) Phosphatase test kit (e.g., Scharer Rapid Phosphatase Test or Fluorophos Test)

b) Buffered substrate solution (contains disodium phenyl phosphate or 4-methylumbelliferyl phosphate)
c) Incubator or water bath
d) Spectrophotometer or fluorometer (depending on the method used)
e) Standard reference samples (positive and negative controls)

Procedure:

1. **Sample Preparation:**
   a) Collect a representative sample of the pasteurized milk.

   b) Mix the milk sample thoroughly to ensure homogeneity.
2. **Reagent Addition**:
   a) Add the specified amount of buffered substrate solution to the milk sample according to the test kit instructions.
3. **Incubation:**
   a) Incubate the mixture at a specified temperature (usually 37°C) for a predetermined time (typically 2 hours for colorimetric methods or 3-5 minutes for fluorometric methods).
4. **Reaction Observation:**
   a) For colorimetric methods, observe any color change in the sample. A yellow color indicates the presence of active phosphatase, suggesting inadequate pasteurization.
   b) For fluorometric methods, measure the fluorescence intensity using a fluorometer. Increased fluorescence indicates active phosphatase.
5. **Result Interpretation:**
   a) Compare the test results with the negative and positive controls. A result matching the negative control (no color change or no fluorescence) indicates successful pasteurization. A result similar to the positive control (color change or fluorescence) indicates the presence of active phosphatase and potentially inadequate pasteurization.

Precautions:

a) All glassware must be cleaned before use. Cleaning should be done by soaking in Chromic acid solution prepared by slowly adding 4 volumes of concentrated H_2SO_4 to 5 volumes of 8% potassium dichromate. After cleaning in chromic acid glassware must be rinsed in warm water and distilled water and finally dried. Glassware used for the test must not be used for any other purpose and must be kept apart from other apparatus in the laboratory.
b) A fresh pipette must be used for each sample of milk. Pipettes must not be contaminated with saliva.

6. The sample of milk should be examined as soon as possible after arrival at the laboratory. If

not examined immediately it must be kept at a temperature between 3°C and 5°C until examined. The sample must be brought to room temperature immediately before being tested.

This method ensures that pasteurized milk meets safety standards by confirming the deactivation of alkaline phosphatase, thus verifying the adequacy of the pasteurization process.

Reference:

- International Dairy Federation (IDF). (1995). *Milk and Milk Products - Detection of Alkaline Phosphatase Activity - Fluorimetric Method Using 4-Methylumbelliferyl Phosphate (ISO 11816-1:1995)*.
- United States Food and Drug Administration. (2021). *Grade "A" Pasteurized Milk Ordinance (PMO)*.

B: The clot-on-boiling test

Principle:

The clot-on-boiling test is a simple and traditional method used to assess the heat stability and quality of milk. This test is particularly useful for detecting milk that has undergone bacterial spoilage or has high acidity, both of which can cause milk proteins to coagulate or clot when boiled. The clot-on-boiling test evaluates the heat stability of milk by heating it to boiling. Milk with high acidity or spoilage tends to clot upon boiling due to protein denaturation. This test helps identify milk that may not be suitable for consumption or further processing.

Materials and Reagents:

Fresh milk sample
Test tubes or small glass containers
Boiling water bath or direct heat source

Procedure:

1. Thoroughly mix the milk before sampling.
2. Collect a representative sample of the milk to be tested.
3. Ensure the sample is well-mixed to achieve homogeneity.
4. Pour 5ml of milk into a clean test tube or glass container.
5. Place the tube in boiling water bath or heat it directly over a flame until the milk reaches boiling.
6. Ensure that the level of the boiling water is higher than the milk level.
7. Stand the test tube of milk in the boiling water for five (5) minutes.
8. Remove the test tube from the water and tilt the tube almost horizontally without shaking the milk inside.
9. Wait until a thin film is formed on the milk.

Result Interpretation:

a) **Negative Result (No Clotting):** If the milk remains fluid and does not show any signs of clotting or coagulation, it is considered to have passed the clot-on-boiling test. This indicates that the milk has acceptable acidity and is free from significant bacterial spoilage.
b) **Positive Result (Clotting):** If the milk shows signs of clotting or coagulation, milk clots or if floccules are seen to be adhering to the sides of the tube when it is returned to the vertical position. It indicates high acidity or bacterial spoilage. Such milk is considered to have failed the clot-on-boiling test and is not suitable for consumption or further processing.

NOTE**:** Colostrum in milk will result in a positive clot-on-boiling test result. The heat stability of the milk is also affected by other factors.

Aschaffenburg and Mullen phosphatase test

(1) The phosphatase test shall be carried out in the manner set out below.
(2) Test each sample as soon as possible after its arrival at the laboratory.
(3) If the sample is not tested immediately on its arrival at the laboratory, keep it at a temperature below 5 °C, but not frozen, until it is tested.
(4) Raise the temperature of the sample to 20-25 °C immediately before it is tested.
(5) Take the following precautions during or in connection with he testing of a sample;
    (a) Except in the case of cultured dairy products, do not test a sample that shows signs of spoiling or souring.
    (b) Use a clean pipette for each sample of milk or cream and ensure that no pipette is contaminated with saliva.
    (c) Do not perform the test in direct sunlight.
    (d) Use only distilled water throughout the test.
(6) Whenever practicable, use reagents of analytical quality for this test. Prepare the buffer substrate solution as follows:
    (a) Buffer solution: Dissolve 3, 5g of anhydrous sodium carbonate and 1,5g of sodium bicarbontate in distilled water and fill up with water to 1l solution in a volumetric flask.
    (b) Keep the solid substrate, disodium p-nitrophenyl phosphate, in a refrigerator.
    (c) Buffer substrate solution:
        i. Place 150mg of the substrate in a standard 100ml volumetric measuring flask and fill to the 100ml mark with the buffer solution.
        ii. Store the solution in a refrigerator and protect from light.
        iii. When distilled water is used for purposes of comparison, the solution must give a reading of less than the standard 10 on the comparator disc AP T W 5 or APTW 7 when viewed in transmitted light through a 5mm cell in the all-purpose comparator.
        iv. Do not use the solution for longer than one week.
    (d) Buffer solution: Dissolve 3, 5g of anhydrous sodium carbonate and 1,5g of sodium bicarbontate in distilled water and fill up with water to 1l solution in a volumetric flask.
    (e) Keep the solid substrate, disodium p-nitrophenyl phosphate, in a refrigerator.
    (f) Buffer substrate solution:
        i. Place 150mg of the substrate in a standard 100ml volumetric measuring flask and fill to the 100ml mark with the buffer solution.
        ii. Store the solution in a refrigerator and protect from light.

iii. When distilled water is used for purposes of comparison, the solution must give a reading of less than the standard 10 on the comparator disc AP T W 5 or APTW 7 when viewed in transmitted light through a 5mm cell in the all-purpose comparator.

iv. Do not use the solution for longer than one week.

(7) Use the following apparatus for the test:

(a) A lovibond all-purpose comparator with a stand for work in reflected light.

(b) A Lovibond comparator disc AP T W 5 or AP T W 7.

(c) Two fused-glass cells, 25mm deep, or test tubes of colourless lass, 13,5mm internal diameter, conforming to B. S. 625, fitted with non-p-nitrophenol containing stoppers, for use in the Lovibond all-purpose 1000 comparator.

(d) A waterbath capable of being maintained at 39 o C + /- 0, 5 o C .

(e) A pipette to deliver 5, 0ml.A supply of 1, 0ml straight-sided pipettes.

(f) A 1l volumetric flask.

(g) A 100ml standard volumetric flask.

(h) After use, empty each tube, rinse it in water, wash well in hot water containing soda, rinse in hot water and then in distilled water and dry, or clean by some other equally effective method.

(i) If, after treatment in accordance with (a) of this subparagraph, a test tube does not appear to be clean, repeat the treatment but, in addition, after rising it in hot water, place it in hydrochloric acid and then rinse it again in hot water and then in distilled water and dry it, or clean it by some other equally effective method.

(j) Clean new glassware by dipping it in a solution of chromic acid consisting of five volumes of 8% (m/v) potassium dichromate and four volumes of concentrated sulphuric acid added slowly and carefully to the mixture of dichromate and water.Keep the solution referred to in (c) of this subparagraph covered and discard it when it turns green.

(8) After cleaning new glassware in the manner described above, rinse it in hot water, then rinse it in distilled water and dry. Pipettes should be rinsed in cold water and then cleaned by soaking for 24 hours in a solution of chromic acid in a 250 ml glass cylinder or other suitable container, and thereafter well rinsed in hot water and then in distilled water and dried, or cleaned by some other equally effective method. Glassware used for the test shall not be used for any other purpose and shall be kept separate from all other apparatus in the laboratory.

(9) The test shall be carried out in the manner as set out below:

(a) Transfer 5ml of the buffer substrate solution to a test tube using a pipette, stopper the test tube and bring the contents to a temperature of 37 °C +/- 0.5 °C.

(b) Add 1 ml of the milk or cream to be tested, replace the stopper of the test tube and mix the contents well by shaking. Incubate the test tube for 2 hours + /- 1 minute at 37 °C ±0.5 °C.

(c) With each series of samples, incubate one control sample prepared from 5ml of buffer substrate solution and 1ml of boiled milk or cream of the same type as that undergoing the test. After incubation, remove the test tube from the water bath and mix the contents well. Place the control sample on the left-hand ramp of the stand and the test sample on the right. Take the readings in reflected light by looking down onto the two apertures with the comparator facing a good source of daylight.If artificial light is needed for matching, use a daylight type of illumination. R evolve the disc until the colour of the test sample matches that of the control sample.

(d) Record readings falling between two standards by affixing a plus or minus sign to the figure for the nearest standard.

The "Aschaffenburg and Mullen phosphatase test" and the classic alkaline phosphatase test are both used to detect the presence of alkaline phosphatase enzyme activity in milk, which is an indicator of proper pasteurization. However, these tests differ in their methods and sensitivity. Below is a detailed comparison:

Classic Alkaline Phosphatase Test

Principle:

a)The classic alkaline phosphatase test measures the enzymatic activity of alkaline phosphatase in milk. This enzyme is deactivated during proper pasteurization, so its presence indicates inadequate pasteurization or post-pasteurization contamination.

Method:

a) **Substrate Used:** The classic test typically uses disodium phenyl phosphate as a substrate.
b) **Reaction:** When the enzyme is present, it hydrolyzes the substrate to produce phenol.
c) **Detection:** Phenol reacts with a color reagent (such as 2,6-dibromoquinone-chlorimide) to form a blue color, which is then measured colorimetrically.
d) **Sensitivity:** The detection limit for the classic test is around 1 microgram of phenol per milliliter of milk, which corresponds to approximately 500 milliunits (mU) of alkaline phosphatase per liter of milk.

Advantages:

a) Simple and widely used method.
b) Cost-effective and requires basic laboratory equipment.

Disadvantages:

a) Less sensitive compared to newer methods.
b) Subjective interpretation of color change can lead to variability.

Aschaffenburg and Mullen Phosphatase Test

Principle:

a) The Aschaffenburg and Mullen phosphatase test is an enhanced version of the classic test, designed to be more sensitive and specific. It also measures alkaline phosphatase activity but uses a different approach for detection.

Method:

a) **Substrate Used:** This test uses p-nitrophenyl phosphate (pNPP) as a substrate.
b) **Reaction:** The enzyme hydrolyzes pNPP to produce p-nitrophenol, which is yellow and can be measured spectrophotometrically.
c) **Detection:** The amount of p-nitrophenol produced is directly proportional to the enzyme activity and is measured at a wavelength of 405 nm using a spectrophotometer.
d) **Sensitivity:** This test has a detection limit of around 0.1 micrograms of p-nitrophenol per milliliter of milk, which corresponds to approximately 10 mU of alkaline phosphatase per liter of milk, making it significantly more sensitive than the classic test.

Advantages:

a) Higher sensitivity allows for the detection of lower levels of alkaline phosphatase.
b) Quantitative results provide more precise and reproducible measurements.
c) Less subjective than colorimetric methods, reducing variability.

Disadvantages:

a) Requires a spectrophotometer, which may not be available in all testing laboratories.
b) Slightly more complex and may require more specialized reagents.

References

i) Codex Alimentarius Commission. (2003). *Codex Standard for Milk and Milk Products (CODEX STAN 206-1999)*. FAO/WHO.

ii) International Dairy Federation (IDF). (1995). *Milk and Milk Products - Detection of Alkaline Phosphatase Activity - Fluorimetric Method Using 4-Methylumbelliferyl Phosphate (ISO 11816-1:1995)*.

iii) United States Food and Drug Administration. (2021). *Grade "A" Pasteurized Milk Ordinance (PMO)*.

Summary
The classic alkaline phosphatase test and the Aschaffenburg and Mullen phosphatase test both serve the same purpose but differ significantly in their methodology and sensitivity. The Aschaffenburg and Mullen test is more sensitive and provides more precise and reliable quantitative data, making it more suitable for modern quality control in milk pasteurization.