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MATHEUS VIEIRA NASCIMENTO

**ELABORAÇÃO DE UM PROTOCOLO DE ESTERILIZAÇÃO DE ESPÉCIMES DE
RESINA ACRÍLICA EM MICRO-ONDAS**

Fortaleza (CE)

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Trabalho de Conclusão de Curso apresentado à
Coordenação do curso Graduação em
Odontologia da Universidade Federal do Ceará
como requisito parcial para a obtenção do
Título de Bacharel em Odontologia.

Orientadora: Profa. Dra. Karina Matthes de
Freitas Pontes

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RESUMO

Experimentos microbiológicos laboratoriais que utilizam resina acrílica muitas vezes necessitam da esterilidade desse material, contudo os métodos mais convencionais de esterilização, como gás óxido de etileno, autoclavagem ou imersão em hipoclorito de sódio são desvantajosos seja por seu custo elevado ou por alterar propriedades da resina. A irradiação por micro-ondas tem sido constantemente reportada na literatura como método seguro tanto no quesito desinfecção como manutenção das propriedades da resina acrílica. Contudo, ainda não se foi estabelecido um protocolo de esterilização padrão que atendesse a normas de controle de esterilidade. O objetivo deste trabalho foi, portanto, estabelecer parâmetros de esterilização com irradiação em micro-ondas para espécimes de resina acrílica, sem prejuízos em suas principais propriedades físico-mecânicas. Para a irradiação no micro-ondas, foram utilizados os tempos de 3 ou 5 minutos associados a potências de 450W ou 650W com os espécimes imersos em 250 mL de água destilada. Na primeira etapa do estudo, os espécimes foram submetidos à formação de biofilme monoespécie de *Staphylococcus aureus* e *Candida albicans*, antes de serem irradiados (n=18). Após irradiação, foi realizado teste de esterilidade em tioglicolato com 14 dias de incubação a 37°C, para checagem da turvação do meio. Na segunda etapa, corpos-de-prova em resina acrílica foram confeccionados para serem submetidos aos testes de rugosidade de superfície (Ra, µm; n=40), microdureza Knoop (kg/mm²; n=40) e estabilidade dimensional (mm; n=40), antes e após os protocolos de irradiação. As análises estatísticas foram realizadas sob intervalo de confiança de 95% ($\alpha=0,05$). Todos parâmetros avaliados (450W/650W por 3 ou 5 minutos) foram capazes de esterilizar as amostras expostas a *S. aureus* e *C. albicans*, exceto o protocolo de 3 minutos a 450W. Não foram constatadas diferenças estatisticamente significativas entre o grupo controle e os parâmetros testados ($p > 0,05$) na rugosidade e na estabilidade dimensional. Na microdureza, somente o parâmetro 450W/3 minutos não ocasionou diferenças estatísticas significativas. Assim, conclui-se os parâmetros 450W/5 minutos e 650W/3 e 5 minutos podem ser considerados seguros na elaboração de um protocolo de esterilização, contudo mais estudos, tanto mecânicos quanto microbiológicos, são necessários para consolidação destes parâmetros.

Palavras-chave: Micro-ondas, esterilização, rugosidade, microdureza, estabilidade dimensional.

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STANDARDIZATION OF MICROWAVE STERILIZATION PROTOCOL FOR ACRYLIC RESIN SPECIMENS

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Abstract

Aims: The purpose of this work was to establish sterilization parameters with microwave irradiation for acrylic resin specimens, without impairment of their main physico-mechanical properties.

Materials and Methods: Microwave irradiation was carried out for 3 or 5 minutes associated with potencies of 450W or 650W with the specimens immersed in 250 mL of distilled water. In the first stage of this study, the specimens were submitted to monospecific biofilm formation of *Staphylococcus aureus* and *Candida albicans*, before being irradiated (n = 18). After irradiation, a thioglycollate sterilization test was performed with 14 days of incubation at 37 ° C, in order to check the turbidity of the medium. In the second stage, samples were tested for surface roughness (Ra, µm; n = 40) as well as Knoop microhardness (kg / mm²; n = 40) and dimensional stability (mm; n = 40) before and after the irradiation protocols. Statistical analyzes were performed under confidence interval of 95% ($\alpha = 0.05$).

Results: All irradiation parameters (450W / 650W for 3/5 minutes) were able to sterilize the samples exposed to *S. aureus* and *C. albicans*, except the protocol 450W/3 minutes. There were no statistically significant differences between the control group and the parameters tested ($p > 0.05$) for roughness and dimensional stability. Only the parameter 450W / 3 minutes did not cause significant statistical differences in microhardness values.

Conclusion: The parameters 450W / 5 minutes and 650W / 3 and 5 minutes can be considered safe values for the elaboration of a sterilization protocol, however further studies, both mechanical and microbiological, are still necessary to consolidate these parameters.

Keywords: microwave, sterilization, roughness, microhardness, dimensional stability.

Introduction

The contamination of acrylic dentures in the oral cavity by microorganisms often leads to lesions and infections, such as stomatitis, angular cheilitis, traumatic ulcers, etc., which compromise not only the durability and adaptability of the device, but also the health of the host^{1,2}. For simulating the conditions found in the oral cavity, acrylic resin samples are vastly used for laboratorial studies and, like acrylic dentures, these samples face challenges concerning convenient and financially affordable sterilization procedures^{3,4}. Several techniques are implemented in the sterilization and disinfection of these materials, especially chemical methods, such as ethylene oxide, chlorhexidine gluconate, and sodium hypochlorite, among others³. Typical laboratory sterilization methods, such as autoclaving, cannot be appointed for acrylic dentures sterilization because upon reaching temperatures above 71°C the material can suffer plastic deformation⁵. However, in addition to having high costs, many of these substances may alter the properties and characteristics of the acrylic sample: color and shape distortion have been reported in the literature⁶⁻⁹.

Considered a more practical and faster approach for the sterilization of acrylic resin samples, microwave irradiation is displayed as an alternative method, however it is still regarded with caution, once it may cause alteration of physical and mechanical properties¹⁰⁻¹⁴. In this regard, three variables appear to play a major role in the outcomes: irradiation time, potency and amount of water for immersion of specimens^{15,11,16,17}. In early studies, irradiation time of 6 minutes was declared efficient in sterilizing both acrylic sample and acrylic dentures^{18,19}. Nevertheless, several authors pointed out that this parameter was responsible for considerable distortions in dimensional stability, flexural strength, surface roughness, microhardness and shear bond strength^{20-22,11,23,24,17,16}. In more recent studies, lower irradiation time (3 – 5 minutes) with the potency varying from 450 to 650 W has shown consistent results with little or no significant mechanical and physical alteration^{15,10,12,25,23,21,26}. Samples that underwent cycles without immersion in water were more prone to shape distortion and less effective in killing the microorganisms¹³, suggesting that quantity of water also influences the end result^{15,27}. In most studies, the amount of water is usually found between

150 and 250 mL. Even in irradiation cycles of less than 5 minutes adjusted to 650 W or less, some changes in the physical and mechanical properties were observed in reservoirs containing 150 mL of distilled water^{28,29}, whereas by using 200 mL better results were obtained.^{30,31,22}

Moreover, these variable parameters were also reported to be capable of eliminating microorganisms associated with denture contamination, such as *Candida albicans*, *Staphylococcus aureus*, *Bacillus subtilis*, etc^{15,27,32-35}.

Nonetheless, literature still lacks a sterilization protocol applying certified methods, such as provided by the Code of Federal Regulations (21 CFR 610.12)³⁶ and the United States Pharmacopoeia (USP, Chapter <71>)³⁷. Some of these testing methods are based on the turbidity in liquid culture media, which indicates viable microorganisms. According to this method, samples should remain in incubation for at least 14 days in a liquid culture media in order to allow slow growing microorganisms to develop, which could be overseen in the other methods with shorter incubation time. For the present work, in order to elaborate a sterilization protocol for acrylic resin samples for laboratorial purposes, in accordance with the above mentioned regulations, fluid thioglycollate medium (FTM) was selected due to its versatile properties that allow several microorganisms to grow.

The aim of this study was to test the reproducibility of the results published in literature by using microwave energy to eliminate microorganisms, using certified sterilization inspection methods, while not significantly changing the material properties in order to eventually elaborate and implement a standardized microwave laboratory sterilization protocol. The working hypothesis is that microwave irradiation is capable of sterilizing the acrylic resin samples without causing any mechanical or physical distortion.

Materials and Methods

Study Design

This research was carried out in two distinct stages: the first phase consisted of microbiological experiments, in which the effectiveness of microwave energy in the sterilization of acrylic resin samples

was tested against two representative microorganisms: *Staphylococcus aureus* (gram + bacteria) and *Candida albicans* (yeast). In the second stage, the effect of microwave energy on surface roughness, Knoop microhardness and dimensional stability of acrylic resins was determined.

For both stages, the following irradiation times and potencies were established:

- 450W for 3 minutes;
- 650W for 3 minutes;
- 450W for 5 minutes;
- 650W for 5 minutes.

Sample Preparation

Both microbiological and mechanical experiments were performed with microwave thermo-polymerizable acrylic resin sample (OndaCryl), commonly used for acrylics of prosthetic bases. The preparation of these samples was achieved by the inclusion of metallic matrices of various shapes and sizes in stone casts, following ISO standards (1567)³⁸ (Table 1).

Table 1 Samples shape and size according to the intended experiment.

| Experiment | Sample shape | Sample size |
|--------------------------------------|--------------|---|
| Antimicrobial activity | Round | 14 x 3 mm (diameter x thickness) |
| Surface Roughness | Rectangular | 64 x 10 x 3,3 mm (length x width x thickness) |
| Microhardness/ Dimensional stability | Round | 14 x 3 mm (diameter x thickness) |

After casting, the metal standards were removed from the muffle and the acrylic resin was manipulated following the manufacturer's recommendations. The finishing and polishing were done in a politriz (Aropol, Arotec Indústria e Comércio), with water sandpapers (Norton Indústria Brasileira) of granulations 220, 400, 600, 1200 and 2400, and afterwards the sample dimensions were confirmed using a digital

caliper (Model CD-6 " CSX-B, Mitutoyo Sul Americana LTDA). The samples were then cleaned, immersed in distilled water and kept in an oven (TE-393/1. Tecnal) at $37 \pm 1^\circ\text{C}$ for 1 week in order to release residual monomer excess. Distilled water was replaced daily.

Microbiological Assay

For the following procedures, all samples were previously sterilized with ethylene oxide and stored for one week in order to allow the release of any residual gas.

The microorganisms tested in this research were *Staphylococcus aureus* (ATCC 25923) and *Candida albicans* (ATCC 10231). The same procedures were executed for each microorganism strain individually. Both microorganisms underwent activation and resuspension process before each experiment and their optical density (O.D.) in a dilution with brainheart infusion broth (BHI, Kasvi) was measured with a spectrophotometer (Ultrospec 1100 pro, Amersham Biosciences) after 18h of incubation in BHI at 37°C . For a dilution corresponding to 10^6 colony forming units (CFU)/mL, the absorbances registered ranged between 0.08 and 0.10 at a wavelength of 652 nm for *Staphylococcus aureus* and 0.28 at a wavelength of 530 nm for *Candida albicans*³⁹.

Sample contamination consisted of their immersion in 1.5 mL of contaminated culture medium broth in individual wells of a sterile 24-well tissue plate (Kasvi), each well containing a sterile acrylic resin disk. After this procedure, the 24-well plate was incubated at 37°C for 48 hours to allow biofilm growth on the sample surface.

Subsequent to incubation, each sample was rinsed inside an individual well of another sterile 24-well plate containing 1.5 mL of sterile distilled water. Each disk was dipped into an individual well three times before being transferred with sterile tweezers into sterile glass pots, each containing 250 mL of sterile distilled water¹⁵. Then, each sample was randomly assigned to the one of following groups^{35,34} as depicted in the table 2 (n=3):

After the appropriate treatments, each sample was rinsed again with sterile distilled water and individually placed in transparent glass test tubes containing 5 ml of sterile fluid thioglycollate medium (FTM, Kasvi).

Table 2 Microwave irradiation time of each experimental group and the potency value for each irradiation time.

| Time / Potency | No irradiation | 450 W | 650 W |
|---|--|------------------------------|------------------------------|
| No irradiation No biofilm growth | Positive Control Group (ethylene oxide sterilization) | -- | -- |
| No irradiation <i>C. albicans</i> biofilm <i>S. aureus</i> biofilm | Negative Control Group | -- | -- |
| 3 minutes irradiation <i>C. albicans</i> biofilm <i>S. aureus</i> biofilm | -- | Experimental Group 1 (E1) | Experimental Group 3 (E3) |
| 5 minutes irradiation <i>C. albicans</i> biofilm <i>S. aureus</i> biofilm | -- | Experimental Group 2 (E2) | Experimental Group 4 (E4) |

The test tubes were vortexed and stored for incubation in a bacteriological oven for 14 days at 35 ° C, being monitored daily for turbidity or non-turbidity observation of the culture medium, following the recommendations from the Code of Federal Regulations (21 CFR 610.12)³⁶ and the United States Pharmacopoeia (USP, Chapter <71>)³⁷.

Mechanical Assays

Samples were individually immersed in a glass vessel containing 250 mL of distilled water and placed in the microwave oven (BMY45, Brastemp) with the power and time of the corresponding experimental group.

Surface roughness

Using a surface roughness reader profilometer (T 1000, Hommel Tester, Hommelwerke, G) five measurements were performed on each sample to obtain the mean surface roughness (Ra, µm), in order to observe if the surfaces of the samples were standardized before starting the experiment¹⁶. Roughness

values were compared before and after irradiation, comparing values within the same group and among different groups (n = 40).

Knoop Microhardness

Microhardness measurements (kg/mm^2) were obtained with a microdriometer (FM-ARS 9000 and FM-100, Future-Tech corp). The specimens were subjected to a calibrated vertical load of 25g for 5 seconds. For each of them, five random indentations were performed and a mean of the control group was calculated for later comparison with the means of the experimental groups²⁹ (n = 40).

Dimensional stability

Samples were marked with diamond tipped burs in peripheral points forming a triangle of approximate sides. Standardized photos were taken using a stereoscopic microscope (Microsystem Wetglar) and a digital camera from a phone (Galaxy S7, f/1.7, 12 MP Samsung Eletronics Co. Ltd). All photographs were taken placed next to a millimeter scale. The segments were measured with ImageJ Launcher software (Research Services Branch), calculating the length (mm) of each segment, determined in the photos obtained before and after the sterilization protocol⁴⁰ (n = 40).

Data analysis

For statistical analysis, the software GraphPad Prism 7.0 (GraphPad Software) was used. The data obtained from the mechanical tests were submitted to the D'Agostino & Pearson normality test. Intragroup comparisons, before and after microwave treatment, were performed by t-paired tests for normal data and Wilcoxon for non-normal data. Intergroup comparisons were performed by one-way ANOVA and Tukey tests for normal data, and Kruskal-Wallis and Dunn for non-normal data. All analyzes considered 95% confidence interval ($\alpha=0.05$).

Results

Microbiological Assay

Twenty-four hours after the microwave experiment with *C.albicans*, the negative group, which did not undergo microwave irradiation, showed substantial microbe growth displayed by the turbidity of the test tube. During the 14 days follow-up, only one test tube of the E1 group (450W for 3 minutes) became turbid after 48 hours of incubation. No further test tube exhibited changes in its visual aspect, including the positive control (samples sterilized with ethylene oxide), assuring that the samples were sterile before the experiment.

In the experiments performed with *S.aureus*, all the negative control (contaminated samples which did not receive microwave irradiation) test tubes exhibited turbidity after 24 hours. Samples from the E1 group also exhibited little signs of turbidity. After 48 hours of incubation, all three samples from this group became visibly turbid, with visual aspect similar to the negative control group. After 14 days, no other group showed signs of turbidity.

Surface roughness

The results for surface roughness displayed on table 3 suggest that samples were standardized and properly randomly distributed among the experimental groups before the experiment ($p= 0.4423$) and remained similar after microwave irradiation ($p= 0.1584$). The results within each group suggested no significant statistical difference within the same group after irradiation ($p=0.6183$, $p= 0.3673$, $p = 0.1523$, $p= 0.2930$, respectively).

Table 3 Mean (\pm SD) values of surface roughness before and after treatment (Ra, μ m).

| | 450W 3min | 450W 5min | 650W 3min | 650W 5min | p |
|---------------|-------------------------|-------------------------|-------------------------|-------------------------|----------|
| BEFORE | 0.173 (\pm 0.038) | 0.182 (\pm 0.036) | 0.175 (\pm 0.022) | 0.203 (\pm 0.051) | 0.4423 |
| AFTER | 0.168 (\pm 0.037) | 0.191 (\pm 0.041) | 0.188 (\pm 0.030) | 0.220 (\pm 0.048) | 0.1584 |
| p | 0.6183 | 0.3673 | 0.1523 | 0.2930 | |

Knoop Microhardness

Table 4 shows the results of the comparisons of all the samples previous to irradiation where no statistical difference among all the samples was observed ($p=0.3402$). However, lower and statistically different hardness values within the same groups (E2, E3 and E4) before and after irradiation were detected ($p=0.038, 0.0048, p=0.0039$, respectively), except for the group with lowest irradiation time and potency (E1).

Table 4 Mean (\pm SD) values of the Knoop microhardness (kg/mm^2) before and after treatment.

| | 450W 3min | 450W 5min | 650W 3min | 650W 5min | p |
|---------------|--------------------------|-------------------------|-------------------------|-------------------------|---------------|
| BEFORE | 16.91 (± 0.62) | 17.14 (± 0.75) | 17.63 (± 1.11) | 17.31 (± 0.57) | 0.3402 |
| AFTER | 16.85* (± 0.71) | 15.66 (± 0.99) | 16.42 (± 0.85) | 16.25 (± 0.75) | 0.0295 |
| p | 0.7756 | 0.0038 | 0.0048 | 0.0039 | |

*versus 450W 5min (one-way ANOVA/ Tukey)

Dimensional stability

Table 5 show that in the intragroup analysis no statistically significant difference was detected for the any of the tested parameters ($p > 0.05$).

Table 5 Mean (\pm SD) values of the internal segments (mm) before and after treatment.

| | 450W 3min | 450W 5min | 650W 3min | 650W 5min | p |
|---------------|--------------------------|------------------------|------------------------|------------------------|-------------------|
| BEFORE | 3.88* (± 0.86) | 4.51 (± 0.98) | 4.93 (± 1.14) | 4.90 (± 0.87) | <0.0001 |
| AFTER | 3.82** (± 0.87) | 4.53 (± 1.08) | 4.97 (± 1.20) | 4.89 (± 0.97) | <0.0001 |
| p | 0.0919 | 0.8471 | 0.4630 | 0.7740 | |

*versus 650W 3 and 5 min (Kruskal-Wallis/ Dunn); **one-way ANOVA/ Tukey.

Discussion

The working hypothesis of this work was partially accepted. Microwave irradiation was capable of sterilizing samples, but not all of the evaluated properties remained unchanged. Temperature, potency,

brand of the resin, polymerization cycle and amount of water seem to play a major role in the outcomes of experimental studies involving microwave sterilization. Higher temperatures could lead to higher diffusion, loss and polymerization of residual monomer molecules^{10,41}. This process could be responsible for increasing internal stress and other changes in mechanical properties⁴¹. Moreover, it is believed that the sterilization of the samples occur by the heating of water molecules inside the microorganism⁴², disorganizing its biological structures.

During microwave irradiation, water started to boil, which has been previously reported^{15,18,32}. It is a crucial factor for the mechanical properties of acrylic resins when considered the fact that temperatures slightly above 100 °C could easily reach the glass transition temperature of the resin, which could lead to dimensional and mechanical distortions⁴³. In many studies^{30,22,11,44,31,5,32,18,33,45}, 200 mL of water was considered a safe amount that would not boil during irradiation. However, especially in the groups exposed to longer irradiation time and higher potency, a pilot study before this work showed that water would eventually reach boiling temperature. Using 250 mL and allowing the microwave oven to cool off between sessions prevented the water from boiling¹⁵.

The most commonly used irradiation/potency parameter in most recent studies has been 3-minute irradiation at 650 W^{32,33,45,35,46,27}. A study from Mima et al.³² showed that the use of shorter irradiation time, such as 2 minutes at 650 W using 200 mL sterile distilled water, was capable of eliminating *C. albicans* from contaminated acrylic specimen. However, the minimal of a 3-minute exposure was necessary to eliminate *S. aureus*, *P. aeruginosa* and *B. subtilis* under the same testing conditions. Another study from Ribeiro et al.³³ suggested that 2-minute irradiation at the same potency would be enough only for reducing colony forming units (CFU) counts; whereas 3-minute irradiation using the same parameters was capable of completely eliminating *Candida* spp., *Staphylococcus* spp. and *Mutans streptococci*. The results of this work showed consistent turbidity in the groups tested with *S.aureus* irradiated with 450 W for 3 minutes, unlike some other studies that considered this parameter capable of eliminating all bacteria^{32,33}. This could

also be attributed to the different amount of water used in these studies, which was 200 mL. The lesser amount could be responsible for better heat distribution and thus resulting in a more effective decontamination of the acrylic samples. However, none of these studies followed the recommendations by the Code of Federal Regulations or the United States Pharmacopoeia, which require 14 days of incubation in order to detect sterility and therefore the lack of longer exposure time could also be responsible for not detecting microbial growth. The work from Sena et al.³⁴ concluded that 3-minute irradiation time at an even lower potency (450 W) was enough to sterilize acrylic contaminated samples with *C. albicans*. However, 200 mL was used for the immersion of the samples, whereas the present research used 250 mL. This could be an explanation for the slightly different results concerning the E1 group (3-minute irradiation at 450 W) where one of the triplicates exhibited turbidity during the experiments with *C. albicans*, thus requiring further investigation.

Surface roughness seems to be a very sensitive property to microwave irradiation.^{30,24,20,23,47} This could be related to the release and dilution of residual monomer, which could eventually cause the sorption of water, leading to a more irregular surface^{41,30}. A surface roughness threshold of $Ra = 0.2 \mu\text{m}$ has been proposed as ideal for avoiding greater bacterial adhesion⁴⁸. The results of this work remained very close to the value of $0.2 \mu\text{m}$ for all parameters tested. Machado et al.³⁰ showed that even 7 cycles of 6 minutes irradiation at 650 W did not alter the surface roughness to values far above $0.2 \mu\text{m}$. Some other studies revealed that 6 minutes of irradiation at a potency varying from 650 to 690 W would cause roughness increase either at the first irradiation or at subsequential repetitions^{20,24,23,47,41}, which suggests that a lower irradiation time would be more secure, since it has been shown in this work that 3 minutes of microwave exposure at 650W was effective for sterilization. The work by Campos et al.²³ also revealed that acrylic resins that undergo microwave polymerization (Onda-Cryl) tend to demonstrate higher discrepancy in surface roughness than resins cured in hot water bath (QC-20) when exposed to microwave disinfection cycles. Nevertheless, despite reports of statistically significant roughness changes, it is still unclear if these results would have any clinical implications²³.

The effects of microwave irradiation on the microhardness of acrylic resin is still very controversial. The results presented in many studies have showed variations in absolute values, often statistically significant ($p < 0.5$)^{30,49,8,16,22,11,28,29,26}. However, some authors consider these variations as clinically insignificant^{8,16,49}, while others suggest that the changes in the surface of the material could lead to easier wear over time due to decrease of hardness^{22,11,28,16,29,26}. The study by Constant et al.²⁸ hinted at higher decrease in hardness values for microwave-polymerized acrylic resins (Onda-Cryl) when compared to acrylic resins cured in hot water bath (QC-20) for samples subjected to a 3-minute irradiation at 650 W protocol, whereas hardness values for QC-20 did not exhibit statistical difference to the non-irradiated group. In this study, only group with the lowest potency and shortest irradiation time (450W/3 minutes) did not show statistically significant difference when compared to the samples before irradiation. All the other groups presented statistically differences when compared to baseline; however, it is still debatable if these changes would be considered clinically relevant.

The dimensional change also seems to follow the pattern observed for the other mechanical and surfaces properties evaluated in this study, which states that brand and polymerization/curing cycle can influence the results^{44,31}. Besides, a study by Polychronakis et al.⁴⁰ showed that microwave disinfection with complete dentures immersed in water exhibited greater dimensional changes when compared to those not immersed, suggesting that disinfection cycles would be more effective if carried out in dry state. However, this could affect the sterilization of the samples, which have been reported to be more effective when samples were immersed in water^{32,18,33}. All tested groups in this study exhibited minor variations after microwave irradiation, which were considered statistically irrelevant ($p > 0.05$). When considered the dimensional change in percentage, all groups analyzed in this work maintained a variation of ranging from 1.54% to 0.02% in the length of the segments. Values ranging up to 1% of dimensional change are considered as clinically acceptable^{50,15}. Studies using longer irradiation time above 5 minutes and or potency values higher than 650 W usually showed higher tendency for dimensional distortions^{40,17,5,15}, whereas shorter irradiation time (below 6 minutes) and potency values ranging from 420 to 650 W stated

that dimensional changes were not relevant.^{51,49,44} . However, it is still debatable whether these changes will in fact be negatively perceived due to variations in size of dentures, which can suffer asymmetric distortions^{17,50}.

The lack of comparison between more thermopolymerizable acrylic resin brands as well as experiments for determining the efficiency of the sterilization protocol for gram-negative microorganisms could be seen as limiting factors of the present study. Therefore, further investigation involving other microorganisms and other material properties, such as flexural strength, are necessary for ensuring the efficacy of these parameters in order to establish a reliable sterilization protocol for acrylic resin specimen.

Conclusion

Within the limitations of this in vitro study, it was concluded that microwave irradiation at 650W for 3min and 450W/650W for 5 min was able to sterilize the acrylic resin specimen for *Candida albicans* and *Staphylococcus aureus* without altering surface roughness and dimensional stability. However, it decreased microhardness values. The parameter 450W 3min did not cause changes in surface roughness, dimensional dimension and microhardness, however, it was not able to sterilize samples contaminated the tested microorganisms.

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