**AGROINDUSTRIAL STRENGTHENING OF QUINOA PROJECT THROUGH THE SCALING OF PROTOTYPES IN RELEVANT ENVIRONMENTS FOR THE INDUSTRY IN THE DEPARTMENT OF CAUCA**

**Identification of Mesophiles NTC 4519**

**Guide Code: 003**

**CYTBIA ASUBAGROIN GIPA RESEARCH GROUP**

**UNIVERSITY OF CAUCA 2021**

Prepared by: Astrid Zoraya Parra Polanco

Directed by: Karen Sofia Muñoz Pabón

1. **AIM**

Describe the methodology used to determine the presence of mesophiles based on NTC 4519 and NTC 4491-1.

1. **BASIS**

Mesophiles are a group of microorganisms that have a higher growth rate at temperatures between 25 and 40 °C, with temperatures between 30 and 40 °C being optimal (Díaz et al., 2017). They present certain relevant characteristics as indicators of food quality; their presence may indicate mishandling of the raw material during processing.

To determine the presence of mesophiles, agar for plate count or PCA is used, sowing is by depth, after incubation for 24 h, the colony count is performed.

The method is applicable for:

a) Products intended for human consumption and animal feed.

b) Environmental samples from the area of ​​production and handling of food for human consumption and animal feed.

1. **MATERIALS**

|  |  |
| --- | --- |
| **MATERIAL** | **AMOUNT** |
| Petri boxes | 3 |
| Spatula | 1 |
| Glass clock | 3 |
| Rack of blue tips 1 mL | 1 |
| erlemeyer | 3 |

Note: Wash, dry and sterilize materials.

1. **REAGENTS**

|  |  |
| --- | --- |
| **agar** | **Quantity** |
| PCA | According to supplier specifications |
| Distilled water |  |

Note: The agar must be prepared according to the specifications of the product data sheet.

1. **TEAMS**

|  |  |
| --- | --- |
| **Team** | **Quantity** |
| Autoclave | 1 |
| Incubator | 1 |
| Kiln | 1 |
| shaker | 1 |
| Colony count equipment | 1 |
| micropipette 1000 μL | 1 |
| Laminar flow cabinet | 1 |

1. **PROCESS**
   1. **Preparation of sample**

Mix 10 g of sample with 90 mL of distilled water in an Erlenmeyer flask, shake in a shaker (MaxQ 4450 orbital Thermo Ficher Scientific USA) for 10 min, then allow to settle. Repeat procedure with the number of samples. Depending on the initial count, dilutions are prepared according to the NTC 4491-1 standard.

* 1. **Agar preparation**

The agar is prepared according to the specifications of the technical data sheet of the container. The amount indicated for the volume is weighed, then the distilled water is added and later it is left to boil on a heating plate with constant agitation. Later, it is sterilized in an autoclave for 20 min at 259 °C and 20 psi.

* 1. **Process**
     1. **Inoculation and incubation**

Using a micropipette, transfer1000 μL of sample from the first decimal dilution (10-1) to the next (10-2); the described procedure is repeated with the additional dilutions, using a different tip for each decimal dilution.

Then an aliquot of 1000 μL of the dilutions is taken and deposited in the agreed Petri dishes for repetitions, then between 10 mL and 15 mL of agar are added.PCAin each Petri dish. Carefully mix the inoculum with the medium and allow it to solidify.

If there is suspicion of microorganism growth, it is recommended to carry out the above process and when the medium solidifies, add 4 more mL of prepared agar, in order to obtain colonies.defined.

The solidified Petri dishes are inverted and packed in vinyl and incubated at 30 °C ± °C for 72 h ± 3 h.

* + 1. **Colony count**

After the specified period for incubation, select the Petri dishes containing colonies and count them in the colony counting equipment, using dim light, try to mark the counted colonies, to avoid confusion.

1. **DISPOSAL OF CHEMICAL AND/OR BIOLOGICAL WASTE.**

After the determination of mesophiles, the boxes are deactivated in an autoclave. The medium is then thrown into a bag indicating biohazard.

**BIBLIOGRAPHY**

Colombian Technical Standard (2018) Microbiology of food and animal feed. Horizontal method for plate count of mesophiles. Plate count technique (NTC 4519)

Díaz, A., Barrio, M., Darré, M., López, M., Cofre, M., Condorí, M., Lazarte, D., Trevisán, V., Peirano, C., Del Bó, C. , Cañate, A., & Alcaide, C. (2017). Microbiological Analysis of Foods; Indicator Microorganisms. Anmat, 3, 1–14. http://www.anmat.gov.ar/renaloa/docs/analisis\_microbiologico\_de\_los\_alimentos\_vol\_iii.pdf