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**MICROBIOLOGY**

# The influence of the microwave oven on the production of solid culture medium and quality of microbial growth

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**Abstract:** Numerous cultivation media currently exist, whether selective, non-selective, enrichment or identification. However, they all have a common goal, which is the growth of microorganisms; the constitution and quality of the culture medium must favor it. For this reason, an important factor that directly affects the quality of a culture medium is its production. Thus, this article investigated the use of a microwave oven in the production of Sabouraud dextrose agar (SDA), and the microbial inactivation compared to the autoclave in a microbiology laboratory. The quality of the medium, time exposure, and sterilization potential were performed using fungal strains of *Candida* spp., *Cryptococcus* spp., *Microsporum* spp., and *Aspergillus* spp. The results showed that the advantages of the use of a microwave oven for the preparation of SDA are practicality, speed, lower energy expense, pH, and constituents preservation of the culture medium, resulting in a richer growth compared to autoclaved SDA. The multivariate analysis of digital images allowed the detection of melanoidins (brownish tone of medium), which are responsible for the negative influence on the microorganisms growth. This research shows the use of the microwave oven as an efficient alternative for the production of the culture medium and maintaining their best quality.

**Key words:** sterilization, microwave oven, sabouraud dextrose agar, mycology, fungal growth, chemometric tools.

## INTRODUCTION

In the 13th century, 400 years before Anton van Leeuwenhoek, a communion bread had a blood-like substance on its surface, which was assimilated to the blood of Christ (Yu 1979). In 1817 Bartholomeo Bizio, by advances in microbiology, showed that this substance was not blood, but a microorganism that he called *Serratia marcescens*. This bacterium colonized bread when stored in a hot, humid place. This is one of the first reports of a bacterium's natural culture (Bonnet et al. 2019).

In 1860 Louis Pasteur creates the first liquid artificial culture medium, where nutrient solutions are boiled for a few minutes and cooled. Noting the lack of growth Pasteur demystifies the theory of spontaneous generation and also finds a way to maintain sterile solutions, used until today (Bonnet et al. 2019) To allow the purification of microorganisms, Robert Koch develops the solid medium. Over the following decades, microbiology was widely evolved, resulting in the discovery and optimization of culture media (Zimbro et al. 2009).

The culture media seek to mimic the natural environment of bacteria with the addition of different elements in the culture medium to cultivate microorganisms previously not

cultivable (Puspita et al. 2012). The cultivation of microorganisms in culture media depends on several important factors such as nutrients, oxygen, humidity, pH, and temperature. Satisfactory growth is proportional to the quality of the constituents and the method of production of the media. Some nutrients include sources of carbon, nitrogen, inorganic phosphates, sulfur, metals, water, and vitamins (Bonnet et al. 2019, Cantarelli et al. 2003, Weenk 1992, Jones et al. 2003).

To maintain the quality of the constituents of the medium, sterilization techniques are of extreme importance, not only for eliminating contaminating microorganisms but also for the preservation of the constituents of the medium. Studies have already shown cases of deterioration of constituents of culture media through autoclaving, resulting in reduced microbial growth compared to other effective ways of producing culture media such as the use of microwave ovens (Shareef et al. 2019, Kothari et al. 2011, Iacoviello & Rubin 2001). The microwave oven has also been used in some industrial processes such as pasteurization of milk and juice, and in bread to reduce microorganisms and increase shelf life (Jaynes 1975, Ohlsson & Bengtsson 2001, Martins et al. 2019).

Because of the advantages of using the microwave oven in our daily lives, this article sought, based on reports in the literature, to evaluate the application of the microwave oven in the production of culture medium in a mycology laboratory. The autoclave was compared to the microwave oven in making a culture medium. The quality of agar production was evaluated using multivariate image analysis (MIA) and qualitative analysis of the growth of fungal species. The quality of the medium as well as the potential for sterilization has also been studied.

## MATERIALS AND METHODS

The tests were carried out using an Electrolux household microwave oven, model ME28S, capacity 28 liters, microwave frequency 2,450 MHz, consumption power 1,600 W. The autoclave used belongs to the brand Prismatec model CS, 30 liters capacity, 2 Kv power, approximately 1.5 Kgf/cm$^3$ pressure, and temperature approximately 121 °C. The study consists of two distinct stages. The first presents assays for the preparation of solid culture medium agar sabouraud dextrose (SDA, Kasvi, Paraná, Brazil). The second addresses the tests carried out on the death of microorganisms using the microwave oven.

### First step

#### *Production and sterility evaluation of SDA by autoclaving and microwaving:*

A 100 mL of sabouraud dextrose agar (SDA) with chloramphenicol (heat stable) (Kasvi, Paraná - Brazil) was produced by autoclaving (15 minutes, 121 °C at 1 ATM) and microwaving (90 seconds, 100% power). Four petri dishes were made, two were subjected to 30 minutes of ultraviolet light (UV) light and the other two did not undergo UV light during the gelling process. The plates were incubated, without inoculation, at 35 °C for four days. After the incubation period, the plates were examined for the presence or absence of contamination.

#### *Influence of time on the sterilization process and the quality of the culture medium*

A 100mL of SDA was produced using different sterilization times (15, 30, and 40 minutes) of autoclaving (121 °C at 1 ATM). The same amount of culture medium (100 mL) was made using the microwave oven in times corresponding to 90, 180, and 240 seconds at 100% power. The pH of each culture medium was measured using

a pHmeter (Kasvi, model K39-0014PA, Paraná, Brazil) before and after both production methods (autoclaving or microwaving). The plates were inoculated with a $10^4$ cells.mL$^{-1}$ of *Cryptococcus* spp., *Candida* spp. and *Microsporum* spp. and the fungal growth was analyzed qualitatively in each prepared agar.

### *Multivariate analysis of images obtained from agar made in microwaves and autoclave*

Digital images of each agar were obtained after being made in the microwave oven (90, 180, and 240 seconds, 100% power) and autoclave (15, 30, and 40 minutes, 121 °C at 1 ATM). The digital images were captured using the Asus Zenfone 5 smartphone (Android v9, Pie operating system, 1.7 GHz octa-core processor, Snapdragon 636, 4 Gb RAM, 12 MP primary camera, and autofocus). Images were acquired with f/1.8 lens aperture in auto mode. Subsequently, the images were analyzed using Matlab® software version 7.10.0.499 (The Math Works, Natick, USA). Three regions of the digital images of each agar were obtained for analysis. The region of interest sampled (100x100 pixels) was transformed into the RGB color histogram. These samples were placed one below the other creating a data matrix with size 18x768. The matrix was subjected to unsupervised HCA (Hierquical Cluster Analysis) and PCA (Principal Component Analysis). For HCA the samples were centered on the mean and analyzed for their single-linkage similarity and Euclidean distance.

## Second step

### *Evaluation of the microbicidal effect of a microwave oven*

A fungal inoculum (5 mL) was prepared in 0.85% sterile saline using fresh cultures of *Candida* spp., *Cryptococcus* spp., and *Aspergillus* spp. approximately $10^3$ cells.mL$^{-1}$ according to the protocols M27-A3 and M38-A2, established by CLSI (2008a, b). A bacterial isolate from *Proteus* sp. was used as a control experiment for chloramphenicol stability of the medium. Aliquots of 25 µL were removed from the tubes containing the inocula before and after autoclaving (15 minutes at 121 °C at 1 ATM) and microwaving (60 seconds, 100% power). The aliquots were plated in SDA. The plates were incubated according to the growth period for each microorganism and read to identify the absence or presence of fungal growth.

### *Effectiveness of the microwave oven in inactivating media containing large concentrations of microbial growth*

Slant SDA tubes containing fungi (*Microsporum* spp., *Candida* spp., and *Cryptococcus* spp.) with abundant growth were subjected to autoclaving and microwaving (15 minutes and 60 seconds respectively). Samples were taken from the tubes and plated before both processes. After being submitted to autoclaving and microwaving, the content of the tubes was poured into the petri dishes. The petri dishes were incubated at 35 °C for *Candida* spp., 32 °C for *Microsporum* spp. and 37 °C for *Cryptococcus* spp. for four days for visual reading.

### *Study of the time of exposure to the microwave oven and microbial inactivation*

Tubes containing 5 mL of an approximately inoculum $10^3$ cells.mL$^{-1}$ (0.85% sterile saline) of *Candida* spp. and *Cryptococcus* spp. An aliquot of 25 uL was plated before and after the first cycle and after each cycle following (10 seconds), completing 1 minute (6 cycles) of microwaving. The times between cycles were approximately 40 seconds, avoiding excessive cooling of the contents present in the tube. The plates were incubated at 37 °C for four days, and read by

evaluating the presence or absence of growth in the different cycles used.

### *Influence of liquid in the microwave oven sterilization process*

A range of colonies of *Candida* spp. was removed and placed on coverslips. These coverslips were subjected to a 1-minute cycle in a microwave oven. Afterward, both coverslips were gently placed on the surface of the SDA and incubated for 2 days at 35 °C for later visualization of the presence or absence of growth.

## RESULTS

The SDA plates made from the autoclave and the microwave oven demonstrated the effectiveness of both methods. Even with and without exposure to UV light during the agar gelling process (approximately 30 minutes), both processes maintained the sterility of the medium after the incubation period. Therefore, no evidence of fungal growth was seen (Supplementary Material - Figure S1 a-d). Nonetheless, there is a difference in color between the media, where SDA made in an autoclave had a darker color related (Maillard reaction products) to SDA made in a microwave oven.

The color difference found between the production methods is confirmed through the production of SDA using different times in autoclave and microwave oven. The most intense color variation was observed at 15, 30, and 40 (Figure 1 2a-c) minutes in autoclaved media. The media manufactured in the microwave oven in 90, 180, and 240 seconds showed a slight visual difference in the shade of the agar (Figure 1 1a-c).

The quality of the agar manufactured at the different times mentioned above was studied by plating strains of *Candida* spp., *Cryptococcus* spp., and *Microsporum* spp. The qualitatively evaluated growth (Figure 2) shows that the longer the time exposure of the agar to heat, the less the development for *Cryptococcus* spp. and *Microsporum* spp. *Cryptococcus* spp. is more affected in the 30 and 40 minutes autoclaved medium. *Candida* spp. shows a less demanding growth profile concerning the quality of the culture medium.

The pH of the culture media was measured before and after the autoclaving and microwave processes in the manufacture of the SDA

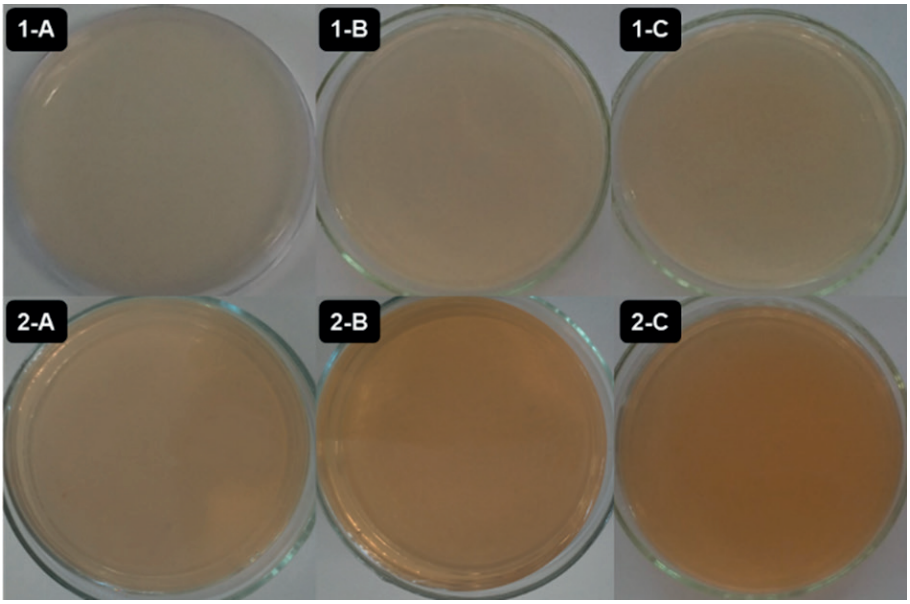


**Figure 1. Sabouraud dextrose agar (SDA) with chloramphenicol made in a microwave oven for 90 (1a), 180 (1b), and 240 (1c) seconds after incubation at 35°C. SDA made in an autoclave for 15 (2a), 30 (2b), and 40 (2c) minutes.**

available in Table I. The most expressive pH variation between the pre and post-production was evidenced by the autoclave, where the acidification of the medium is proportional to the exposure time. As for the SDA made in a microwave oven, the variation on acidification of the medium was minimal. Only the medium subjected to the longest microwaving production time (four minutes) showed a slightly acidification, less than the variation found in the medium made by autoclaving. Therefore, the use of the microwave oven provided excellent pH preservation.

The multivariate analysis developed through the analysis of hierarchical groupings (HCA) of the color of the different media production times showed a similarity profile between the images obtained from the same agar (Figure 2S). Principal component analysis (PCA) shows that two principal components (PC1 x PC2) were able to capture 40% of the color variance of the agars (Figure 3).

PC1 allows us to identify the separation of samples between agar made in microwave oven and autoclave. PC2, on the other hand, allows separating the time profile applied to both production methods. Therefore, the unsupervised analysis (HCA and PCA) allowed the verification of the visual differences in color found in the different agars. When analyzing the loadings obtained by PC1 x PC2 (Figure S3), the regions 368 to 370 and 595 to 598 are components of the colors green and blue and allow to separate the samples of microwaves of 90 and 240 seconds, while colors represented by red are present in regions 131 to 134, allowing the separation of autoclave samples.

Samples of microwave agar 180 seconds also show the separation by red (137 to 139) and green colors (374 to 376). When we analyze the PC1xPC3 score plots (figure S4), PC1 once again manages to separate the two agar preparation techniques, and PC3 also segments the times. When viewing the loadings for PC1 x PC3 (figure S5), the same contributions of green and blue in PC1 were found. However, it seems more evident that the difference in the shade of red (106-114) shows us a more extended arrangement of the autoclaves.

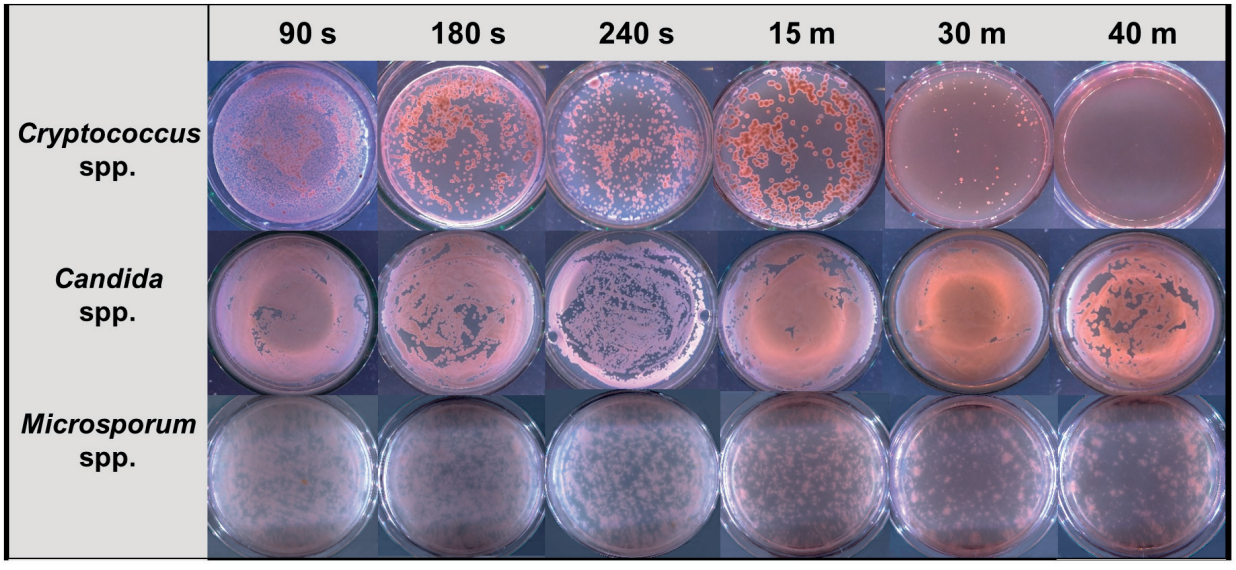


**Figure 2. Fungal growth performance in different agars made by microwave oven (90, 180, and 240 seconds) and autoclave (15, 30, and 40 minutes).**

The general analysis of the main components indicates that the brownish effect presented by the samples is the factor that allows PC1 to perform the separation. The color analysis of lighter profiles (RGB - red, green, and blue) are characterized by the sum of complete channels, for example, the white color where red, blue, and green are 255, 255, 255 respectively, or the absence of color, such as black where all channels are absent (0, 0, 0). Soon colors like yellow have the frequencies of channels 255, 255, and 0 where the sum of red (255), green (255), and absence of blue (0) form the yellow color.

In the transition from light brown tones (eg.: 245, 222, 179) to darker brown tones (eg.: 244, 164, 96), the blue channel decreases, followed by the green channel. This profile was also observed in our analyzes since the blue channel has his participation modified according to the darkening of the agar associated with the gradual increase in the presence of melanoidins and directly proportional to the time of exposure to heat.

After evaluating the quality of the medium manufactured in a microwave oven, its microbicidal effect was compared to autoclaving. It was observed that the fungal suspensions subjected to 60 seconds in a microwave oven resulted in the death of all tested fungi compared to the aliquots removed before exposure (controls), which showed satisfactory growth (Figure S6). The *Proteus* spp., used for the control test of chloramphenicol stability, was inhibited as expected in both media. The same occurs when we test the action of the microwave oven on large amounts of fungal growth (slant tubes containing fungi with abundant growth). Slant agars with abounding fungal growth, which were subjected to autoclaving and microwaving, did not show growth after the 4-day incubation period (Figure S7) of the poured contents. Thus, it is possible to see that microwaving can also inactivate a large amount of microorganisms.

After verifying that 60 seconds of exposure of the microorganisms to the microwave oven is sufficient to cause their death, other times were tested using a fungal inoculum. The aliquots removed from the tubes (in duplicate) before and after the first cycle and after each cycle thereafter (10-second) in the microwave oven (Figure S8) show that *Cryptococcus* spp. (1a-f) was more sensitive when compared to *Candida* spp. (2a-f), which was effectively killed only after 40 seconds of exposure.

Given the proven microbicidal effect of the microwave oven in a liquid medium, fresh colonies of *Candida* spp. were deposited on

**Table I. pH measurement of the SDA medium before and after autoclaving and microwaving.**

| Method/time | pH values* | |
|---|---|---|
| **Autoclave** | **Pre-production** | **Post-production** |
| 15 min | 6.1±0.1 | 5.6±0.1 |
| 30 min | 6.1±0.1 | 5.4±0.1 |
| 40 min | 6.1±0.1 | 5.3±0.1 |
| Microwave oven | Pre-production | Post-production |
| 90s | 6.1±0.1 | 6.1±0.1 |
| 180s | 6.1±0.1 | 6.0±0.1 |
| 240s | 6.2±0.1 | 5.8±0.1 |

***The pH measurement of the SDA medium was performed in triplicate for each container.**

the surface of a dry coverslip and exposed for 1 minute in a microwave oven. The coverslips incubated in SDA plates showed growth after the incubation period. The importance of water in the microbicide effect of microwaving will be discussed in more detail in the discussion session. (Figure S9).

## DISCUSSION

During this study, we show that the use of the microwave oven in the production of a solid culture medium in a research laboratory is effective, fast, practical, economical, and maintaining the quality of the medium. In addition, its use has also been studied on the ability to inactivate contaminated materials with a high and low microbial load. The autoclave was used as a comparison control to use the microwave oven to prove that both methods are effective, but present different aspects of use, which provides different experiences in the laboratory routine.

The data obtained allowed us to infer that the microwave oven is equally effective in the autoclaving process in the production of sterile SDA culture medium since both maintained sterility with or without the aid of UV light during the gelling process. However, it is crucial to highlight the difference found in the color of the media, where the autoclaved SDA has a darker color, indicating a possible Maillard reaction of the culture medium compared to the SDA made by the microwave oven.

Some studies have evaluated the autoclaving process in the production of solid culture medium and have also found the darkening of the autoclaved medium to occur (Bhattacharjee et al. 2009, Kothari et al. 2011, Kim & Lee 2003). This effect can be explained by the Maillard reaction that occurs due to complex non-enzymatic interactions between amino acids or protein and a reducing sugar

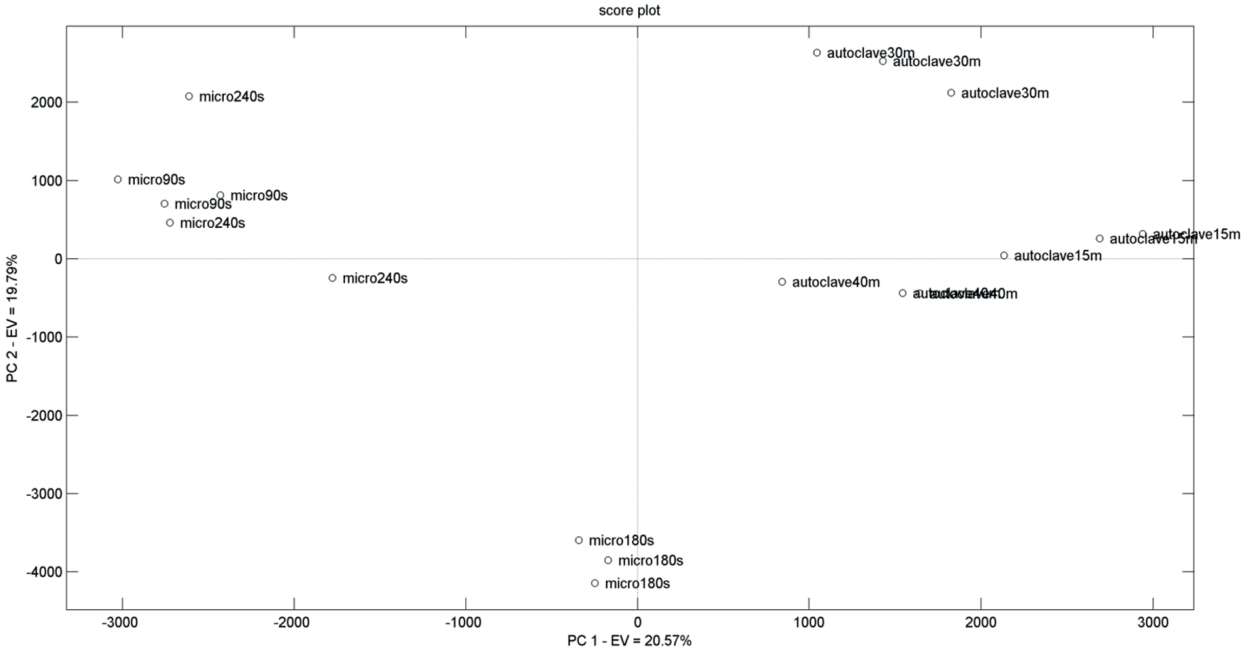


**Figure 3. Score plot PCA1 x PCA2 of the sampled images. Micro90s, Micro180s, and Micro240s: agar made in a microwave for 90, 180, and 240 seconds, respectively. Autoclave15m, Autoclave30m, and Autoclave40m: agar made in a autoclave for 15, 30, and 40 minutes, respectively.**

during heating, giving rise to brown polymeric compounds called melanoidins (Rabbani & Thornalley 2012). In addition, articles have shown that the presence of melanoidins can interfere with the growth or even inhibit of microorganisms (Rufián-Henares & de la Cueva 2009, Song et al. 2016, Mu 2016, Rufián-Henares & Morales 2007). In our study, we also observed this behavior in strains of *Microsporum* spp. and *Cryptococcus* spp. Helou et al. (2013) showed in their study that bacteria in a medium rich in products from the Maillard reaction show an increase in the lag phase of growth and, consequently, a decrease in growth rates as an adaptation mechanism. Baqai & Hafiz (1992) also used the autoclave and microwave oven to make a MacConkey medium for the cultivation of six different bacterial genera, with a more abundant growth in the medium prepared in a microwave oven. Therefore, culture media containing significant amounts of sugars are more likely to be degraded by autoclaving due to high temperatures and prolonged exposure time.

Multivariate image analysis (MIA) is an alternative that has been widely used in several areas of science, such as food, chemical, biomedical and environmental analysis (Herrero-Latorre et al. 2019, Cabrita et al. 2012, Antonelli et al. 2004, Botelho et al. 2014, Nattkemper 2004, Hemmateenejad et al. 2010). In our work, MIA allowed the manufactured agar to be successfully separated according to each characteristic, so we were able to associate brown tones in a darkening scale with the presence of melanoidins. These appear to be proportional to the acidification of the medium, degradation of the constituents, and consequently, there is a reduced growth probability, mainly affecting microorganisms whose metabolism requires a richer culture medium to achieve optimum growth.

As seen in our results, the thermal degradation of glucose is related to acidification of the culture medium, therefore, the greater the degradation suffered by glucose, the lower the pH of the culture medium. Woo et al. (2015) evaluated the thermal degradation of glucose solutions, noting that acidification occurred at all times and temperatures tested, due mainly to the increase in organic acids. The same acidification profile was found in our autoclaved media, as the microwave oven showed excellent pH conservation even in the largest exposure times. This fact is due to the inability of the microwave oven to exceed 100 °C (sea level) due to ambient atmospheric pressure compared to the 2 ATM reached by the autoclave.

Although the association between temperature and time of exposure represents a strong influence on the final pH of the culture medium, we can observe that the differences in the growth of the tested fungi can be directly correlated with the degradation of the other constituents of the culture medium. An example of this behavior is *Cryptococcus* spp. which showed a reduction in visual growth in SDA manufactured by the microwave oven during 180 seconds of heating, where the pH was practically not changed after production.

When we observe the behavior of *Candida* spp. in the different culture mediums we see a homogeneous growth at all times of production, regardless of the form of production. Thus *Candida* spp. demonstrates greater tolerance to pH variations, as well as to degradation products present in culture media with higher exposure to heat. This behavior of greater tolerance to pH variations is evidenced by Heaney et al. (2020) presenting in their study the growth capacity of some species of the genus *Candida* in pH ranges 2 to 13.

With the absence of growth of *Cryptococcus* spp. on autoclaved agar for 30 and 40 minutes,

we can see that this genus has a relatively fastidious growth character. Yoneyama et al. (2007) studied the effect of solutions containing different concentrations of autoclaved D-glucose and reports that *Salmonella*, *Escherichia coli*, and *Listeria* sp. suffered from bactericidal effect to the increase of the concentration of degraded D-glucose, however, the strains of *Vibrio* sp. were the most affected, thus highlighting the influence of the culture medium on growth characteristics.

Another significant constituent present in the culture medium studied is chloramphenicol, used to prevent contamination by bacteria. Ramos & Pilawa (2016) evaluated the effect of temperature on the degradation and generation of free radicals by chloramphenicol during thermal sterilization and found that temperatures equal to or higher than 120 °C culminate in the higher formation of free radicals and degradation. In our study, we used a *Proteus* sp. and we showed that the microwave oven was not able to degrade the chloramphenicol present in the medium since there was no bacterial growth present in both media demonstrating stability in its concentration sufficient to inhibit bacteria.

Shareef et al. (2019) studied the production process of culture medium by autoclave and microwave oven, as well as the microbicidal effect on strains of *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Penicillium* sp., Observing the elimination of all microorganisms with 4 minutes of use at 50 Hz power. In addition, they also found different shades of culture media autoclaved and manufactured in a microwave oven, also seen in our study.

Kang & Kato (2014) found a germicidal effect (99%) after 20 seconds of treatment in a microwave oven at a power of 1000 W for samples of *Cladosporium herbarum* and *Fusarium solani*, except for *Bacillus subtilis* spores. A study by Latimer & Matsen (1977) showed that *B. subtilis* spores were inactivated with 5 minutes of exposure, and the other tested bacteria were entirely eliminated within 45 seconds. These findings demonstrate that the effectiveness of the elimination of microorganisms using the microwave oven is dependent on the exposure time and the power of the device.

When we tested the action of the microwave oven for 60 seconds only on the pure colonies of *Candida* spp. removed from the culture medium and deposited on coverslips, we find the growth of the remaining cells. This finding indicates the importance of the presence of water for the performance of the microbicidal effect. Dixon et al. (1999) submitted tablets of base materials used in the production of dentures to the microwave oven in the presence and absence of water. Only materials that were submerged in water were completely sterilized. Another study also noted the importance of the liquid medium in the microwave sterilization process (Jeng et al. 1987) used *B. subtilis* spores in borosilicate vials in the absence of liquid and reaches 90% spore reduction viable only after 45 minutes of exposure, thus highlighting the essential activity of water in the microbicidal effect.

There are two main protocols for standardization and quality control for the production of microbiological culture media, they are ISO 11133-1:2014 and CLSI M22-A3 (2004). However, these two documents have restricted access, they are only available for purchase. Although each manufacturer has its internal quality control for the culture media, there is still a lack of consensus on the preparation methods. Cantarelli et al. (2003) raised the question of applying the M22-A2 guideline (NCCLS 1996) corresponding to quality control for commercially prepared culture media is sufficient in the laboratory routine. In their study, they report that commercially manufactured

culture media do not provide good microbial growth, even under quality control, compared to homemade culture media. Although most quality control protocols are comprehensive mainly for culture media used in bacteriology, an Australian protocol (Guidelines for the Quality Assurance of Medical Mycology culture media 2012) was developed in 2012 for media used in mycology. This protocol arises due to the lack of consensus and standardization on the production and control of media.

A study used five different marks of Sabouraud Gentamicin-Chloramphenicol Agar Media to evaluate the growth of 124 fungi and concluded that only one brand provided great growth for all tested fungi (Brun et al. 2001). These reports show us that the absence of a well-established universal standardization for evaluating the quality of growth of microorganisms in the culture media ends up generating difficulties and inconsistencies within the microbiological analyzes. False results can occur when we use production techniques that compromise the growth of microorganisms, resulting in erroneous results in determining susceptibility by disc-diffusion, incorrect quantification of colony-forming units, as well as making it difficult to isolate and identify some pathogens in clinical samples (Wang et al. 2019, Fowotade et al. 2018).

One of the main limitations of the use of the microwave oven in the sterilization of materials is the various constitution of these, consequently, metals and some types of plastics cannot be placed in the microwave oven due to the risk of damaging the equipment as well as the material. Another problem is the capacity, usually, the microwave oven has up to 38 liters of space, thus being a disadvantage compared to the autoclave. However, the use of the microwave oven in this study proved to be a fast, practical, and economical alternative in terms of energy consumption for making culture media, as well as for inactivating microorganisms.

In addition, the ability to reach temperatures close to 100 °C quickly, and consequently the shorter time of exposure to heat allows the preservation of the constituents, thus providing better microbial growth. This study presents a perspective to adaptation and implementation of this technology to the laboratory routine as well as its improvement seeking to optimize the processes of sterilization, confection, and quality of culture media. Besides, the color analysis performed in this study can be a helpful tool as an indicator of agar quality is an efficient and accurate alternative that can be applied in industrial quality control for commercial culture media.

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## REFERENCES

ANTONELLI A, COCCHI M, FAVA P, FOCA G, FRANCHINI GC, MANZINI D & ULRICI A. 2004. Automated evaluation of food colour by means of multivariate image analysis coupled to a wavelet-based classification algorithm. Analytica Chimica Acta 1: 3-13. DOI:10.1016/j.aca.2004.01.005.

BAQAI R & HAFIZ S. 1992. Microwave oven in microbiology laboratory. J Pak Med Assoc 1: 2-3. PMID: 1545479.

BHATTACHARJEE MK, SUGAWARA K & AYANDEJI OT. 2009. Microwave sterilization of growth medium alleviates inhibition of Aggregatibacter actinomycetemcomitans by Maillard reaction products. J Microbiol Methods 2: 227-230. DOI:10.1016/j.mimet.2009.06.004.

BONNET M, LAGIER JC, RAOULT D & KHELAIFIA S. 2019. Bacterial culture through selective and non-selective conditions: the evolution of culture media in clinical microbiology. New Microbes New Infect 34: 100622. DOI:10.1016/j.nmni.2019.100622.

BOTELHO BG, DE ASSIS LP & SENA MM. 2014. Development and analytical validation of a simple multivariate calibration method using digital scanner images for sunset yellow

determination in soft beverages. Food Chemistry 159: 175-180. DOI:10.1016/j.foodchem.2014.03.048.

BRUN S, BOUCHARA JP, BOCQUEL A, BASILE AM, CONTET-AUDONNEAU N & CHABASSE D. 2001. Evaluation of five commercial Sabouraud gentamicin-chloramphenicol agar media. Eur J Clin Microbiol Infect Dis 10: 718-723. DOI: 10.1007/s100960100577.

CABRITA MJ, AIRES-DE-SOUSA J, DA SILVA MDRG, REI F & FREITAS AMC. 2012. Multivariate statistical approaches for wine classification based on low molecular weight phenolic compounds. Aust J Grape Wine Res 2: 138-146. DOI:10.1111/j.1755-0238.2012.00182.x.

CANTARELLI VV, INAMINE E, BRODT TCZ, SECCHI C, PEREIRA FDS, AMARO MC, BATALHA AA & LIGIERO SD. 2003. Quality control for Microbiological culture media. Is it enough to follow the NCCLS M22-A2 procedures? Braz J Microbiol 34: 8-10. DOI: 10.1590/S1517-83822003000500003.

CLSI - CLINICAL AND LABORATORY STANDARDS INSTITUTE M22-A3. 2004. Quality Control for Commercially Prepared Microbiological Culture Media. Approved Standard – 3rd ed., Wayne, PA.

CLSI - CLINICAL AND LABORATORY STANDARDS INSTITUTE M27-A3. 2008a. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. Approved Standard – 3rd ed., Wayne, PA.

CLSI - CLINICAL AND LABORATORY STANDARDS INSTITUTE M38-A2. 2008b. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi. Approved Standard – 2nd ed., Wayne, PA.

DIXON DL, BREEDING LC & FALER TA. 1999. Microwave disinfection of denture base materials colonized with Candida albicans. J Prosthet Dent 2: 207-214. DOI:10.1016/s0022-3913(99)70250-7.

FOWOTADE A, FAYEMIWO SA, BONGOMIN F, FASUYI TO, AIGBOVO OA & ADEGBORO B. 2018. Internal and external quality control in the medical microbiology laboratory. Afr J Clin Exper Microbiol 3: 238-250. DOI: 10.4314/ajcem.v19i4.1.

GUIDELINES FOR THE QUALITY ASSURANCE OF MEDICAL MYCOLOGY CULTURE MEDIA. 2012. Culture Media & Mycology Special Interest Groups, Australian Society for Microbiology.

HEANEY H, LAING J, PATERSON L, WALKER AW, GOW NAR, JOHNSON EM, MACCALLUM DM & BROWN AJP. 2020. The environmental stress sensitivities of pathogenic Candida species, including Candida auris, and implications for their spread in the hospital setting. Med Mycol 6: 744-755. DOI: 10.1093/mmy/myz127.

HELOU C, MARIER D, JACOLOT P, ABDENNEBI-NAJAR L, NIQUET-LÉRIDON C, TESSIER FJ & GADONNA-WIDEHEM P. 2013. Microorganisms and Maillard reaction products: a review of the literature and recent findings. Amino Acids 46: 267-277. DOI:10.1007/s00726-013-1496-y.

HEMMATEENEJAD B, MOBARAKI N, SHAKERIZADEH-SHIRAZI F & MIRI R. 2010. Multivariate image analysis-thin layer chromatography (MIA-TLC) for simultaneous determination of co-eluting components. The Analyst 7: 1747-1758. DOI:10.1039/c0an00078g.

HERRERO-LATORRE C, BARCIELA-GARCÍA J, GARCÍA-MARTÍN S & PEÑA-CRECENTE RM. 2019. Detection and quantification of adulterations in aged wine using RGB digital images combined with multivariate chemometric techniques. Food Chem 3: 100046. DOI:10.1016/j.fochx.2019.100046.

IACOVIELLO MP & RUBIN SA. 2001. Sterile preparation of antibiotic-selective LB agar plates using a microwave oven. Biotechniques 5: 963-965. DOI: 10.2144/01305bm08.

ISO 11133-1. 2014. Microbiology of food and animal feeding stuffs – Practical guidelines on performance testing of culture media.

JAYNES HO. 1975. Microwave pasteurization of milk. J Milk Food Technol 7: 386-387. DOI: 10.4315/0022-2747-38.7.386.

JENG DK, KACZMAREK KA, WOODWORTH AG & BALASKY G. 1987. Mechanism of microwave sterilization in the dry state. Appl Environ Microbiol 9: 2133-2137. DOI:10.1128/AEM.53.9.2133-2137.1987.

JONES RN, KRISHER K & BIRD DS. 2003. Results of the survey of the quality assurance for commercially prepared microbiology media. Update from the College of American Pathologists Microbiology Surveys Program (2001). Arch Pathol Lab Med 6: 661-665. DOI:10.5858/2003-127-661-rotsot.

KANG Y & KATO S. 2014. An electromagnetic simulation study of the distribution of the power absorbed in evaporative humidifier elements. HVAC&R Research 8: 899-907. DOI: 10.1080/10789669.2014.954918.

KIM KW & LEE SB. 2003. Inhibitory effect of Maillard reaction products on growth of the aerobic marine hyperthermophilic archaeon Aeropyrum pernix. Appl Environ Microbiol 7: 4325-4328. DOI:10.1128/aem.69.7.4325-4328.2003.

KOTHARI V, PATADIA M & TRIVEDI N. 2011. Microwave sterilized media supports better microbial growth than autoclaved media. Res Biotechnol 5: 63-72. ISSN: 2229-791X.

LATIMER JM & MATSEN JM. 1977. Microwave oven irradiation as a method for bacterial decontamination in a clinical microbiology laboratory. J Clin Microbiol 4: 340-342. DOI: 10.1128/jcm.6.4.340-342.1977.

MARTINS CPC ET AL. 2019. Microwave processing: current background and effects on the physicochemical and microbiological aspects of dairy products. Compr Rev Food Sci Food Saf 1: 67-83. DOI:10.1111/1541-4337.12409.

MU K. 2016. Evidence to indicate that maillard reaction products can provide selective antimicrobial activity. Integr Food Nutr Metab 4: 330-335. DOI: 10.15761/IFNM.1000154.

NATTKEMPER TW. 2004. Multivariate image analysis in biomedicine. Journal of Biomedical Informatics 5: 380-391. DOI:10.1016/j.jbi.2004.07.010.

NCCLS - NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS M22-A2. 1996. Quality Control for Commercially Prepared Microbiological Culture Media; 2nd ed., Pennsylvania – USA.

OHLSSON T & BENGTSSON N. 2001. Microwave technology and foods. Adv Food Nutr Res 43: 65-140. DOI: 10.1016/s1043-4526(01)43003-8.

PUSPITA ID, KAMAGATA Y, TANAKA M, ASANO K & NAKATSU CH. 2012. Are uncultivated bacteria really uncultivable? Microbes Environ 4: 356-366. DOI:10.1264/jsme2.me12092.

RABBANI N & THORNALLEY PJ. 2012. Glycation research in amino acids: a place to call home. Amino Acids 4: 1087-1096. DOI:10.1007/s00726-010-0782-1.

RAMOS P & PILAWA B. 2016. Free radical formation in chloramphenicol heated at different temperatures and the best thermal sterilization conditions - application of EPR spectroscopy and UV spectrophotometry. Pharm Dev Technol 10: 1016-1023. DOI:10.1080/10837450.2016.1265555.

RUFIÁN–HENARES JÁ & DE LA CUEVA SP. 2009. Antimicrobial Activity of Coffee Melanoidins–A Study of Their Metal-Chelating Properties. J Agric Food Chem 2: 432-438. DOI:10.1021/jf8027842.

RUFIÁN–HENARES JA & MORALES FJ. 2007. Antimicrobial activity of melanoidins. J Food Qual 30: 160-168. DOI: 0.1111/j.1745-4557.2007.00112.x.

SHAREEF SA, HAMASAEED PA & ISMAEIL AS. 2019. Sterilization of Culture Media for Microorganisms Using a Microwave Oven Instead of Autoclave. Raf J Sci 1: 1-6. DOI:10.33899/rjs.2019.159390.

SONG R, YANG P, WEI R & RUAN G. 2016. Antioxidative, Antibacterial, and Food Functional Properties of the Half-Fin Anchovy Hydrolysates-Glucose Conjugates Formed via Maillard Reaction. Molecules 6: 795. DOI:10.3390/molecules21060795.

WANG H, LI Y, FAN X, CHIUEH TS, XU YC & HSUEH PR. 2019. Evaluation of Bruker Biotyper and Vitek MS for the identification of Candida tropicalis on different solid culture media. J Microbiol Immunol Infect 4: 604-611. DOI: 10.1016/j.jmii.2017.11.002.

WEENK GH. 1992. Microbiological assessment of culture media: comparison and statistical evaluation of methods. Int J Food Microbiol 2: 159-181. DOI: 10.1016/0168-1605(92)90113-h.

WOO KS, KIM HY, HWANG IG, LEE SH & JEONG HS. 2015. Characteristics of the Thermal Degradation of Glucose and Maltose Solutions. Prev Nutr Food Sci 2: 102-109. DOI:10.3746/pnf.2015.20.2.102.

YONEYAMA N, HARA-KUDO Y & KUMAGAI S. 2007. Effects of heat-degraded sugars on survival and growth of Vibrio parahaemolyticus and other bacteria. J Food Prot 2: 373-377. doi:10.4315/0362-028X-70.2.373.

YU VL. 1979. Serratia marcescens: historical perspective and clinical review. N Engl J Med 16: 887-893. DOI:10.1056/NEJM197904193001604.

ZIMBRO MJ, POWER DA, MILLER SM, WILSON GE & JOHNSON JA. 2009. Difco & BBL Manual: Manual of Microbiological Culture Media, 2nd ed., BD Diagnostics - Diagnostics Systems.

## SUPPLEMENTARY MATERIAL

**Figure S1. Sabouraud dextrose agar (SDA) with chloramphenicol after incubation for 4 days at 35 °C. a: SDA produced by autoclaving and subjected to 30 minutes of UV light. b: SDA produced by autoclaving without using UV light. c: SDA made in a microwave oven submitted to 30 minutes of UV light. d: SDA produced in a microwave oven without using UV light.**
**Figure S2. Analysis of hierarchical groupings by the pattern of sample points according to the single-linkage algorithm and Euclidean distance of the images obtained from the microwave agar (1, 3, and 4 minutes) and autoclave (15, 30, and 40 minutes).**
**Figure S3. PCA1 x PCA2 loadings of the sampled images showing the distribution of variables. The numbers represent the distribution of the color histogram, where red belongs to channels 1-255, green 256-512, and blue 513-768.**
**Figure S4. Score plot PCA1 x PCA3 of the sampled images. Micro90s: agar made in microwave for 90 seconds. Micro180s: agar made in a microwave for 180 seconds. Micro240s: agar made in microwave for 240 seconds. Autoclave15m: autoclaved agar for 15 minutes. Autoclave30m: autoclaved agar for**

**30 minutes. Autoclave40m: autoclaved agar for 40 minutes.**
**Figure S5. PCA1 x PCA3 loadings of the sampled images showing the distribution of variables. The numbers represent the distribution of the color histogram, where red belongs to channels 1-255, green 256-512, and blue 513-768.**
**Figure S6. Inocula before and after sterilization procedure. a: Control of inoculum growth containing *Aspergillus* sp. b: Inoculum of *Aspergillus* sp. after 15 minutes of autoclaving. c: Inoculum of *Aspergillus* sp. after 1 minute of exposure to the microwave. d: Control of inoculum growth containing *Candida* sp. e: *Candida* sp. after 15 minutes of autoclaving. f: *Candida* sp. after 1 minute of exposure to the microwave. g: Control of growth of the inoculum containing *Cryptococcus* sp. h: inoculum of *Cryptococcus* sp. after 15 minutes of autoclaving. i: Inoculum of *Cryptococcus* sp. after 1 minute of exposure to the microwave.**
**Figure S7. The use of autoclave and microwave oven for inactivation of high microbial load. The numbers 1, 2, and 3 represent *Cryptococcus* spp., *Candida* spp., and *Trichophyton* spp. respectively. a: samples taken from tubes before autoclaving and microwaving to control the growth of microorganisms. b: contents of plated tubes after autoclaving. c: Content of the plated tubes after using a microwave oven.**
**Figure S8. Analysis of the time of exposure of the fungal inoculum to the microwave oven. 1: *Cryptococcus* spp., 2: *Candida* spp., a, b, c, d, e, and f exposure for 10, 20, 30, 40 50, and 60 seconds respectively. Upper side: replicate 1, bottom: replicate 2.**
**Figure S9. Colonies of *Candida* spp. exposed to 60 seconds in a microwave oven without a liquid medium. a: control growth of *Candida* spp. before microwaving. b and c: coverslips plated with *Candida* spp. colonies after 60 seconds microwaving.**

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