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

**Identification of fungi and yeasts NTC 5698-2**

**Guide Code: 002**

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1. **AIM**

Describe the methodology used to determine the presence of fungi and yeasts NTC 5698-2 and NTC 4491-1.

1. **BASIS**

Fungi and yeasts are widely distributed in the environment, they can be present as normal flora of a food, or as contaminants in processing equipment.Certain species of fungi and yeasts are used in the preparation of some foods; however, as contaminants, they can cause two main changes; aesthetic, due to the visible formation on the surface of the food and another deeper one, the result of metabolism can generate the change in pH and particular aromas.

The method is based on inoculating a known amount of sample, in a specific selective medium, taking advantage of the ability of microorganisms to use agar polysaccharides as nutrients.

1. **MATERIALS**

|  |  |
| --- | --- |
| **MATERIAL** | **AMOUNT** |
| Petri boxes | 3 |
| Spatula | 1 |
| Glass clock | 3 |
| Rack tips 1000μL | 1 |
| rack tips 100μL | 1 |
| erlemeyer | 3 |

Note: Wash, dry and sterilize materials.

1. **REAGENTS**

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

1. **TEAMS**

Clearly and descriptively detail the equipment and quantity for the development of the method or determination.

|  |  |
| --- | --- |
| **Team** | **Quantity** |
| Autoclave | 1 |
| Incubator | 1 |
| Kiln | 1 |
| shaker | 1 |
| Colony count equipment | 1 |
| micropipette 1000 μL | 1 |
| micropipette 100μL | 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 at 150 rpm and 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**

PDA 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.

Pour PDA agar into Petri dishes so that it gels, once gelling is complete, take a 100 μL aliquot of the dilutions and place them in the agreed Petri dishes for repetitions, shake vigorously in the box with the help of glass beads Petri dish, repeat the process with each of the repetitions.

The solidified Petri dishes are inverted and packed in vinyl and incubated at 30 °C ± 24 to 72 h. Carry out monitoring during the incubation of the fungi and yeasts.

* + 1. **colony count**

After the specified period for incubation, select the Petri dishes containing colonies ≤ 300 colonies and count them in the colony counting equipment, using dim light, taking care to mark the counted colonies, to avoid confusion.

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

After the determination of fungi and yeasts, the boxes must be 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 fungi and yeasts. Plate count technique (NTC 5698-2)