INTERSTATE STANDARD

GOST 26669-85

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FOOD-STUFFS AND FOOD ADDITIVES
PREPARATION OF SAMPLES FOR MICROBIOLOGICAL ANALYSES

Продукты пищевые и вкусовые

Подгтовка проб для микробиологичексих анализов

Reference number: GOST 26669-85
Technormativ IS LLC, translation from Russian, 2013

The contents:

This standard applies to food-stuffs and food additives and stipulates the preparation of test samples for microbiological examination.

The terms and definitions mentioned in this standard are given in Annex 1**.**

(Revised version, Rev. No 1).

1. EQUIPMENT, REAGENTS AND MATERIALS

1.1. The following equipment, reagents and materials are used for the preparation of test samples for microbiological examination:

water-bath;

homogenizer, laboratory blender or porcelain mortar according to the GOST 9147 (*Laboratory porcelain ware and apparatus. Specifications*);

membrane filtration device;

gas or alcohol burner according to the GOST 25336 (*Laboratory glassware and equipment. Basic parameters and dimensions*);

metal funnels;

punch;

bottle opener;

can opener;

scissors, scalpel, pincers according to the GOST 21241 (*Medical pincers. General technical requirements and test methods*), spatula, spoon;

stencil (template);

test tubes according to the GOST 25336;

vessels according to the GOST 25336;

pipettes according to the Standard technical documentation;

rubber corks;

glass beads;

rectified ethyl alcohol according to the GOST 5962 (*Rectified ethyl alcohol. Specifications*)[1]; 70%;

polythene bags;

detergent;

sodium chloride according to the GOST 4233 (*Reagents. Sodium chloride. Specifications*);

peptone for bacteriological purposes according to the GOST 13805 (*Dry fermentation pepton for bacteriological objects. Specifications*).

All equipment and surfaces of the devices being in the direct contact with the product are to be sterilized in one of the ways specified in the GOST 26668 (*Food-stuff and food additives. Preparation of sampling for microbiological analyses*).

1.2. The preparation of peptone-salt solution

The peptone-salt solution is prepared in the following way: 8.5 g of sodium chloride and 1.0 g of peptone are dissolved in 1 dm^3 of distilled water by simmering. The received solution is infiltrated if necessary through the paper filter, then the level of pH at 7.0±0.1 is set, and the solution is bottled into the flasks, test tubes or other dish, corked up and sterilized at a temperature (121±1) °C for the next 30 min.

The solution is kept in the dark place at a temperature (4±2) °C during no more than 30 days under the conditions that exclude water evaporation.

[1] On the territory of the Russian Federation the GOST R 51652-2000 (*Rectified ethyl alcohol of food raw material. Specifications*) is applied.

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The temperature of the peptone-salt solution should correspond to the temperature of the examined product.

1.3. The preparation of the peptone water.

The method of peptone water preparation is analogous to that of the peptone-salt solution but without sodium chloride adding.

2. THE PREPARATION OF TEST SAMPLES FOR THE EXAMINATION

2.1. The test sample package is subject to inspection in regards of correspondence of the title represented on the lithographic imprint or on the label to the title specified in the accompanying documents.

2.2. All dirt on the test sample package should be cleaned. If the test samples of the product received for the examination are sealed, then the impermeability of the package should be verified. The impermeability of the preserves is determined in accordance with the GOST 8756.18 (*Canned food products. Methods for determination of appearance, tightness of package and inner surface condition of metallic package*), the tightness of the polymeric package containing food as well as the preserves sealed by a lid with a flexible membrane (push button) are to be verified by sight. The surface of the flexible membrane should be bent inwards. Tightly sealed glass, metal or polymeric package containing food should be washed up with a detergent, then rinsed with clean water and dried out. Non sealed package containing food should be wiped with a tampon moistened in ethyl alcohol.

Directly before the microbiological examination the preserves are subject to thermostatting.

The following preserves is subject to thermostatting:

tightly sealed, outside defect-free, designated for the determining of the industrial sterility of the preserved food and the microbiological stability of the preserves;

with lolled-up ends and springer swell in a tightly sealed package, designated for revealing the causes of these defects appearance.

The preserves designated for the revealing of botulinic toxins in it, the swells with the signs of microbiological deterioration and untight are not subject to thermostatting.

For the vital activity of mesophilous aerobic, facultatively anaerobic and anaerobic microorganisms to show up the preserves are thermostatted at a temperature 30 °C - 37 °C in a package with a volume up to 1 dm^3 inclusive during not less than 5 days, in the package the volume of which is more than 1 dm^3 – not less than 7 days.

For the vital activity of thermophilous aerobic, facultatively anaerobic and anaerobic microorganisms to show up the preserves in a package of whatever volume are thermostatted at a temperature 55 °C - 62 °C during not less than 3 days. During the thermostatting the preserves are inspected on a daily basis. The preserves with the defects of package having showed up are removed from the thermostat immediately after discovering and exposed to the room temperature during 24 hours, afterwards the condition of the package and if possible the food appearance are marked. The preserves in a package which takes a normal form after cooling at the room temperature is considered to be defect-free and the thermostatting is continued.

After thermostatting of the preserves and cooling during 24 hours down to the room temperature the condition of the package and if possible the food appearance are marked.

The defects of the preserves are listed in Annex 2.

2.3. The microbiological examination of the product test samples looking outside like the normal ones is to be carried out in a box meeting the conditions of asepsis. The package of the test sample containing product looking suspicious or spoiled should be opened in a separate room.

The box preparation procedure is set forth in Annex 3.

2.2, 2.3. **(Revised version, Rev. No 1).**

2.4. The test samples of frozen food before preparation of the sub-sample should be defreezed at a temperature (4±2) ºC. The sub-sample is taken immediately after defreezing but not later than 18 h since the defreezing started.

The product test sample is permitted to be defreezed at a temperature 18 °C - 20 °C during 1 h.

The product test samples of homogenios structure are permitted to be defreezed in the thermostat at a temperature 35 °C provided that the complete defreezing is reached in no more than 15 minutes.

2.5. Opening the product test sample packge

2.5.1. Directly before opening the package with the product test sample contained in the end-user package, free-flowing or having liquid phase ones are mixed by tenfold turning upside-down or circular rotation.

2.5.2. The test sample package of the product (except the preserves) should be wiped with a tampon moistened in 70% ethyl alcohol; alcohol is burnt or removed by way of free vaporization. Then the package is opened, the spout of the metal or glass jar is annealed, afterwards you select the mass (volume) of the product in a quantity necessary for the preparation of one or more sub-samples.

2.5.3. The package with a test sample (made of foil paper bags, polymeric materials or paper) is opened at a place pretreated with a tampon, moistened in alcohol. The procedure of opening the package with the test sample is carried out in such a way to eliminate the possibility of contamination the product, surrounding items and environment.

2.5.4. The surface of the preserves looking like normal ones should be pretreated by ethyl alcohol before opening in one of the following ways:

in case of glass jar the lid is pretreated, in case of metal jar the end of the jar opposite to the labeled one is pretreated.

The surface of the lid is wiped with an alcoholic tampon, and then the tampon is placed on the surface and burnt before opening the preserves;

rubber bottle hoods and castellated lids, beckelite and plastic latch mechanisms are treated similarily, only that the tampon is not burnt;

metal lid (end) depending on the purpose of the examination is opened or punctured with a punch for 1 – 4 times in immediate proximity to the burning tampon. The dimension of the whole (the diameter and length) should be 1 – 3 cm.

The selected sub-samples are immediately sowed in culture media or transferred to a peptone-salt solution for the preparation of dilution;

before opening the bottles or tubes with screw-tops the pretreated lid or bouchon is unscrewed. The bottle edges or tube membrane are burnt in the burner flame; the membrane is punctured with a sterile scalpel.

Before opening the bottle corked up with a castellated or foil cork the latch mechanism is burnt in the burner flame, the cork is removed by a sterile opener, the bottle edges are burnt in the burner flame again.

By opening the bottles with the rubber latch mechanism, the last preptreated by ethyl alcohol is removed without preliminary burning; afterwards the ottle edges are burnt in the burner flame.

2.5.5. The preserves looking defective are paced on the metal tray. Directly before the selection of the sub-samples of the product the surface of the lid (end) is treated in a way specified in the paragraph 2.5.2, but without ethyl alcohol burning. The pretreated lid (or end) is covered by an overturned sterile metal funnel so that the funnel entirely covered the surface. The lid (end) is carefully punctured by a sterile punch trough a narrow opening of the funnel forming in such a way a needle-like hole.

Instead of metal funnel you are permitted to use a polythene bag. Aftet the lids (end) pretreating the preserves are placed into the polythene bag wiped with ethyl alcohol beforehand, so that the bottom of the bag covered the surface to be opened. The bag should br tightly tied up from the bottom side. Then carefully with a slight push of the punch you should make simultaneously a hole in the preserve lid and in the polythene bag tightly pressed to the lid.

When the can with the product stops producing gas and the product, you should remove the funnel and the bag, wipe the lid with a sterile tampon again, widen the hole with a punch and immediately afterwards select the sub-sample of the product for sowing or preparation of dilution.

2.6. The selection of the sub-samples and preparation of of the initial dilution

2.6.1. Depending on the indicators to be determined one or more sub-samples are selected from each product test sample for the preparation of dilution and/or sowing in culture media.

2.6.2. The mass (volume) of the sub-sample designated for sowing in culture media and/or the preparation of its dilution should be fixed in the Standard technical documentation for the certain product kind or examination methods.

2.6.3. The sub-sample for the sowing is selected by gravimetric or volumetric method immediately after opening the product test sample. The opening procedure is carried out under conditions eliminating the contamination of the product with microorganisms in immediate proximity to the burner flame and by sterile

tool.

2.6.4. The product sub-sample is selected in such a way that it contained all the components and in the proportion similar to that of the examined test sample.

2.6.5. For the preparation of the product sub-sample dilution you may use the peptone-salt solution. It is permitted to prepare the initial dilutions of the products having mass concentration of NaCl more than 5% using peptone water, the initial dilutions of meat, fish and diary products – using physiological solution.

The mass (volume) of the product sub-sample designated for the preparation of the initial dilution or homogenate should amount to not less than (10±0.1) g/cm^3.

The ratio between the mass (volume) of the product sub-sample and the volume of the peptone-salt solution for the initial and successive dilutions shall constitute:

1:9 – for tenfold dilution (for the products containing a large quantity of fat without surfactants 1:10);

1:5 – for sixfold dilution;

1:3 – for fourfold dilution;

1:1 – for twofold dilution.

If there is a need to dilute the sub-samples of the products containing the large quantity of fat it is permitted to use surfactants (sodium bicarbonate etc.) having no antimicrobial activity.

For preparation of the sub-samples dilution of the product with high osmotic pressure it is permitted to use peptone or distilled water.

(Revised version, Rev. No 1).

2.6.6. The initial dilution of the product sub-sample is prepared meeting the conditions of asepsis in one of the following ways:

solving the product;

diluting the product having liquid phase;

making a suspension of powders, spreads and surfaces of the product parts contaminated with germs;

homogenizing the solid products.

2.6.7. The sub-samples of test samples of liquid and sticky products are selected with a sterile pipette having cotton stopper by putting the pipette in the product depth.

The part of the product sticking to the pipette surface is remained to drop to the pipette nib. The forming drop is removed by touching to the internal side of the dish or consumer package over the product surface.

Sticky products are removed from the pipette surface with a sterile tampon.

The product sub-sample is transported to reservoir with peptone-salt solution for the preparation of initial dilution so that the pipette did not touch the peptone-salt solution surface. Another sterile pipette is used for thorough mixing of the product with the peptone-salt solution by way of tenfold filling and extrusion of the mixture.

Working with the sticky products it is advisable for its fast mixing with the peptone-salt solution to put into the reservoir a few glass beads.

2.6.8. The liquid product rich in carbon dioxide ($C0_2$), is transported to a sterile conical vessel closed with a cotton stopper or to other reservoir and is heated on the bain-marie with frequent circular motion mixing at a temperature from 30 °C till 37 °C until the gas bubbles stop to escape.

The sub-sample of the product test sample is selected and pretreated as described in the paragraph 2.6.7.

2.6.9. The sub-samples of the test samples of powdery and free-flowing products are selected with a sterile spoon or spatula from dfferent spots of the product (if necessary before the weighd dose selection 2 cm of the upper layer of the product are removed using a sterile spoon), then the sub-sample is transported to a preliminary weighed sterile reservoir with a top and weighed. The peptone-salt solution is added to the sub-sample in a quantity requisite for the preparation of the initial dilution. The mixture is mixed or stirred by twentyfivefold circular motion with a radius of 30 cm until the product of a homogenious structure is obtained.

If the powdery product is not water-soluble then leave the suspension obtained after adding the peptone-salt solution for 10 minutes to settle and then again strongly shake it for 1 minute.

2.6.10. The sub-samples of the test samples of the products swelling in the water are selected and the initial dilution is prepared according to the requirements of the standard technical documentation for the certain kind of product.

2.6.11. The sub-samples of the test samples of solid water-soluble products are selected using spatula or spoon after its grinding, milling or comminution under aseptic conditions and then treat as described in paragraph 2.6.9.

The sub-sample of the test sample of water-insoluble solid products be homogenized in cases specified in the Standard technical documentation. By homogenization of the product the total number of rotations of a homogenizer should amount to 15-20 thousand. The number of rotations of the homogenizer should not be less than 8000 and more than 45000 rotations per minute.

If after the product homogenization a heterogeneous mass is obtained, it should be settled during 15 minutes and for sowing and (or) dilution preparation the supernatant is used.

It is permitted to homogenize unsterilized product by its comminuting in a sterile saddlestone meeting the aseptic requirements until a homogenious structure is obtained.

(Revised version, Rev. No 1).

2.6.12. The sub-sample of the test samples of the spreads are selected after its thorough mixing using a spoon or glass rod and then are pretreated as described in the paragraph 2.6.9.

2.6.13. The sub-sample of the test samples of liquid fats are selected using a pipette heated by flaming. After filling the pipette with the product the residues of the product are removed fromt the surface of the pipette using a sterile tampone.

The product from the pipette is transported to the reservoir with ground glass stopper and diluted with a requisite quantity of the peptone-salt solution, heated up to 40 °C-45 °C; in case of revealing the psychrophilic microorganisms the temperature should not exceed 37 °C. The fat residues sticking to the pipette are washed off with peptone-salt solution, a few times filling and emptying the pipette.

2.6.14. The sub-sample of the test samples of solid fats are selected by cutting the product with a knife or wire into several parts. In case of need the top layer is removed.

The sub-sample of the product is selected from the surface of the slice in different spots using a scalpel and is transported to the weighed reservoir with a top.

The certain quantity of the sub-sample is transported to an open-mouthed ware with a ground glass stopper. The fat residues sticking to the ware walls are rinsed off by a certain quantity of the peptone-salt solution, heated up to 40 °C - 45 °C, added to the ware in amount requisite for the preparation of the initial dilution.

The sub-sample of the solid fats can be selected by volume. The fats are rendered in an open-mouthed ware on a bain-marie at a temperature no more than 45 °C; in case of revealing the psychrophilic microorganisms the temperature should not exceed 37 °C.

After mixing of a melted fat it is transported using a warm pipette to an open-mouthed ware with a ground glass stopper, containing the requisite quantity of the peptone-salt solution for the preparation of the initial dilution. The peptone-salt solution is preheated up to 40 °C - 45 °C; in case of revealing the psychrophilic microorganisms the temperature should not exceed 37 °C.

2.6.15 The sub-samples of the whipped or mushy products, containing a large quantity of fats, after mixing with a glass rod are selected with a spoon to the weighed ware and add the peptone-salt solution, preheated up to 40 °C-45 °C, in a quantity requisite for the preparation of the initial dilution.

2.6.16 Determining of the microbial contamination of the product test samples is carried out by washout using the cotton tampons.

The sterile cotton tampon is moistened in the peptone-salt solution, and then it is used for wiping in different places the surface of various parts of the examined products of total area 100 cm^2.

The area of the examined surface is measured with the help of stencils having the holes of the appropriate dimension.

The tampon is placed in a test tube, containing 10 cm^3 of peptone-salt solution. The content of the test tube is thoroughly mixed with the help of the pipette. The obtained suspension is considered to be the

initial dilution.

(Revised version, Rev. No 1).

2.7. The preparation of a twofold dilution

2.7.1. The first twofold dilution of a sub-sample is the initial one; the initial dilution is prepared according to the paragraph 2.6. It is the base for the preparation of the following dilutions.

2.7.2. The following second dilution is prepared from one part of initial dilution and nine parts of the peptone-salt solution by way of mixing in a test tube.

If the mixing of the initial dilution was carried out with the help of pipette, then the same pipette is used for adding 1 cm^3 of initial dilution to 9 cm^3 of the peptone-salt solution trying not to touch the surface of the dilution with a pipette.

2.7.3. The third ant further dilutions are prepared similarily.

2.7.4. The interval between preparation the sub-samples of the product, its dilutions and sowing in culture media should not exceed 30 minutes.

Annex 1

(Reference)

THE TERM USED IN THE STANDARD AND ITS DEFINITION

Term	Definition
Sub-sample	A part of the test sample of certain mass, volume, designated for preparation of homogenate, initial dilution or direct sowing in culture media.
Initial dilution	A sub-sample of the product, diluted by the solution to the requisite concentration, which may constitute two - (2^{-1}), four (4^{-1}), six (6^{-1}), but often tenfold (10^{-1}) dilution
Microbiological stability of preserves	Correspondence of the quality indicators of the preserves to the requirements, established by the Standard technical documentation for certain kinds of products concerning microbiological indicators
Full preserves	The preserves microbiological stability of which does not depend on the storage time at the tepmperature, specified for this kind of product in the Standard technical documentation
Industrial sterility of preserves	The absence in the preserved food of microorganisms, able to develop at a storage temperature, determined for this kind of preserves, as well as microorganisms and microbial toxins dangerous for human health
Normal appearance of preserves (during microbiological examination of quality)	The preserves without defects of package, closure and preserved food
Defects of preserves	Each separate nonconformance of appearance of the preserves, condition of package or closure or quality of the preserved food to the requirements of the Standard technical documentation
Lolled-up ends preserves	The preserves in a package, with one end lolling up in case of pressing on the opposite end, but after stopping pressure returning to the initial state, as well as the preserves in a package, having swollen because of disregard the temperature of storage, but taking normal appearance at a room temperature
Springer swell	The preserves in a package with a constantly swelling bottom (lid), able to take the normal state (accomponied by swelling of the opposite end). After the pressure removing the bottom (lid) takes the previous swelling state.
Swells	The preserves in the swelling package, unable to take the normal appearance

Term	Definition
The tightness of preserve sealing	The state of the package and the closure providing protection of the preserves from penetration of microorganisms during sterilization (pasteurization), storage and transportation
Thermostatting of preserves	Holding the preserves during a certain period of time at a temperature favourable for the developing of microorganisms in the product
Tongue	Local roll of the bottom part of the lid hook on the metal can or local squashing of the bottom part of the tube clasp
Barb	Local overturn of the joint with sharp projecting of the lid hook from under the joint Cutting off the top or the bottom surface of a seam, accompanied by removing the ware and the part of a sheet metal from the seam surface
Undercut	The absence of the hook catching
False seam	Excessive thickening of the bottom of the seam until the bottom part is squashed
Rolling seam (roll)	

(Revised version, Rev. No 1).

Annex 2

(Reference)

PRESERVED FOOD DEFECTS

The defects of the preserved food are considered to be:

signs of developing of microorganisms, fermentation, mold growth, sliming etc., visible by the naked eye;

sediment on the bottom of the can or on the border of the surface product with the package (a «ring»);

liquid phase turbidity;

coagulation;

turning sour;

odour or taste extraneous, unusual for the product;

discoloration.

The defects of the package appearance with the product, packed in it, are considered to be:

signs of untightness, visible by the naked eye: rifts, through crack, leaks or traces of the product, escaping the can;

swell;

springer swell;

lolled-up ends preserves;

improper design of a tin seam (tongues, barbs, undercuts, false seam, rolling seam);

rust, the removing of which leads to the formation of cavities;

deformation of package, ends or longitudinal seam of a tin in the form of sharp edges and «ticks»;

skew of the lid on a glass jar, undercutting of the lid corrugation along the seamed area, protrusive rubber gasket («loop»);

crack or split of the glass near the seam, incomplete lid fitting with respect to the jar neck;

deformation (impression) of the lid of the glass jar, cuasing the failure of the seam;

convexed flexible membrane (push button) on the lead.

Annex 3

(Reference)

THE PROCEDURE OF THE CLEAN BENCH PREPARATION

The preserves are opened at a clean bench, specifically adjusted to microbiological examination. There should be no surfaces in the clean bench inaccessible for humid disinfection, and no air motion caused by draughts. The walls, florr and ceiling should be coated with a material or coloured with a paint, resistant to damp treating by disinfectant solutions. For air sterilization the clean bench is equipped with ultraviolet lamps based on 1,5-2,5 watt for 1 m^3.

There should be only microbiologist, carrying out the examination, in the clean bench and assistant, if necessary.

There should be a table and a stool in the clean bench. Unnecessary things except those requisite for carrying out the examination of the preserves are not permitted.

On the table there should be:

alcohol or gas burner;

jar with a ground stopper, containing alcohol;

can closed with the lid, containing previously prepared thick sterile cotton tampons with a size 3 x 3 cm or cotton rings;

cans with disinfectant solution (3cm layer height) for placing of the used pipettes or tubes;

small metal or enameled tray, used for placing the used cans;

sterile pipettes or tubes, using for selecting a test probe.

In the drawer of the table the auxiliary tool should be kept: the pincer and the punch. The punch should have the form of a spear with a cross section as rhomb with diagonals 1 x 1,5 cm or a cross section of a right triangle.

In case of opening a big amount of the cans the punch, attached to the rack is used. In this case the opening is carried out by pressing the punch onto the can lid with the help of the lever.

Before opening the can the punch is flamed in the tampon flame.

The clean bench is washed and disinfected before carrying out the examination (not earlier than 24 h before the commencement) and after its finishing. The disinfection is made by wiping all the surfaces with chloric or other disinfectant solutions subject to the corresponding manual. Germicidal lamps are switched on in the clean bench 45 minutes before the work commencement for (30±5) minutes.

At a present time for carrying out the microbiological examination the laminar clean benches (UHP air shielded box) are used. The laminar clean benches are produced by Uzhgorod medical facilities «Laminar», the clean benches of the type BPV 1200 are produced in Hungary, the clean benches of the type TVG-S II 1.14.1 – by a company Babcock - BSH (Germany).

Annexes 2, 3. **(Implemented in addition, Rev. No 1).**

INFORMATION DATA

1. DEVELOPED AND ORIGINATED by the State agrindustrial committee of the USSR

2. APPROVED AND IMPLEMENTED by the Resolution of the State standard committee of the USSR dated 04/12/85 No. 3810

3. The standard fully complies with ST SEV 3014-81

4. The standard contains the international standards ISO 6887-83 (E) and ISO 7218-85

5. SUPERSEDES the GOST 10444.0-75

6. LIST OF REFERENCE STANDARD TECHNICAL DOCUMENTATION

Notation of the STD reffered to	Number of a pargraph
GOST 4233-77Reagents. Sodium chloride. Specifications	1.1
GOST 5962-67 (*Rectified ethyl alcohol. Specifications*)	1.1
GOST 8756.18-70 (*Canned food products. Methods for determination of appearance, tightness of package and inner surface condition of metallic package*)	2.2
GOST 9147-80 (*Laboratory porcelain ware and apparatus. Specifications*)	1.1
GOST 13805-76 (*Dry fermentation pepton for bacteriological objects. Specifications*)	1.1
GOST 21241-89 (*Medical pincers. General technical requirements and test methods*)	1.1
GOST 25336-82 (*Laboratory glassware and equipment. Basic parameters and dimensions*)	1.1
GOST 26668-85 (*Food-stuff and food additives. Preparation of sampling for microbiological analyses*)	1.1

7. The duration limit is cancelled by the Resolution of the State standard committee of the USSR dated 23/01/91 No. 38

8. The REVISED VERSION with the Revision No 1, approved in September, 1989 (IUS 12-89)