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Microbiological evaluation of anatomical organs submitted to glycerinization and freeze-drying techniques

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ABSTRACT

Alternatives conservation techniques are being requested with the proposal of formaldehyde substitution. Formaldehyde results in excessive anatomical specimens' weight and it can cause serious health problems to the manipulator, such as cancer. However, it provides an efficient germicide and fungicide action depending on concentration. The substitute techniques are glicerinization and freeze-drying which have advantages such as non-production of smells, lightness of the organs and dispenses the use of fixatives in conservation. As well as both intrinsic and extrinsic factors interfere in microbial growth, microbiological analyzes are essential to detect possible deteriorative microorganisms in organs and concluding effectively the technique used. Formalinized, glycerinated and freeze-drying organs were collected in three different times which were intercalated by two months, except formalinization that had one evaluation. The procedure required the use of sterilized swabs wetted in peptone water and molds measuring 5,0 cm x 10,0 cm positioned on two different piece's local resulting in 100 cm^2 of area, to spread plate of total moulds, mesophiles (except in freeze-drying), psychrophilic (only in freeze-drying) and *Pseudomonas* sp (except in formalinization). All the plates were counted and compared between each technique's evaluations by variance analyzes. Both alternatives techniques resulted in zero or in very low microbial quantity to cause health problems as well as it preserve pieces morphology. All values of all analyzes resulted below 1/ml, showing that glicerinization and freeze-drying techniques are so as efficient as formaldehyde.

1. Introduction

The mainly objective of anatomical laboratories is the study of anatomical organs and systems, that can be synthetics or cadaverous. This becomes easy the practical learning, as well as improve applicative, assimilative and comprehensive abilities of students. Anatomical pieces conservation concern people for over five thousand years due to the fact that natural cadaverous pieces are necessary for teaching, what become that method as the most usable in worldwide [3,6,12].

According to the author, there is a great number of anatomical techniques' variety, some of them differing some methodology steps as different details on procedure, substitution, reduction or addiction of some used materials that can evidence some specific system. Those topics can also be applied at conservation methods.

Anatomical techniques' variety facilitates practical learning, applicative skills, assimilative and understanding in addition to academic scientific character. Currently there are many techniques to help in animal tissue preservation for study [21].

In the early days of conservation techniques utilization, the dehydration was the main way to keep bodies and anatomical pieces preserved, sometime later, the perfused fixers substances began to be used in the bodies circulatory system. In anatomy, the fixation is the most important step before dissect and study a piece because it will ensure that all tissues remain fixed and protected by decomposition [22].

The anatomical pieces conservation is made with solutions that prevent fungi and any other microorganism colonization. The main

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solutions fixers are formaldehyde, phenol, ethyl alcohol and glycerin [23].

The most conservative technique used is formalinization, which requires the use of formaldehyde (10%). Its use has advantages because of the low price (R$ 0.52/L) and the efficient bactericidal and fungicide action. On the other hand, it can lead to negatives factors, such as high weigh, pieces dimming and stiffness after prepared [10]. Besides, formaldehyde can cause serious health problems to manipulator, such as tumors [2], and to aquatic fauna [20].

According to [8], to reduce these risks, gas masks are highly recommended during the handling of cadavers placed in formalin baths. Also, to reduce formaldehyde concentration in cadavers, anatomical organs must be thoroughly rinsed in water before they are handled. [18] reported that ancient microbiology and the characterization of ancient pathogens have emerged within the expanding field of ancient DNA. For the author, linked to the amount of microorganism present in the organism at the time of death, sample age, burial environment, DNA degradation and oxidation are factor that will determine whether or not infectious agents from the past remain detectable.

In a study of [8] made with cadavers that were fixed and preserved by a solution based on nitrite pickling salt, ethanol and glycols, it showed that embalming animal cadavers with those products results in cadavers free of mold and colonized by low numbers of bacteria. This method carries no health risk, is environmental friendly, cost effective and also results in cadavers close to their original physiological appearance. However, further studies are necessary to provide more data on the microbiological safety.

Two other alternative techniques are glicerinization and freeze-drying, which involves association of absolute alcohol and glycerin or pieces dehydration by freezing and unfreezing cycles, respectively [9].

The mainly advantages of using glycerin includes no malignancy to health, pieces malleability after prepared, no production of smell and no damage to the environment, what guarantee students wellness. However, its disadvantages include the product's high price, which is around R$ 5.36/L of solution [10].

Similarly to glicerinization, freeze-drying's advantages include lightness and no production of smell on prepared organs. Furthermore, this technique is cheap, once it does not need fasteners when prepared. The mainly disadvantage on its use is reduction of organs volume, as well as loss of morphology and malleability when compared with fresh pieces.

Not only pieces storage (Extrinsic factor) but also its natural conditions (Intrinsic factor) are relevant for growing and development of microorganisms, except virus. So, the way on how organs are conserved and preserved influences directly on presence and multiplication of moulds and bacteria, that use nutrients present on viscera to supply their energetic demand for proliferation.

Water availability (Intrinsic factor) is related to free form water and allows biochemical functions, helping on microbial metabolism [17]. Relative moisture of air and gas concentration on environment (extrinsic factor) influence on pieces water activity and thus, on microbial growing.

During time, microorganisms present on anatomical organs can deteriorate that samples and therefore they become dispensable for anatomical study. According to [1], the mainly spoiling microorganisms presented in meat sample are bacteria, such as *Pseudomonas* spp., *Acinetobacter/Moraxella, Shewanella putrefaciens, Brochotrix thermosphacta, Lactobacillus* spp. and also some species from *Enterobacteriaceae*, besides moulds and yeasts.

Alternatives techniques cited have been showing effective conservation, however its microbiological proprieties are unknown.

Thus, the study in question aims to verify the effectivity of alternative techniques performed by Ref. [3] method (Fig. 1), focusing on the presence of spoiling microorganisms for each technique.

2. Materials and methods

For microbiological analyzes of anatomical organs, it were used stomach of equines from Animal Anatomy Laboratory, located at Department of Veterinary Medicine of Faculty of Animal Science and Food Engineering – USP, Pirassununga Campus. The formalinized organs were maintained in formaldehyde 10% and glicerinizated as well as freeze-dying organs were produced according to the protocol described by Ref. [3]. All anatomical pieces have maintenance each five years.

2.1. Sampling

Sampling of one anatomical organs conserved by two alternatives techniques and the conventional technique (formaldehyde) was performed using sterilized swabs moistened in peptone water (0.1%) and molds of 5.0 cm × 10.0 cm. Samples were collected in two different points in order to reach an area of 100 cm^2. Following, the sample was transferred to an identified dilution tube containing 10 mL of peptone water (0.1%) and submitted to serial dilutions.

All the procedures were in accordance of Bioethical committee of animal experimentation at Faculty of Animal Science and Food Engineering, USP, Pirassununga, Brazil.

2.2. Storage

All samples were stored at room temperature (25 °C) after processing.

2.3. Microbiological analyzes

The formalinized samples were evaluated once, just after removed from vat, performing the water activity (Aw) measurement, spread plate of total mesophiles and moulds presence analyzes.

Glicerinizated and freeze-drying samples were submitted to three collection intercalated by two months between them, always measuring the water activity. Total mesophiles counts was restricted to glicerinizated samples while total psychrotrophics counts to freeze-drying. Both techniques included water activity evaluation, as well as moulds and *Pseudomonas* spp. presence.

2.4. Evaluation of water activity (Aw)

For water activity measurement, it was used an equipment AquaLab brand, Decagon Devices, Inc., Pullman, Wash, USA.

2.5. Total mould counting

Moulds were analyzed following the method described by Silva et al. (2001), by spread plate and using Dichloran Glycerol Agar (DG18) for each glicerinizated and freeze-drying samples and Dichloran Rose Bengal Chloramphenicol Agar (DRBC) Agar, according to water activity obtained. Incubation was performed at 25 °C for 5 days. After counting, results were expressed as colony-forming unit (CFU) per gram of sample, following the protocol preconized by Refs. [15] and [16].