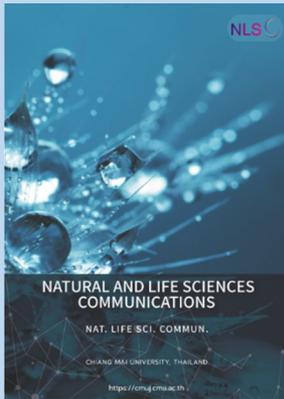


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# Color Change of The Iris During Ocular Prosthesis Fabrication and After Accelerated Aging

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## ABSTRACT

This study aims to investigate the color change of the brown iris of the three-layer acrylic resin ocular prosthesis during the fabrication process and after accelerated aging. Four groups of ocular specimens were created using combinations of heat-cured (H) and chemical-cured (C) acrylic resin: HHH, HCH, CCH, and CCC (n=10 each). The iris color was measured with a spectrophotometer after iris painting with inverted painting technique, fabrication, and UVB-accelerated aging for 504 and 1,008 hours. The CIE Lab\* data were used to calculate the color difference (Delta E;  $\Delta E$ ) between each period. The data were analyzed using descriptive statistics, one-way ANOVA and Tukey's HSD post hoc test at a 95% confidence level. All groups showed no significant differences in color change after fabrication. However, significant differences were observed following UVB-accelerated aging for 504 and 1,008 hours. The HHH group had the lowest  $\Delta E$  ( $1.07 \pm 0.37$  and  $1.37 \pm 0.18$ ), significantly different from the CCC group with the highest  $\Delta E$  ( $1.85 \pm 0.59$  and  $2.17 \pm 0.28$ ). The HCH ( $1.27 \pm 0.67$  and  $1.80 \pm 0.42$ ) and CCH ( $1.24 \pm 0.31$  and  $1.75 \pm 0.67$ ) groups showed no significant differences. Additionally, both groups were not significantly different from the HHH and CCC groups. The fabrication process did not affect the iris color, despite material differences. The prostheses made from chemical-cured acrylic resin showed significant color changes over time, while heat-cured acrylic resin in all layers offered superior long-term stability. However, using heat-cured acrylic in the cornea layer and chemical-cured acrylic in the rest is acceptable if fabrication time is a concern.

**Keywords:** Accelerated aging, Artificial eye, Artificial iris, Color change, Color stability, Ocular prosthesis

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## INTRODUCTION

The accuracy and stability of the color are the desired properties of an ocular prosthesis. The color change during the fabrication process could result in the prosthesis being rejected on the delivery visit. The polymerization of acrylic resin is probably the cause of these changes (Reis et al., 2008; Canadas et al., 2010; Goiato et al., 2010a; 2011; Mundim et al., 2012; Moreno et al., 2015).

The service life of an ocular prosthesis can range from one to five years (Goiato et al., 2009; Haddad et al., 2011). The noticeable iris color change is one of the reasons for replacing the new prosthesis (Goiato et al., 2009; Mundim et al., 2012). The color change is attributed to the gradual deterioration of polymers and environmental exposure (ERPF, 1953; Fernandes et al., 2009; Goiato et al., 2010a, 2010b; 2011; Mundim et al., 2012).

The conventional method of fabricating an acrylic resin ocular prosthesis in three layers is commonly used. In the process, the iris layer is completed first, then the base layer, and finally the cornea layer (Patil et al., 2007; Raizada and Rani, 2007). Various techniques can be utilized to color the iris, including painting directly onto an iris cap (inverted painting), pressing acrylic resin onto a painted paper disk, and covering a painted paper disk with an iris cap (Bannwart et al., 2013; Goiato et al., 2014). Among these techniques, the inverted painting technique exhibits the least color change (Bannwart et al., 2013).

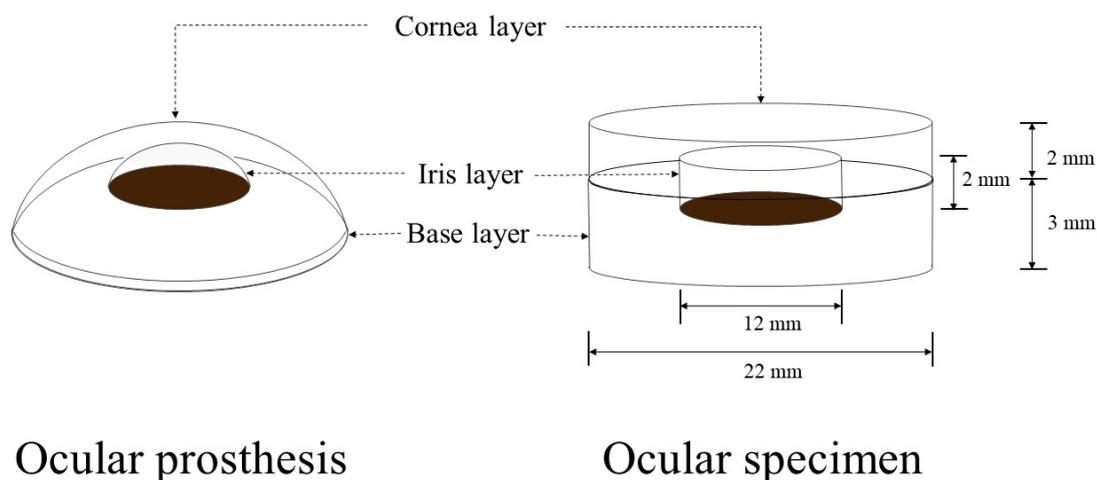
Heat-cured acrylic resin is the most common material used to fabricate ocular prostheses due to its excellent physical properties, including clarity, color stability, and minimal porosity. A high-temperature water bath polymerization procedure can lead to color changes (Reis et al., 2008; Fernandes et al., 2009; Canadas et al., 2010; Moreno et al., 2015). Therefore, chemical-cured acrylic resin may be used as an alternative. However, no existing studies have investigated the changes in iris color in ocular prostheses made of chemical-cured acrylic resin. This material is available at a dental lab and is easy to handle and process. In addition, it does not require high temperatures for polymerization, which is supposed to affect the color of the iris (Zafar, 2020). Due to the advantages of chemical-cured acrylic resin, switching from heat-cured to chemical-cured acrylic resin may potentially decrease the processing time and increase color stability for ocular prostheses.

This study aims to investigate the color change of the brown iris of the three-layer acrylic resin ocular prosthesis made with various combinations of heat-cured and chemical-cured acrylic resin during the fabrication process and after accelerated aging. The null hypotheses state that there are no significant differences in the color change of the iris during the fabrication process or after accelerated aging, regardless of the acrylic resin combinations.

## MATERIALS AND METHODS

### Specimen design and material used

The G\* Power program (Version 3.1.9.6) was used to calculate the specimen size of this study. Four groups of the three-layer ocular specimens (n=10) were made using various combinations of heat-cured acrylic resin (H) and chemical-cured acrylic resin (C). The specimen design was based on the composition of an ocular prosthesis as shown in Figure 1. Each group was identified by the sequence of acrylic resin layers from the base to the cornea: HHH, CCH, HCH, and CCC as presented in Table 1. The materials used are listed in Table 2.



**Figure 1.** An ocular specimen was designed based on an ocular prosthesis. Flat surface was chosen to achieve consistent color measurement.

**Table 1.** Group of specimens and the order of acrylic resin.

Group code	HHH	HCH	CCH	CCC
Cornea layer	Heat-cured	Heat-cured	Heat-cured	Chemical-cured
Iris layer	Heat-cured	Chemical-cured	Chemical-cured	Chemical-cured
Base layer	Heat-cured	Heat-cured	Chemical-cured	Chemical-cured

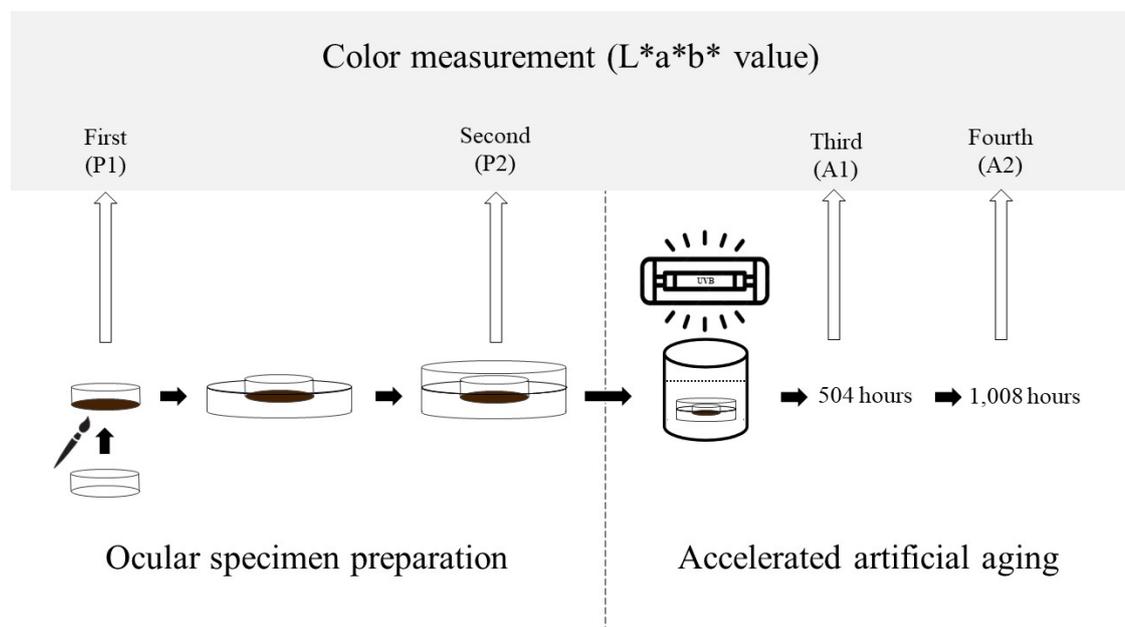
**Table 2.** The materials used to fabricate ocular specimens.

Material	Trade name	Manufacturer	Composition
Heat-cured PMMA	Meliodont® Heat Cure	Kulzer GmbH, Hanau, Germany	Polymethylmethacrylate, methylmethacrylate, dimethacrylate, 1,4-butandiol dimethacrylate
Chemical-cured PMMA	Meliodont® Rapid repair	Kulzer GmbH, Hanau, Germany	Polymethylmethacrylate, methyl methacrylate, tetramethylene dimethacrylate, 2-(2H-Benzotriazol-2-yl)-4-methylphenol
Oil paint (brown color, burnt umber)	Winton oil color	Winsor&Newton, United Kingdom	Linseed Oil, calcined natural earth, iron oxides with manganese silicates

### Experimental procedures and data gatherings

The summary of specimen preparation, aging process, and color measurements is presented in Figure 2. The specimen fabrication followed the conventional method, with the inverted painting on the iris layer. It started with the iris layer, then the base layer, and finally the cornea layer. The first color measurement (pre-processing: P1) was taken through the outer surface of the iris layer which had already been painted. The iris layer was then processed to form an ocular specimen by adding the base and cornea layers, respectively. The second measurement (post-processing: P2) was taken through the outer surface of the cornea layer. The color differences were assessed to determine color changes induced by fabrication process.

In the accelerated aging process, the specimens were simulated to natural degradation by ultraviolet B (UVB) rays. The third and fourth color measurements were taken after accelerated aging for 504 hours (A1) and 1,008 hours (A2) respectively. The color differences were assessed to determine color changes induced by the aging process.



**Figure 2. Methodology workflow.**

## Specimen preparation

### Acrylic resin processing

Specimens were created using three layers of colorless acrylic resin, starting with the iris, followed by the base, and finally the cornea, as presented in Figure 2. Each layer was prepared as follows:

Lost-wax casting technique was used to produce each layer of specimens. Casting wax, formed by brass mold, was used to create dental stone mold for specimens. The colorless acrylic resin was applied into the dental stone mold in a metal flask and compressed with a hydraulic press machine at the pressure of 2 bars. The acrylic used depended on each group as specified in the table 1.

Then, Acrylic resin in a metal flask was processed in accordance with the manufacturer's instructions. The polymerization process of heat-cured acrylic resin was conducted in water bath at 74°C for 8 hours, followed by an additional 1 hour at 100°C. Meanwhile, the polymerization process of the chemical-cured acrylic resin was carried out in 60°C water for 15 minutes. The specimens were removed from the flask after cooling down to room temperature.

The specimens were hand-shaped and polished using abrasive papers under running water, with grid numbers 320, 600, 1000, 2000, 3000, 5000, and 7000, respectively. The iris layer was polished on only one side, while the other side was prepared for painting. Once the iris layer was completed, color was applied to the iris, and the base and cornea layers were constructed using the same method as described until the specimen was finished.

### Iris painting

Brown oil paint was applied directly to the non-polished side of the iris layer by the inverted painting technique. A size 12 flat-tip brush was used for the application. Three coats of paint were applied to each specimen, with each coat allowed to dry before applying the next coat. Once the last coat was applied, the specimens were

naturally dried for 72 hours to ensure complete drying. The painting process was performed by the same individual, under identical conditions, and in the same direction for consistency. The color on each specimen was measured using vernier calipers to ensure uniformity.

### Aging process

The UVB accelerated aging system for non-metallic objects (ASTM G-53, American Society for Testing Materials Norma 53) was used to simulate natural deterioration. During accelerated aging, the ocular specimens were immersed in distilled water and exposed to UVB chamber. The cycle of exposure was 8 hours with UVB and 4 hours without UVB. This process was repeated until reaching durations of 504 and 1,008 hours, equivalent to 6 months and 12 months of use, respectively.

### Color analysis

The iris color of each specimen was measured using a reflective spectrophotometer (Rayplicker, Borea, France). The measurements were taken at the center of the iris using a spectrophotometer with a circular measuring probe, 25 mm in diameter. The specimen was placed in the center of a cardboard sheet with a grid for distance reference. A plastic ring, compatible in size with the spectrophotometer, was used to maintain a consistent vertical and horizontal distance for accurate positioning. Each specimen was measured three times, and the average value was then used for color analysis. The color value was converted to CIE  $L^*a^*b^*$ , and then, the color difference ( $\Delta E$ ;  $\Delta E$ ) was calculated by the formula.

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Delta E was used to assess the color changes of the specimens over time: pre and post processing (P1-P2), after accelerated aging for 504 hours (P2-A1), and after accelerated aging for 1,008 hours (P2-A2).

### Statistical analysis

The  $L^*$ ,  $a^*$ , and  $b^*$  values and Delta E served as key parameters in the statistical calculations. The normality of all data was confirmed using the Shapiro-Wilk normality test. Descriptive statistics were conducted to compare the difference of the  $L^*$ ,  $a^*$ , and  $b^*$  values. One-way ANOVA was used to evaluate the significant differences in color change of the specimens in the different stages. Tukey's HSD post hoc test was used to compare differences in the obtained values. The results were analyzed at a 95 percent confidence level or an alpha level of 0.05 ( $P \leq 0.05$ ).

## RESULTS

### Interpretation of $L^*$ , $a^*$ , and $b^*$ values

The  $L^*$ ,  $a^*$  and  $b^*$  values of each group were interpreted.  $L^*$  refers to brightness,  $a^*$  describes the color position between green and red, and  $b^*$  indicates the color position between blue and yellow. A summary of the data is provided in Table 3.

All groups demonstrated decreased  $L^*$  values, or became darker, following specimen processing. In contrast, an increase in brightness was observed after 504 and 1,008 hours of aging. For  $a^*$  values, all groups showed a continuing shift towards green tone from processing to aging. As indicated by the  $b^*$  values, the specimen containing chemical-cured acrylic resin exhibited a consistent increase in yellow shade following the aging process. However, due to the substantial change in  $L^*$ ,  $a^*$ ,

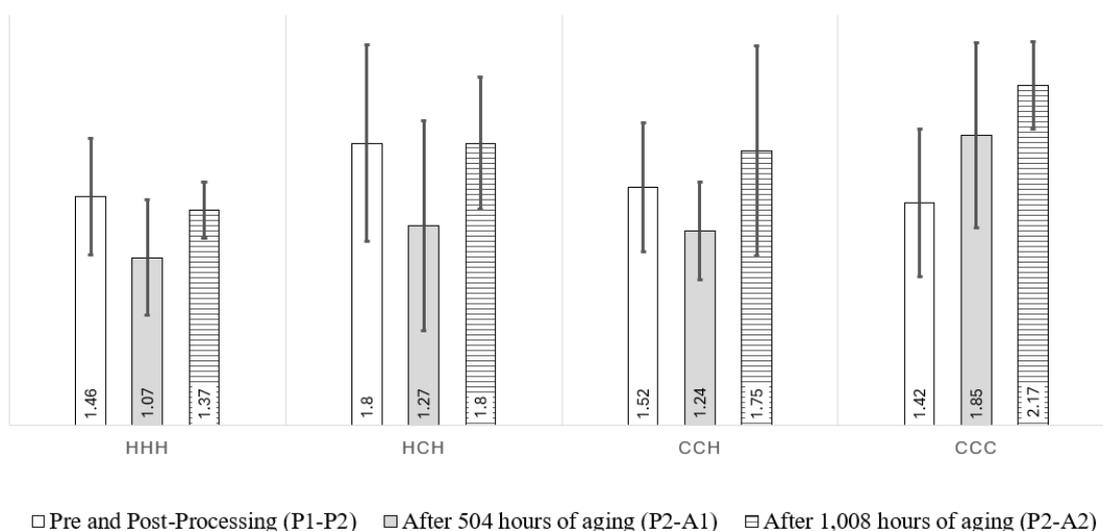
and b\* values for each group, the specimen was found to have slightly changed in color.

**Table 3.** The mean and standard deviation of L\*, a\*, and b\* values for each group.

Value	Stage of record	Mean of L* a* b* values measured at various times (SD) n = 10 / group			
		HHH	HCH	CCH	CCC
L*	Pre-processing	8.78 (0.07)	9.31 (0.27)	8.61 (0.09)	9.94 (0.11)
	Post-processing	7.76 (0.090)	7.97 (0.19)	7.51 (0.17)	8.76 (0.16)
	504 hours of aging	8.09 (0.12)	8.55 (0.23)	7.97 (0.21)	7.97 (0.27)
	1,008 hours of aging	8.85 (0.10)	9.15 (0.18)	8.49 (0.20)	9.05 (0.19)
A*	Pre-processing	2.10 (0.122)	1.98 (0.08)	2.16 (0.08)	2.04 (0.13)
	Post-processing	2.08 (0.151)	1.59 (0.22)	1.58 (0.13)	1.93 (0.11)
	504 hours of aging	1.30 (0.119)	1.43 (0.18)	1.64 (0.09)	1.46 (0.19)
	1,008 hours of aging	1.48 (0.12)	1.06 (0.12)	1.43 (0.16)	0.83 (0.09)
B*	Pre-processing	2.46 (0.19)	2.42 (0.15)	1.96 (0.08)	2.25 (0.11)
	Post-processing	2.10 (0.13)	2.47 (0.19)	2.17 (0.12)	2.07 (0.11)
	504 hours of aging	2.43 (0.17)	2.39 (0.18)	2.47 (0.16)	3.00 (0.21)
	1,008 hours of aging	2.31 (0.11)	2.47 (0.15)	3.12 (0.26)	3.59 (0.10)

**Analysis of color differences with Delta E**

The mean and standard deviation of Delta E for each group are presented in Figure 3 and Table 5. The Delta E of the CCC group tended to increase, while the values of the other groups decreased after 504 hours of aging and then increased after 1,008 hours of aging.



**Figure 3.** Bar graphs illustrate the mean Delta E and standard deviation of each group.

The data was analyzed using one-way ANOVA and Tukey HSD multiple comparison analysis. A P-value of less than or equal to 0.05 was significant. One-way ANOVA was conducted to assess the differences in Delta E between the group of specimens at the defined stages (Table 4).

**Table 4.** One-way ANOVA for comparing Delta E across four groups of specimens at the defined stage.

		<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>Sig.</i>
P1-P2	Between Groups	0.871	3	0.290	1.241	0.309
	Within Groups	8.416	36	0.234		
	Total	9.286	39			
P2-A1	Between Groups	3.444	3	1.148	4.449	0.009
	Within Groups	9.288	36	0.258		
	Total	12.732	39			
P2-A2	Between Groups	3.196	3	1.065	5.744	0.003
	Within Groups	6.676	36	0.185		
	Total	9.872	39			

The findings indicated that there were no statistically significant differences in Delta E between the groups at the stage of pre- and post-processing (P1-P2). However, notable differences were noted when comparing each group at the following stages. After 504 hours of aging (P2-A1), the CCC group had a significantly higher Delta E compared to the HHH group. After 1,008 hours of aging (P2-A2), the CCC group still demonstrated a significantly higher Delta E compared to the HHH group. There were no significant differences between the HCH and CCH groups. Additionally, both groups were not significantly different from the HHH and CCC groups. (Table 5)

**Table 5.** The mean and standard deviation of Delta E for each group, and the statistical differences between groups.

	Stage of record	Mean ΔE of the specific stage of record (SD)			
		Fabrication technique (n = 10 /group)			
		HHH	HCH	CCH	CCC
<b>Fabrication process</b>	Pre-processing	1.46 <sup>a</sup>	1.80 <sup>a</sup>	1.52 <sup>a</sup>	1.42 <sup>a</sup>
	-	(0.37)	(0.63)	(0.414)	(0.47)
<b>Aging process</b>	Post-processing (P1-P2)				
	Post-processing	1.07 <sup>a</sup>	1.27 <sup>ab</sup>	1.24 <sup>ab</sup>	1.85 <sup>b</sup>
	-	(0.37)	(0.67)	(0.31)	(0.59)
	504 hours of aging (P2-A1)				
<b>Aging process</b>	Post-processing	1.37 <sup>a</sup>	1.80 <sup>ab</sup>	1.75 <sup>ab</sup>	2.17 <sup>b</sup>
	-	(0.18)	(0.42)	(0.67)	(0.28)
	1,008 hours of aging (P2-A2)				

Note: Different lowercase letters denote significant difference in row ( $P \leq 0.05$ ).

## DISCUSSION

This study demonstrated that the conventional method of fabricating a three-layer acrylic resin ocular prosthesis, using a combination of heat-cured and chemical-cured acrylic resin and painting the iris layer with the inverted painting technique, accepted the first hypothesis but rejected the second hypothesis. The color of the brown iris showed no significant change during fabrication. However, significant color changes were observed following accelerated aging.

### **Color changes affected by polymerization during fabrication process**

This study showed that there were no statistically significant differences in Delta E values among the various combinations of three-layer acrylic resin groups during the fabrication process. Previous studies revealed the differences in the physical properties of heat-cured and chemical-cured acrylic resin (Bohra et al., 2015; Zafar, 2020). However, regarding results, the various combinations of acrylic resin did not affect the color change of the brown iris of the ocular specimen in this study. The absence of significant differences in color changes between groups can be attributed to the painting technique and the color used for coloring the iris.

According to previous studies, the inverted painting technique is known for its minimal impact on the color change of artificial iris (Moreno et al., 2015). The method of coloring the iris in this study involved applying three coats of paint underneath the iris layer, and the following base layer was processed directly afterward. The iris paint only contacted the base layer. So, the color change occurred on the final coat of paint, which directly interacted with the acrylic monomer during the fabrication of the base layer. However, the change in the iris color was not noticed due to the concealment by the first and second coats of paint. Moreover, this method eliminates the need for adhesives, a factor known to cause color destabilization and subsequent color changes (Bannwart et al., 2013; Moreno et al., 2015). Hence, the color of the iris remains consistent or may undergo only minimal change upon completion of the fabrication process.

High temperature during the polymerization process can lead to color change in the iris (Reis et al., 2008; Fernandes et al., 2009; Canadas et al., 2010; Moreno et al., 2015). Intense heat or repeated exposure to heat resulted in the deterioration of both the paint and acrylic resin (Sweeney et al., 1972; Canadas et al., 2010; Goiato et al., 2010). However, the lack of significant difference in color changes between groups might be the result of the brown oil paint used. Oil paint exhibits improved color stability due to the presence of the opacifier (zinc oxide) and mineral components (linseed oil), which enhance its resistance to temperature change (Bannwart et al., 2013). Zinc oxide exhibits excellent thermal stability, allowing it to withstand high temperatures (Yawong et al., 2005). Linseed oil is a drying oil, meaning it undergoes polymerization when exposed to air (oxidation), forming a cross-linked film. This polymerized film can withstand heat (Sarjono et al., 2020).

Moreover, in this study, the acrylic resin process was conducted according to the manufacturer's instructions with the appropriate application of heat so the process might not significantly impact the color of the iris during the fabrication process.

### **Color changes affected by accelerated aging**

Both the acrylic resin and the paint employed in fabricating ocular prostheses are categorized as polymers (Moreno et al., 2015). While using the ocular prosthesis, individuals may be exposed to ultraviolet rays from natural and artificial light sources (Reis et al., 2008; Mundim et al., 2012). Ultraviolet rays significantly impact the properties and durability of polymers. Polymers contain components that can absorb ultraviolet rays, which cause the breaking of the covalent bonds of the polymer chains (Fernandes et al., 2009; Anusavice et al., 2013; Santos et al., 2016). This results in

the photodegradation of the polymer and the loss of its desirable properties. Nonetheless, this process occurs gradually over time (Bannwart et al., 2013).

The iris color of each specimen exhibited a progressive change as time passed. After 504 and 1,008 hours of aging, the HHH group had a significant difference in Delta E compared to the CCC group. The HHH group showed the least color changes. Subsequently, the HCH, CCH, and CCC groups exhibited increasingly more pronounced color changes, with the CCC group demonstrating the most significant change. The discoloration of the acrylic resin in the iris and cornea layers affected the color change in the iris. These processes can be described by the result of  $L^*$ ,  $a^*$ , and  $b^*$  values. After the aging process, the chemical-cured acrylic resin showed an increase in yellowish tint and turbidity, which corresponds to an increase in  $L^*$  and  $b^*$  values. In contrast, the color of heat-cured acrylic resin remained clear, and the  $L^*a^*b^*$  value did not change significantly either. Therefore, the groups that contained chemical-cured acrylic resin exhibited a greater degree of color change.

Chemical-cured acrylic resin contains tertiary amines as activators and has a higher amount of residual monomer compared to heat-cured acrylic resin. The gradual release of residual monomers and the oxidized tertiary amines can cause discoloration of acrylic resin (Mundim et al., 2012). The residual monomer of chemical-cured acrylic resin affects the chemical bonds of the paint, leading to exchanges or ruptures of the bonds. Instability in these bonds could promote a higher degree of color change (Goiato et al., 2011; Mundim et al., 2012; Moreno et al., 2015). This process is initiated during the polymerization of the prostheses and is further intensified by exposure to ultraviolet rays during their usage (Goiato et al., 2011; Mundim et al., 2012). Tertiary amines are prone to oxidation, and the resulting oxidized amines can lead to discoloration of acrylic resin (Dogan et al., 1995; Zafar, 2020). HCH and CCH showed no significant differences, despite differences in the materials used for the base layer. This was due to both groups having an iris layer made from chemical cured acrylic resin and a cornea layer made from heat cured acrylic resin. Consequently, color changes occurred in the same direction following the accelerated aging process. The color was measured from the cornea layer to the iris layer, reflecting only the color of the iris. Thus, variations in the base layer's alterations might have occurred, but they had no effect on the color measurement.

### **Clinical implications**

According to the National Bureau of Standards (Ruyter et al., 1987; Liberman et al., 1995; Andreotti et al., 2014), Delta E below 1 indicates a minimal color change, while Delta E between 1 and 3 is deemed clinically acceptable, and Delta E exceeding 3.3 is considered clinically noticeable. In this study, the Delta E of each group was within the range of 1 to 3, indicating that the color changes observed in all specimen groups were considered clinically acceptable.

The HHH group showed minimal color change after processing, and good color stability over time. In contrast, the CCC group exhibited the most color change after the aging process, making it unsuitable for long-term use. Regarding specimen fabrication, the CCH group differed from the CCC group only in the cornea layer, in which heat-cured acrylic resin was used instead of chemical-cured acrylic resin. Remarkably, the outcomes of the CCH group were favorable, as this group had minimal color change after processing and acceptable color stability after the aging process. It could also be suggested that the cornea layer considerably affects the long-term color stability, and using heat-cured acrylic resin for the cornea layer of ocular prostheses is recommended because of its superior transparency and fewer pores (Canadas et al., 2010; Fernandes et al., 2010). Therefore, if the duration of the procedure is a concern, an ocular prosthesis with heat-cured acrylic resin in the cornea layer and chemical-cured acrylic in the remaining layers can be applied.

## LIMITATIONS

The limitations of this study were that the specimen fabrication procedures may not be entirely consistent with protocols used by other researchers, potentially leading to color changes with different fabrication techniques. Additionally, the flat surface of the specimen may cause different color changes compared to the curved surface of the actual prostheses. The accelerated artificial aging process was tested for only 1,008 hours (equivalent to 12 months), a longer experimental duration may result in differences in the observed color changes.

## CONCLUSION

The fabrication process of an acrylic-resin ocular prosthesis did not affect the color changes of the brown iris created by the inverted painting technique, despite material variances. However, the color of ocular prostheses changes over time, particularly for those made from chemical-cured acrylic resin. The three-layer ocular prosthesis made from heat-cured acrylic resin was suggested for long-term use due to its superior color stability. Nevertheless, using heat-cured acrylic in the cornea layer and chemical-cured acrylic in the rest is acceptable if fabrication time is a concern.

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## CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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