



Color stability of dental restorative materials submitted to cold temperatures for forensic purposes



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ABSTRACT

In the *post-mortem* examination of the dental arches of accident victims in cold locations, dental restorative materials can be found. Cold temperatures can be capable of causing color changes of aesthetic materials, such as composite resin (CR) and glass ionomer cement (GIC). The aim of this study was to evaluate the effect of the cold action on the color stability of CR and GIC restorations, in order to discriminate them and enable the adequate comparison between *antemortem* and *post-mortem* data. Sixty bovine teeth (30 CR and 30 GIC) were prepared (6 × 6 × 2 mm) and separated into groups (n = 10). The color readouts were taken by a portable spectrophotometer, before and after of cold action (2.5 °C, –20 °C, –80 °C) inside of freezers. There were color alterations in the coordinates (ΔE , ΔL^* , Δa^* e Δb^*) for both materials. The authors concluded that cold was capable of producing changes in color in the two esthetic materials, with similar intensities between the two, at all the temperatures studied, when analyzed at 7 days. After being submitted to cold for 30 days, the changes were more significant for CR, allowing it to be differentiated from GIC after 30 days, at all the temperatures tested. Therefore, the test proposed in the study was shown to be practical, feasible and capable of helping Forensic Odontology with the identification of victims.

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Innumerable accidents occur in snowy locations as a result of collisions, aircraft crashes, severe blizzards, cold waves, earthquakes, avalanches, or accidents in cold chambers and other situations in which the victim's death results from low temperatures or harmful factors in environments where exposure to cold continues to act on the body even after the victim's death.¹ In Iceland, researches about natural disasters have demonstrated that 193 persons had suffered fatal accidents resulting from avalanches.² The Galtuer avalanche in February 1999, which killed 31 people, was the worst in Austria since 1953.³ A database analysis of accidents in the Swiss Alps pointed out that avalanches with fatal victims are more numerous than the same type of incidents with survivors.⁴

In these cases, discovery and rescue of victims may require

prolonged periods of time due to the climatic and geographic conditions of the location itself.¹ The bodies may not be found whole, either because of the action of time, mechanical shocks or for other reasons. Canadian researchers showed that over the course of two decades, 204 persons died in accidents due to avalanches. The predominant causes of death were a combination of asphyxia and severe traumas. In 87 victims it was possible to perform an external exam of the cadaver, in the majority, however, this was not possible because of their multiple traumatic injuries.⁵

Every time it is necessary to identify bodies in an advanced state of decomposition, skeletal, mutilated, dismembered, fragmented, among other conditions, in which dactyloscopy (fingerprinting) would possibly be discarded by the impossibility of obtaining records on the body,⁶ Forensic Odontology becomes the most feasible, practical and fast method of choice when seeking a positive identity, or exclusion of a suspected victim.⁷

Some softwares like CAV-ID (Computer Aided Victim Identification), WinID, IDIS (Intelligent Dental Identification System), ADIS (Automated Dental Identification System), Plass Data system and Toothpics can accelerate the human identification process,⁸ mainly

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in remote or hard-to-reach regions, sending the *post-mortem's* data immediately after accidents happen.

However, the teeth are the most resistant structures of the human body, even more so than bone tissue, after exposure to extreme temperatures⁹ and frequently, they are the only findings capable of being analyzed, because both the teeth and restorative materials used in performing dental treatments have high resistance to the action of injurious agents.¹⁰ Thus, cold temperatures are not capable of destroying them, but may cause changes in the dental restorative materials when these are present in the victim's oral cavity.

But there is no database of *ante-mortem* records of dental color, similar fingerprints. So, the software may, often, be not well indicated for this kind of analysis and comparisons. Color change in esthetic materials may contribute to investigations for confirming submission to cold temperatures, offer information about the approximate temperature to which they were submitted, and about the period in which they were exposed to these conditions.

This is why it is important to study the possible color changes that have occurred in esthetic materials widely used in Dentistry, such as composite resin (CR) and glass ionomer cement (GIC),¹¹ with the purpose of distinguishing between them, thus seeking support for adequate comparison of *post-mortem* and *antemortem* data, and consequently make a greater contribution to elucidating the identification of a victim.

Thus, the aim of this study was to evaluate the effect of cold temperatures on the color stability of these restorative materials, in an endeavor to simulate their behavior when present in the teeth of victims' who died by freezing or other reasons in extremely cold environments.

1. Materials and methods

Sixty sound bovine incisive teeth were prepared (6 × 6 × 2mm) in the central region of the buccal surface. After this, the teeth were randomly separated into two groups, according to the restorative material used (Table 1).

After restoring the teeth, initial color readouts were taken using a portable spectrophotometer (VITA Easyshade[®], Bad Säckingen, Germany), that consisted of a digital tip 6 mm in diameter, with 19 optical fibers and spectrophotometric sensors that emit bands of light. The color readouts were taken by measuring the reflected light from the restoration, according to the CIE L*a*b*¹² scale.

The CIE L*a*b* scale (Commission Internationale de L'Éclairage) consists of three Cartesian coordinate axes in which L* (gray scale) indicates color luminosity (ranging from 0 – black to 100 – white); a* indicates the amount of red (positive values) and green (negative values) and b* indicates the amount of yellow (positive values) and blue (negative values) of color.¹²

For the color readout, the restored teeth were placed on a standard white background (White Standard Sphere for 45°, 0° Reflectance and Color Gardner Laboratory Inc. Bethesda,

Geretsried, Germany). The tip of the equipment was kept perpendicular and in contact with the surface of the restoration. Three measurements were taken in each tooth, and the average obtained was considered the initial color readout.

The teeth restored with each material were randomly separated into groups (n = 10) according to the temperature to which they were submitted: 2.5 °C (frost free refrigerator, RFG 700 GE[®], Campinas, SP, Brazil), –20 °C (vertical freezer, CVU18 Consul[®], Joinville, SC, Brazil) or –80 °C (Ultra Freezer, AL 374 - 80 V, American Lab[®], Charqueada, SP, Brazil).

The internal temperatures to which the samples were submitted were measured using an infrared thermometer (MT-360, Minipa[®], Sao Paulo, SP, Brazil), portable digital, that performs non-contact temperature measurements with the assistance of a laser sight, to identify the location of measurement. After 7 and 30 days of exposure to cold, the samples were removed from the freezer immediately after new color readouts were taken according to the methodology described. The color changes (ΔE) of the materials were calculated by the formula¹³:

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

Where $\Delta L^* = L_f^* - L_i^*$; $\Delta a^* = a_f^* - a_i^*$; and $\Delta b^* = b_f^* - b_i^*$, being L_i^* , a_i^* and b_i^* referred to as the initial readouts; and L_f^* , a_f^* and b_f^* as final readouts for color coordinates. The color values (ΔE) and the changes in coordinates (ΔL*, Δa* and Δb*) were analyzed according to Two-way ANOVA, repeated measures, Bonferroni, p < 0.05, to compare all the materials and the temperatures tested since the materials could present similar alterations at different temperatures. The variation factors considered for comparison were temperature and time.

2. Results

The comparisons of ΔE readout mean values may be visualized in Fig. 1. There was no statistically significant difference (p > 0.05) in color change when the materials were submitted to low temperatures for 7 days.

After 30 days, the authors observed that the temperature to which the material was submitted was not significant for its color change because there was no difference (p > 0.05), irrespective of the temperature to which the two materials were subjected. However, there was a greater color change in CR at the three temperatures tested, a significant result (p < 0.05) in comparison with GIC.

Comparative analysis between 7 days and 30 days for each material showed there was greater color change (p < 0.05) in CR after 30 days, at all temperatures tested. For GIC, there was no statistically significant difference (p > 0.05) at any of the temperatures analyzed (Fig. 1).

The comparisons of ΔL* readout mean values may be visualized in Fig. 2. After 7 days, there was no statistically significant change

Table 1
List of materials used, commercial brands, manufacturers, colors and restorative methods.

| Category | Commercial Name | Manufacturer | Color Restorative Method (clinical steps) |
|----------------------|------------------------------|---|--|
| Composite Resin | Filtek ^{MR} Z250 XT | 3M ESPE [®] , Sumare, SP, Brazil | 1. Acid etching (37% phosphoric acid, Alpha Etch DFL [®] , Rio de Janeiro, RJ, Brazil) for 15 s, washing, and drying; 2. Bonding system application (Adper Single Bond 2, 3M ESPE [®] , Sumare, SP, Brazil) and photo-activation (Ultralux EL Dabi Atlante [®] , Ribeirao Preto, SP, Brazil) for 10 s; 3. Material was inserted in increments and photo-activated for 20 s; 4. Finishing and polishing (File Discs Sof LexTM Pop On, 3M ESPE [®]). |
| Glass Ionomer Cement | Ketac TM Fil Plus | 3M ESPE [®] , Sumare, SP, Brazil | 1. Powder/liquid (1:1) agglutination up to 1 min; 2. The material was applied in increments while it still had a humid shine until the cavity was filled. |

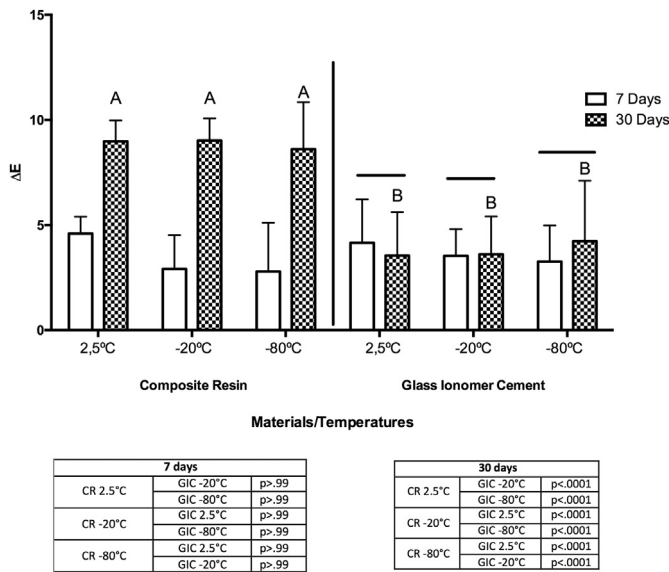


Fig. 1. Graphic representation of the comparison of ΔE changes of materials when submitted to different temperatures (One-way ANOVA, Bonferroni test, $p < 0.05$). Horizontal lines over bars indicate no statistically significant difference ($p > 0.05$) between 7 and 30 days. For all the comparison between 7 days, $p > 0.05$. Different letters in the comparison between materials after 30 days, ($p < 0.05$). CR = Composite Resin. GIC = Glass Ionomer Cement. Tables below the graph indicate the differences between different materials submitted to different temperatures.

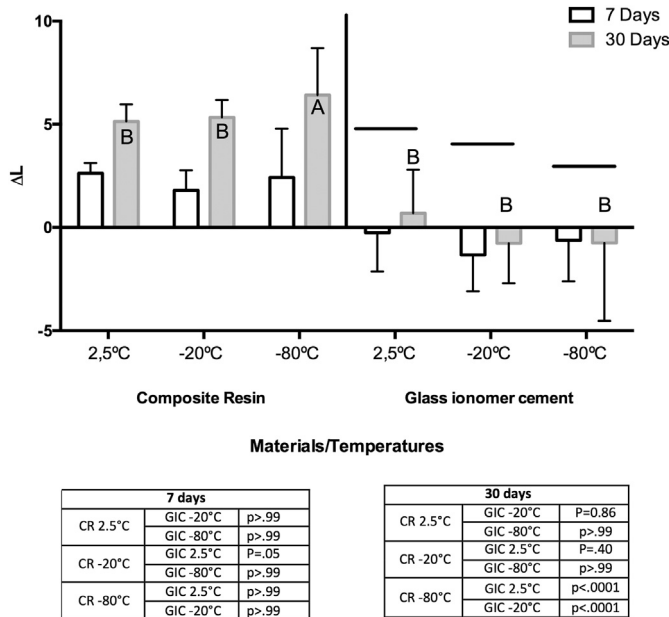


Fig. 2. Graphic representation of the comparison of ΔL changes of materials when submitted to different temperatures (One-way ANOVA, Bonferroni test, $p < 0.05$). Horizontal lines over bars indicate no statistically significant difference ($p > 0.05$) between 7 and 30 days. Different letters indicate significant difference ($p < 0.05$) between materials at the same temperature, after 30 days. CR = Composite Resin. GIC = Glass Ionomer Cement. Table below the graphic indicates the differences between different materials submitted to different temperatures.

($p > 0.05$) at any of the temperatures evaluated for any material.

At 30 days of submission to cold, CR showed changed in the coordinate L^* at significant levels ($p < 0.05$) when submitted to a -80°C , in comparison with the other temperatures tested, which presented no difference ($p > 0.05$) among them. For GIC, there was

no statistically significant change ($p > 0.05$) at any of the temperatures analyzed.

Comparative analysis between 7 days and 30 days of exposure to cold, for each material, indicated that there was a greater change in the coordinate L^* for CR after 30 days, at all the temperatures tested, differing statistically ($p < 0.05$) from the values shown when it was submitted for 7 days. For GIC, there was no statistically significant difference ($p > 0.05$) when the time during which it was submitted to low temperatures was compared (Fig. 2).

The comparisons of Δa^* readout mean values may be visualized in Fig. 3. After 7 days, a greater change occurred for CR after submission to 2.5°C , a result differing ($p < 0.05$) from that which occurred after submission to -80°C , but similar ($p > 0.05$) to that observed after submission to -20°C . For GIC, the greatest change also occurred after submission to 2.5°C , a statistically significant result ($p < 0.05$) in comparison with the other two temperatures tested, which showed no difference ($p > 0.05$) between them.

Change in coordinate a^* for CR submitted to -80°C was similar ($p > 0.05$) to that found for GIC at -20°C and -80°C . For all the other comparisons, there was a significant difference ($p < 0.05$) in the changes that occurred, with the alteration in CR being positive and that in GIC, negative.

After 30 days, there was no significant difference ($p > 0.05$) for GIC with regard to the temperatures to which it was submitted. CR showed greater change after being submitted to -20°C ; a result similar ($p > 0.05$) to that of 2.5°C , but differing from -80°C , which showed a lower change in this coordinate. When the behavior of the materials was compared with regard to all the temperatures tested, there was a significant difference ($p < 0.05$) for all the comparisons.

When comparing the esthetic materials considering the time during which they were submitted to low temperatures, the authors observed that for both material, the greatest changes in coordinate a^* occurred after 30 days, at all the temperatures tested, with significant difference ($p < 0.05$) in comparison with 7 days

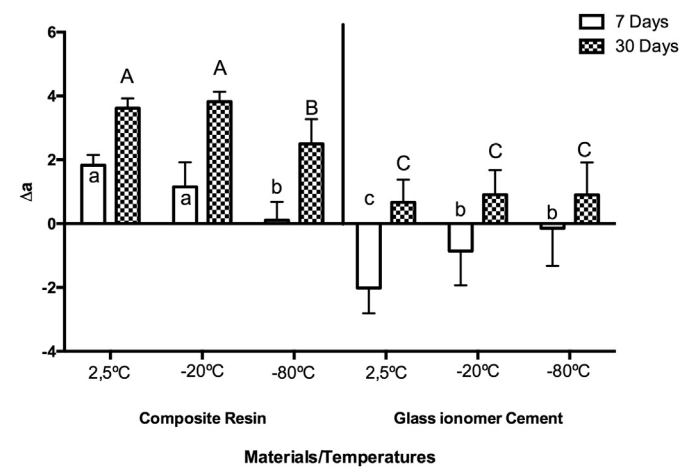


Fig. 3. Graphic representation of the comparison of Δa changes of materials when submitted to different temperatures (One-way ANOVA, Bonferroni test, $p < 0.05$). Different letters, lowercase at 7 days and uppercase at 30 days, indicate significant difference ($p < 0.05$) between materials at the same temperature. CR = Composite Resin. GIC = Glass Ionomer Cement. Table below the graphic indicates the differences between different materials submitted to different temperatures.

(Fig. 3).

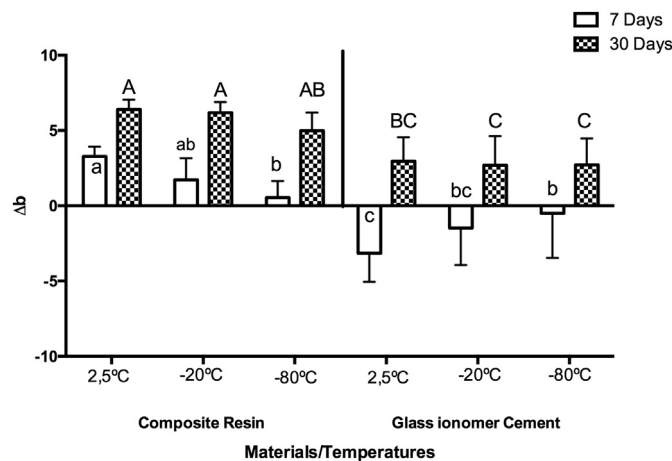
The comparisons of Δb^* readout mean values may be visualized in Fig. 4. The change after 7 days was greater, the higher was the temperature to which the materials were submitted, with both materials presenting greater changes on being submitted to 2.5 °C; a result similar ($p > 0.05$) to that found for materials submitted to -20 °C and differing statistically ($p < 0.05$) from materials submitted to -80 °C.

In spite of presenting a similar behavior with regard to the temperatures to which they were submitted, CR presented change in coordinate b^* , as opposed that presented by GIC; that is, while the coordinate showed a positive change for CR, negative values were found for GIC, being statistically significant results ($p < 0.05$) for all the temperatures tested.

By the different comparisons between materials and temperatures, the authors observed that the results of CR submitted to -20 °C presented no difference ($p > 0.05$) in comparison with GIC submitted to -80 °C. The same occurred for GIC (-20 °C) in comparison with CR (-80 °C); and between CR and GIC at -80 °C.

After 30 days, the temperatures at which the materials were submitted were not significant ($p > 0.05$) for change in coordinate b^* . However, the difference ($p < 0.05$) between the materials at all the temperatures tested was maintained, being higher for CR. The behavior of CR when submitted to -80 °C was similar ($p > 0.05$) to that of GIC submitted to 2.5 °C. For all the other comparisons between the different temperatures, there was a significant difference ($p < 0.05$).

When comparing the change in coordinate b^* for each material considering the time during which they were submitted to low temperatures, the authors verified that for both materials, the greatest changes occurred after 30 days, being statistically significant results ($p < 0.05$) in comparison with those found after 7 days, irrespective of the temperature to which the materials were submitted (Fig. 4).



| 7 days | | |
|----------|-----------|-------------|
| CR 2.5°C | GIC -20°C | $p < .0001$ |
| | GIC -80°C | $p < .0001$ |
| CR -20°C | GIC 2.5°C | $p < .0001$ |
| | GIC -80°C | $p > .05$ |
| CR -80°C | GIC 2.5°C | $p < .0001$ |
| | GIC -20°C | $p = .12$ |

| 30 days | | |
|----------|-----------|-------------|
| CR 2.5°C | GIC -20°C | $p < .0001$ |
| | GIC -80°C | $p < .0001$ |
| CR -20°C | GIC 2.5°C | $p = .0006$ |
| | GIC -80°C | $p = .0002$ |
| CR -80°C | GIC 2.5°C | $p = .12$ |
| | GIC -20°C | $p = .04$ |

Fig. 4. Graphic representation of the comparison of Δb changes of materials when submitted to different temperatures (One-way ANOVA, Tukey test, $p < 0.05$). Different letters, lowercase at 7 days and uppercase at 30 days, indicate significant difference ($p < 0.05$) between materials at the same temperature. CR = Composite Resin. GIC = Glass Ionomer Cement. Table below the graphic indicates the differences between different materials submitted to different temperatures.

3. Discussion

The aim of this study was to evaluate the action of cold, by simulating the effects of cooling and freezing on human bodies, on the color stability of esthetic dental restorative materials. The authors started with the null hypothesis that there would be no difference in the property studied in each material, irrespective of the low temperatures and time during which the materials were submitted to them. The results demonstrated that the hypothesis tested could not be accepted because the cold was capable of producing significant ($p < 0.05$) change in color of the materials, particularly in CR after 30 days of submission to cold.

The temperatures of 2.5 °C, -20 °C and -80 °C were selected for performing the tests, due to the possibility of precisely maintaining these temperatures using refrigerators. The time the materials remained in the cold simulated situations in which bodies or human bones have been found in short spaces of time, of approximately one week, up to more prolonged periods of time, of around 30 days under conditions of extreme cold.

The color analysis in different periods of time may contribute to the estimate of the time of death; that is, the time elapsed between death and necroscopic exam of the oral cavity of the cadaver. Definition of the time of death may be important for establishing the *causa mortis* medically and legally, and also show evidence of whether the victim suffered *antemortem* or *post-mortem* lesions or displacements, thus contributing to the experts' investigations.¹⁴

When several days have passed after death, it is impossible to estimate the time of death by means of analyzing body cooling, *post-mortem* rigidity, and the appearance of hypostasis, because it does not concern case of recent death.¹⁵ However, the presence of dental remains involved with esthetic restorative materials may make not only the instrumental color analysis an auxiliary method of identification but also a low cost, practical and accessible chronathanatological technique, mainly in far locations with intense winter conditions, in which the transport of cadavers may be difficult and delayed, and it is necessary to establish a field Forensic Medicine Institute.

In cases of human bones discovered in cold places, low temperatures act directly on dental remnants. In non-fully rotted corpses, the dermal, muscular, and fatty tissues of the face may protect the dental tissues from the direct action of the cold.¹⁶ Thus, there may be a difference between the ambient temperature and the internal temperature of the oral cavity. The impossibility of simulating the soft tissue protection is a limiting factor in this study.

However, following death, the metabolic activities cease in the body, leading to tissue hypoxia and acidification, and consequent cellular autolysis.¹⁷ Thus, the corporal capacity of thermoregulation becomes null, causing the body temperature to equilibrate with the external temperature progressively. Furthermore, the exposure to intense cold for an extended period will lead to cooling or freezing of soft tissues, and to the lost of their thermal barrier function.¹⁸

The materials selected for this study were CR and GIC, because of the diversity of their indications, making them widely used in Dentistry.¹¹ Therefore, when esthetic restorative materials are present during the *post-mortem* exam of the dental arches of victims of accidents involving low temperatures, these materials will probably be CR or GIC.

As a substrate for the restorations, bovine teeth were used. The authors selected bovine teeth in preference to human teeth because the former are more easily obtainable; it is possible to acquire a sufficient quantity in healthy conditions, and because it would not cause any harm to the proposed test, since the aim of this study was to evaluate the changes that occur in the materials, and not specifically in the dental structures. Apart from the bioethical question

involved, it is easier to standardize bovine tooth samples, and there is less risk of infection.^{19,20}

Bovine teeth show a similarity to the human dental tissues, particularly concerning enamel, with: very similar orientation of the prisms; equivalent percentage by weight of calcium; and protein matrix composed of the same aminoacids.¹⁹ Concerning dentin, there is a consensus in the literature that in the superficial cavities, 2 mm deep, bovine dentin has been shown to be feasible for use in adhesion studies, providing adequate bond strength when compared with human dentin.²⁰

Several authors have analyzed color changes by visual inspection of teeth^{16,21,22} and dental materials^{23–26} resulting from the action of heat. In this study, the authors evaluated the action of cold on esthetic restorative materials, because there are no reports in the literature of studies of this factor on the physical properties of these materials, particularly their color stability for forensic purposes.

Color stability may be evaluated by visual inspection or instrumental analysis.²⁷ Visual analysis is a subjective method that depends on the individual criterion of each observer, generating different interpretations of color changes that occurred. During the necroscopic exam, various factors such as: the position of the object observed; lighting of the room, fatigue and emotional state of the members of the forensic team may interfere with the visual analysis of color.¹¹ With the purpose of avoiding errors of interpretation, in this study, the authors used instrumental color analysis by means of a portable spectrophotometer that performed precise numerical color readouts.²⁸

The intrinsic color of composites may be changed by the action of various physical-chemical conditions, such as visible light, UV irradiation, changes in humidity and temperature.^{29,30} The chemical additives of composites, particularly those that undergo reaction, such as initiators and ultraviolet filters, may degrade the color components³¹ by the action of extreme temperatures. Some studies^{32,33} have evaluated the different causes of color change in CR and concluded that the chemical instability of the material may lead to endogenous color change.

Usually, filler particles are united in the resin organic matrix by means of the silane bonding agent that forms a matrix/filler interface. Frequently, γ -methacryloxypropyltrimethoxysilane is used as bonding agent.³⁴ The action of low temperatures on CR restorations present in the dental tissues of cadavers may cause a reaction of degradation of this matrix/filler particle interface, and cause changes in color due to the change in the way light is dispersed by the particles.³⁵ The greater color change that occurred at 30 days of exposure to cold observed in CR when compared with the analysis at 7 days demonstrated that this degradation continued to develop with the passage of time.

The monomer TEGDMA, present in the composition of CR, has a greater predisposition to water sorption, increasing the solubility of the polymer formed and the color instability.³⁶ The humidity present when materials are submitted to cold for prolonged periods of time (30 days) may have influenced the more intense change in color than in shorter periods (7 days) of exposure to a humid environment.

By analysis of coordinate L* alone, it is not possible to estimate whether or not CR was submitted to cold in exposures of up to 7 days. For GIC, this estimate was not possible even at extreme temperatures of -80°C and for prolonged periods of up to 30 days.

The lower the temperature to which materials were submitted, the greater was the stability of CR in the saturation of red, in both the period of 7 days and 30 days. Lower temperatures (-80°C) may have caused rapid freezing of the matrix and blockage, or at least reduction in the movement of the electrons as a species of solidification of the covalent bonds and stiffening of the polymer chains.

A lower level of mobility of the molecules provide the matrix with a higher degree of organization, and lower level of byproduct formation, leading to greater color stability.³⁷

For GIC, contrary to that which occurred for CR, the reduction in saturation of red in 7 days may have occurred due to water sorption, and consequent “whitening”, particularly at 2.5°C , generating the formation of a tone of color with paler characteristics. In a similar manner as occurred with CR, freezing of the hydrogel matrix of GIC at -80°C may have resulted in a lower water sorption capacity, and thus greater color stability in coordinate a*. After 30 days, the saturation of red remained stable, on an average. Prolonged periods (30 days) of submission to cold may have generated this same effect, with a lower level of water sorption and greater stability in this coordinate.

Analysis of coordinate b* for CR in 7 days demonstrated “yellowing”, which was more discrete, the lower was the temperature to which CR was submitted. The “yellowing” of CR may have resulted from the degradation of the photoinitiator camphorquinone.³¹ Rapid freezing of the polymer chains at -20°C , and predominantly at -80°C , could have generated a lower level of camphorquinone degradation and thereby, greater stability in coordinate b*.

In GIC, after 7 days there was a reduction in the saturation of yellow that led to a more opaque appearance of the restoration. In the same way as in CR, the lower was the temperature to which GIC was exposed, the greater was the stability of coordinate b*. GIC is a more hydrophilic material than CR. Therefore, a higher level of water sorption may have resulted in discoloration to the white and opaque tonality, particularly at 2.5°C , a temperature at which the material is only cooled, without attaining freezing point.^{30,34}

After 30 days, the increase in yellow saturation of CR was more intense than it was in 7 days. This may have occurred by the gradual degradation of camphorquinone with the passage of time. However, extremely low temperatures appear to stabilize this degradation, seeing that at -80°C the change in coordinate b* was more discrete when compared with -20°C and 2.5°C . In GIC, the degree of “yellowing” was similar at the three temperatures evaluated. The smaller changes in coordinate b* in the time intervals tested was also verified at -80°C , confirming the greater stability of this coordinate, the lower was the temperature to which the material was submitted.

In this study, the authors sought to use a portable, easy-to-handle appliance that could serve Forensic Odontology as an auxiliary method of identifying victims. This was due to its capacity to distinguish between different esthetic dental restorative materials by means of color readouts, and thus enabling a *post-mortem* oral exam to be performed without mistakes about the type of material observed, for appropriate comparison with the *ante-mortem* information obtained from clinical dental record charts of victims.

4. Conclusion

The authors concluded that cold was capable of producing changes in color in the two esthetic materials, with similar intensities between the two, at all the temperatures studied, when analyzed at 7 days. After being submitted to cold for 30 days, the changes were more significant for CR, allowing it to be differentiated from GIC after 30 days, at all the temperatures tested. When submitted to -80°C , it was possible to distinguish between the materials after 7 days, considering the alteration in all the color coordinates involved. Therefore, the test proposed in the study was shown to be practical, feasible and capable of helping Forensic Odontology with the identification of victims. As regards analysis of the time of death, the method proposed could not be used as a

chrono-thanatological technique, because it was not possible to estimate the time during which CR and GIC were submitted to the action of cold temperatures.

Conflict of interest statement

None.

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The authors deny any conflicts of interest related to this study.

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