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NATURAL DENTIFRICES: HOW CAN PROLONGED TOOTHBRUSHING INFLUENCE ENAMEL COLOR AND SURFACE ROUGHNESS?

Murilo Guimarães CAMPOLINA[1], Lia DIETRICH[2], Alexia da Mata GALVÃO[3], Hugo Lemes CARLO[2], Maria Antonieta Veloso Carvalho de OLIVEIRA[4], Gisele Rodrigues da SILVA[2]

[1] Post-graduation Program in Dentistry, School of Dentistry, Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brazil.
[2] Department of Operative Dentistry and Dental Materials, School of Dentistry, Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brazil.
[3] Department of Endodontics, School of Dentistry, Anhanguera College, Uberlândia, Minas Gerais, Brazil.
[4] Department of Endodontics, School of Dentistry, Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brazil.

Corresponding author:
Gisele Rodrigues da Silva
giselerosilva@ufu.br

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Abstract
This study evaluated the color change (ΔE00) and surface roughness (Ra) of enamel submitted to prolonged toothbrushing with natural non-fluoride dentifrices. Five groups of bovine teeth (n=10) were brushed with a control dentifrice: (C) Colgate Total 12 – Colgate; and four natural dentifrices: (R) Restore – Jeunesse; (OG) OnGuard – doTerra; (OC) OzonCare – Philozon; and (N) Natural activated charcoal – Suavetex. Mechanical toothbrushing simulated one month (T1), six months (T2), one year (T3), and two years (T4) of product use. ΔE00 and Ra were measured before and after toothbrushing. Two-way repeated measures ANOVA and Tukey's test were performed. Dentifrice ($p<0.001$), toothbrushing time ($p=0.004$), and their interaction ($p=0.031$) influenced color change (ΔE00). Natural dentifrices promoted similar color change and Ra to the traditional product. After six months of simulated toothbrushing, OG promoted more color changes than N and R. Only OG and R reached ΔE00 above the acceptability threshold. OC and N dentifrices generated Ra above the limit of clinical roughness from T2. Toothbrushing with natural toothpaste for up to two years causes similar enamel color changes and roughness to the traditional product.

Keywords: Dentifrices. Natural products. Oral health. Toothbrushing. Toothpaste. Whitening.

1. Introduction

Alternative healthcare products, commonly called natural (Mazur et al. 2022), have been increasingly used because of their supposedly less industrialized formulations. Dentifrices stand out among these items, characterized mainly by different tastes and colors, the absence of components such as fluoride and alcohol, and the inclusion of essential oils and natural elements such as ozone (Dietrich et al. 2021) and activated charcoal in their compositions (Eisenberg et al. 1998; Goldstein et al. 2000; Jacobsen et al. 2001; Brooks et al. 2017; Mathias-Santamaria et al. 2022; Emídio et al. 2023; Tomás et al. 2023).

Dentifrices often comprise abrasives (Giles et al. 2009; Ganss et al. 2011; Roselino et al. 2015), detergents, humectants, binders, preservatives, dyes, flavorings, and water (Cury et al. 2014). Their primary function is to remove residues and bacterial plaque that adhere to the tooth surface, gums, and periodontal tissues (Rashrash et al. 2017; Welz et al. 2018; Sanchez et al. 2020). The fluoride in their composition helps destabilize the dental biofilm, enabling physicochemical effects by reducing dental demineralization and promoting remineralization through saliva, which is relevant for controlling caries in an acidic oral environment (pH<5.5) (Rashrash et al. 2017; Welz et al. 2018; Sanchez et al. 2020).

The increasing use of natural products (Piekarz et al. 2017; Valones et al. 2019; Mazur et al. 2022) is attributed to a demand for less invasive aesthetic procedures and is reinforced by disseminated social media information (Welz et al. 2018). The desire for whiter teeth stands out among these aesthetic procedures (Carvalho et al. 2022). The industry promotes whitening dentifrices as an alternative to the traditional protocol performed and supervised by a dentist using hydrogen or carbamide peroxide. The problem is that the supposed whitening effect (Law et al. 2022) of these dentifrices usually causes an over-abrasive action (Bollen et al. 1997; Devila et al. 2020). That may increase enamel surface roughness, damaging the tooth structure and making teeth more vulnerable to an acid attack and bacterial deposition, which dentifrices should prevent.

Natural dentifrices suggest whitening effects, structure strengthening, and more dental health benefits than conventional products. However, they lack essential compounds for dental protection, such as fluoride. Considering these products are freely commercialized and consumed, it is worth understanding their behavior concerning tooth enamel, particularly color change and surface roughness. The null hypothesis of this study was that natural dentifrices do not cause more enamel color change or surface roughness than conventional fluoride toothpaste over a two-year toothbrushing simulation.

2. Material and Methods

This research followed the bioethical principles of the Declaration of Helsinki and considered the CRIS guideline (Checklist for Reporting *In vitro* Studies) (Krithikadatta et al. 2014) for its performance.

Sample size calculation

The sample size was calculated based on a previous pilot study. A minimum treatment difference of 0.98 with an expected standard deviation of 0.6 was considered for the primary outcome (color change - ΔE00). Ten samples per group were required, with an 80% test power and a 95% significance level. The calculation used the statistical software package SigmaStat version 12.5 (Systat Software Inc., San Jose, CA, United States).

Tooth preparation and handling

This study used 50 crowns of bovine teeth without cracks and structural defects. The samples were cleaned with scalpel blades and then underwent prophylaxis with a rubber cup (Microdont, São Paulo, SP, Brazil) and pumice stone (Biodinama, Ibiporã, PR, Brazil). Horizontal cuts separated the crowns from the roots. Then, the crowns were embedded in polyester resin (Maxi Rubber, Campinas, Brazil). The buccal surface was wet-ground progressively with #600, 1200, 1500, and 2000-grit silicon carbide papers (AROPOL-VV, Arotec, Cotia, Sao Paulo, Brazil) before being polished with alumina suspension at 0.3 µm (Buehler, Lake Bluff, IL, United States) and a felt cloth (Buehler) to obtain a polished and uniform surface. Initial enamel color and roughness were measured.

Figure 1 indicates tooth preparation and handling.

The samples were randomly divided into five groups (n=10), according to the dentifrice of each toothbrushing protocol:

(C) Control: Colgate Total 12 - Colgate-Palmolive, São Paulo, SP, Brazil;
(R) Restore – Jeunesse, São Paulo, SP, Brazil;
(OG) OnGuard – doTerra, Pleasant Grove, Utah, USA;
(OC) OzonCare – Philozon, Balneário Camboriú, SC, Brazil;
(N) Natural activated charcoal – Suavetex, Uberlândia, MG, Brazil.

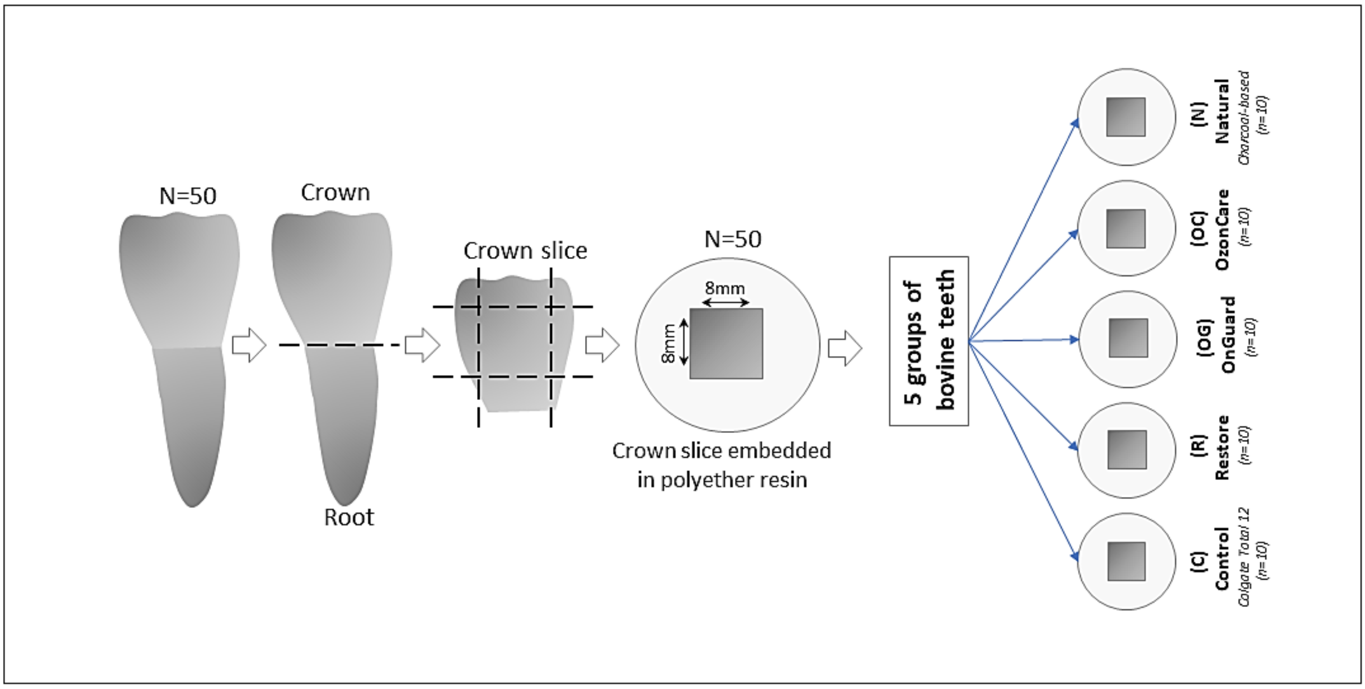

Figure 1. Schematic illustration of the experimental design.

Table 1. shows the dentifrices, their components, and manufacturers.

DENTIFRICE	COMPONENTS	MANUFACTURER
Colgate Total 12	Glycerin, Water, Hydrated Silica, Sodium Lauryl Sulphate, Arginine, Flavor, Cellulose Gum, Zinc Oxide, Poloxamer 407, Tetrasodium Pyrophosphate, Zinc Citrate, Benzyl Alcohol, Xanthan Gum, Cocamidopropyl Betaine, Sodium Fluoride, Sodium Saccharin, Acid Phosphoric, Sucralose, Titanium Dioxide.	Colgate-Palmolive, São Paulo, SP, Brazil
Restore	Sodium bicarbonate, citric acid, hydroxyapatite, sodium cyclamate, sodium saccharin, xylitol, potassium citrate, melaleuca alternifolia (tea tree) extract, fragrance, limonene, linalool	Jeunesse, São Paulo, SP, Brazil
OnGuard	Glycerin, Water, Hydrated Silica, Hydroxyapatite, Xylitol, Calcium Carbonate, Cellulose Gum, Mentha piperita (Peppermint) Essential Oil, Citrus sinensis (Wild Orange) Essential Oil, Eugenia caryophyllata (Clove) Essential Oil, Oil Cinnamomum zeylanicum Essential Oil (Cinnamon Bark), Eucalyptus Essential Oil, Rosemary Essential Oil, Stevia, Wintergreen Essential Oil, Myrrh Essential Oil, Sarkosyl, Carrageenan.	doTerra, Pleasant Grove, Utah, USA
OzonCare	Glycerin, Water, Sarkosyl, Xanthan Gum, Peppermint Oil, Ozonated Olive Oil, Xylitol, Titanium Dioxide, Benzyl Alcohol, Calendula Flower Extract, Eucalyptus Oil, Menthol, Sodium Benzoate, Tahiti Lime Essential Oil, Pomelo Oil, Tea Tree Oil, Sucralose, Potassium Sorbate.	Philozon, Balneário Camboriú, SC, Brazil
Natural activated charcoal	Glycerin, Sodium Benzoate, Carrageenan, Hydrated Silica, Lauryl Glycoside, Xanthan Gum, Stevioside, Xylitol, Flavorings, Water, Powdered Charcoal, Sodium Bicarbonate. Organic Ingredients: Bamboo Extract, Pomegranate Extract, Salvia Extract.	Suavetex, Uberlândia, MG, Brazil

A researcher not involved in the implementation and evaluation processes performed randomization and allocation. The color and roughness evaluators who statically analyzed the data were blinded for the coding methodology.

Toothbrushing protocols

After the primary data analysis, the toothbrushing protocols started on the MEV3T-10XY brushing machine (Odeme Dental Research, Luzerna, SC, Brazil). The samples were submitted to mechanical toothbrushing cycles with a vertical load of 300 g on the heads of soft-bristle toothbrushes (Colgate-Palmolive, São Paulo, SP, Brazil), with controlled temperature (25 ± 1°C) and a linear movement at 2 Hz (120 cycles/min).

All samples were immersed in a paste containing the dentifrice of each group and water at a 1:1 ratio (weight/volume). The literature describes that 73,000 mechanical brushing cycles correspond to five years of toothbrushing for a healthy person (Roselino et al. 2015). Therefore, the simulated times were (T1) one month, (T2) six months, (T3) one year, and (T4) two years, with specimens subjected to 1,217, 7,300, 14,600, and 29,200 toothbrushing cycles, respectively.

Every 300 cycles, the toothpaste solutions were replaced with new ones. Before the simulations, baseline values (T0) were calculated for color and roughness measurements. The specimens were ultrasonically washed for ten minutes and measured for roughness and color.

After each toothbrushing period, the samples were cleaned in an ultrasonic tank (Cristófoli Biossegurança, Campo Mourão, Paraná, Brazil) for ten minutes. After cleaning, the color and roughness were analyzed to verify changes over time.

Color assessment

Sample colors were measured with a spectrophotometer (Ci64UV, X-Rite, Grand Rapids, MI, USA) and evaluated according to the color system established by the *Commission Internationale de L'Eclairage* (CIE) based on color dimensions measured by L* (white/black axis), a* (red/green axis), and b* (yellow/blue axis) values. The color was measured initially (T0) and at T1, T2, T3, and T4, and the specimens were placed on metal support to standardize measurements in the same area (4 mm in diameter).

Measurements were taken in triplicate with standard D65 illuminant (wavelength from 400 nm to 700 nm) with specular light included (SPIN mode), 2° observation angle, against a white background (L* white = 95.2; a* white = 21.2; and b* white = 50.3 - ColorChecker grayscale, X-Rite, Grand Rapids, MI, USA). The data were selected for analysis from the obtained mean values.

The total color change was calculated considering the initial analysis using the ΔE_{00} formula (Miotti et al. 2017; Luo, et al. 2001; Paravina et al. 2015). The CIEDE2000 color change (ΔE_{00}) was calculated with $\Delta E_{00} = ((\Delta L / K_L S_L))^2 + ((\Delta C / K_C S_C))^2 + ((\Delta H / K_H S_H))^2 + R_T ((\Delta C \Delta H / S_C S_H))^{1/2}$; where ΔL, ΔC, and ΔH are luminosity, chroma, and hue differences between color measurements; K_L, K_C, and K_H are the parametric factors that influence viewing and lighting conditions; R_T is the hue and chroma interaction differences in the blue region; S_L, S_C, and S_H are weighting functions for color difference adjustments considering L*, a*, and b* coordinate location variations (Paravina et al. 2015).

Surface roughness

After measuring the initial values (T0), the surface roughness of samples was analyzed after each simulated toothbrushing time (T1, T2, T3, and T4) using the Surf Test SJ-301 roughness meter (Mitutoyo Corporation, Japan). Measurements were taken at a constant speed of 0.25 mm/s and measuring force of 4 mN, and the cut-off value was adjusted to 0.25 mm. The obtained numeric parameters were the surface roughness (Ra) means.

Statistical analysis

The two-way repeated measures ANOVA and Tukey's test analyzed color (ΔE_{00}) and roughness (Ra) data. Toothbrushing time was a repetition factor. Statistical analysis was performed using the Jamovi 2.0 statistical software package (dev.jamovi.org). The significance level was $\alpha = 0.05$ for all data analyses.

3. Results

Figure 2 shows color change values represented by ΔE_{00}, comparing toothbrushing time and dentifrices. Dentifrices ($p<0.001$), toothbrushing time ($p=0.004$), and their interaction ($p=0.031$) influenced color change (ΔE_{00}). Natural dentifrices promoted similar color changes to traditional products. After six months of simulated toothbrushing, OG promoted more color change than N and R. Only OG and R reached ΔE_{00} above the acceptability threshold (Paravina et al. 2015).

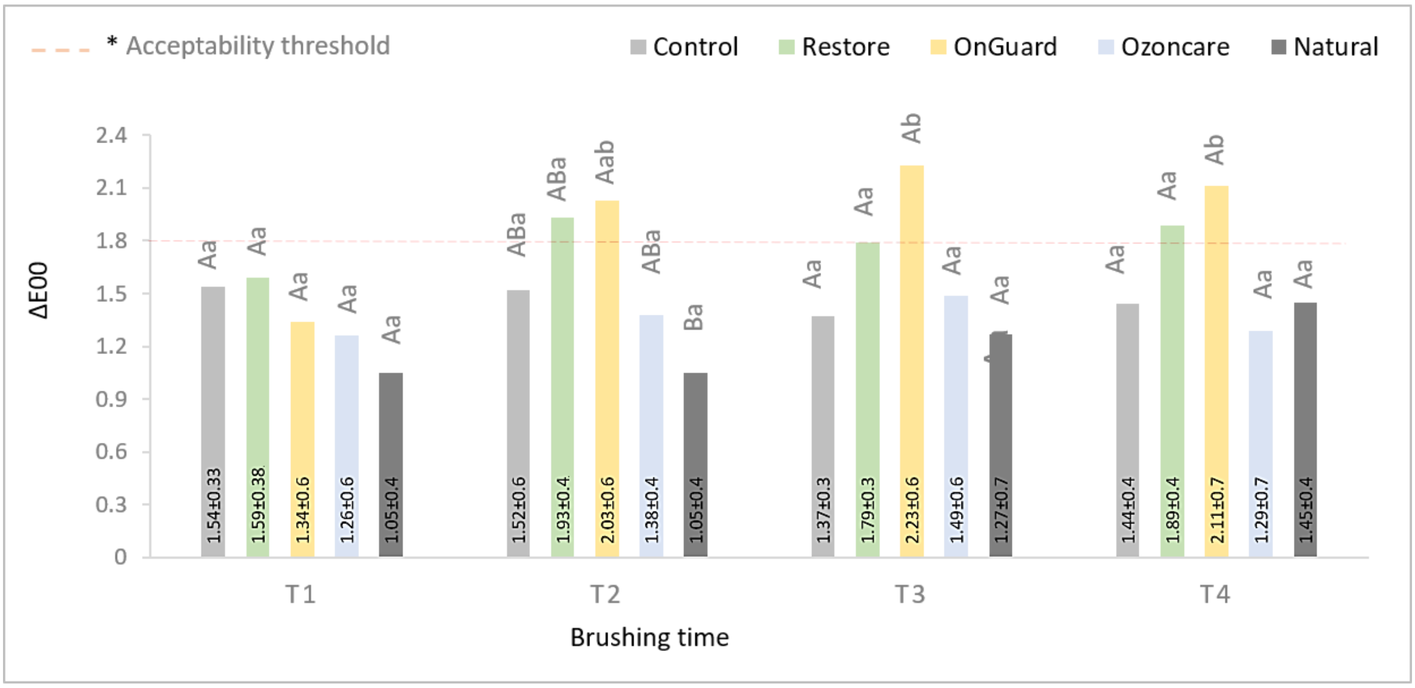

Figure 2. Mean (± standard deviation) of color change represented by ΔE00 comparing brushing time and dentifrices (n=10). T1- 30 days; T2- 6 months; T3- 1 year; T4- 2 years. Different letters (lower case compare time; upper case compare dentifrices) indicate statistical differences by Tukey's test (P<0.05). The threshold of color acceptability was defined according to Paravina et al. 2015.

Figure 3 indicates CIELAB parameters. L* is the most concerning from an aesthetic point of view (dark to light). After toothbrushing, L* values were practically constant. Higher b* values suggest tooth yellowing. Teeth brushed with OG and N dentifrices showed higher b* results.

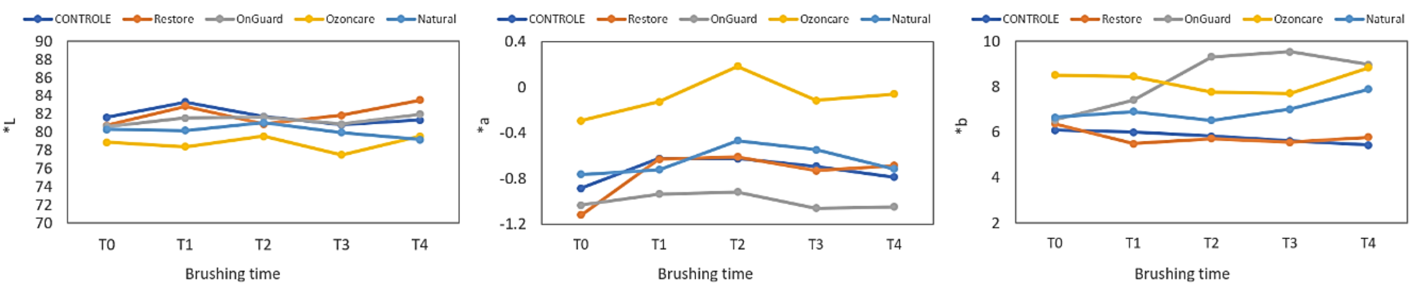

Figure 3. L*, a*, and b* color parameters of tooth enamel after simulated brushing with different dentifrices for up to 2 years. (L*: White/Black; a*:/Green; b*: yellow/blue).

Figure 4 presents mean and standard deviation values of enamel surface roughness. Dentifrices (p=0.025) and toothbrushing time (p<0.001) significantly differed, but their interaction did not (p= 0.205). Ra values were higher than the initial ones after two years of simulated toothbrushing.

All natural dentifrices caused similar enamel surface roughness to the control group. Natural dentifrice comparisons showed that R had a lower ability to change roughness than N (p=0.017).

4. Discussion

This study evaluated the effect of long-term toothbrushing with alternative dentifrices with natural formulations on teeth. The null hypothesis was accepted. Natural toothpastes behaved similarly to traditional fluoride products regarding color change and surface roughness. Visual thresholds are relevant qualitative indicators in dentistry to evaluate and interpret clinical outcomes and compare treatments. The color change limit is ΔE00 higher than 1.77, indicating an acceptable color difference (Paravina et al. 2015).

Roughness values above 0.20 represent a highly rough tooth surface, more susceptible to biofilm accumulation and caries formation (Bollen et al. 1997).

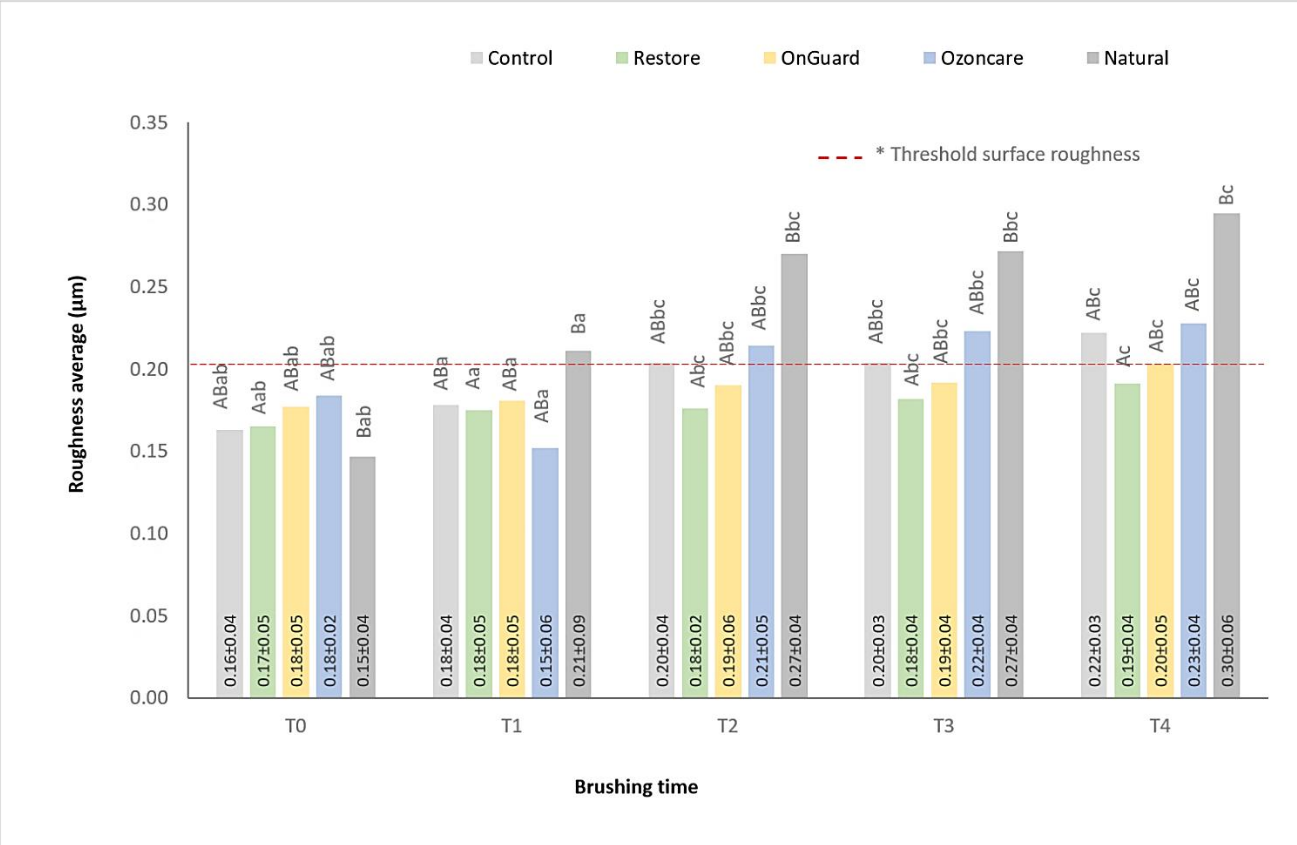

Figure 4. Mean (± standard deviation) of roughness average (Ra) comparing brushing time and type of dentifrices (n=10). T0- Before brushing. T1- 30 days; T2- 6 months; T3- 1 year; T4- 2 years. Different letters (lower case compare time; upper case compare the type dentifrices) indicate statistical differences by Tukey's test (P<0.05). Enamel-healthy clinic threshold for surface roughness was indicated according to Bollen et al. 1997.

Onguard and Restore dentifrices achieved color changes above the acceptability threshold (Paravina et al. 2015). Regarding surface roughness, none of the dentifrices differed much from the control toothpaste. However, the Natural activated charcoal and OzonCare toothpaste generated roughness changes above the safety threshold (Bollen et al. 1997), suggesting higher abrasiveness.

The abrasives in dentifrice formulations primarily clean and remove bacterial biofilm and food remnants that adhere to the tooth surface and nearby tissues (Cury et al. 2014; Devila et al. 2020). These compounds also have cosmetic functions to control color and remove stains (Cury et al 2014; Devila et al. 2020). The dentifrices that promise a whitening effect aim to improve this aesthetic function, usually increasing the number of abrasives in their formulas. However, the literature demonstrates that whitening dentifrices harmfully increase surface roughness and promote structural wear on teeth and restorative materials (Cury et al. 2014; Devila et al. 2020).

The RDA (Relative Dentin Abrasivity) and REA (Relative Enamel Abrasivity) indices of dentifrices estimate their abrasive power (Ganss et al. 2011; Giles et al. 2009; Kamiya Coppini et al. 2022). Dental companies rarely provide these data to certify the safety of their products. This lack of information hinders dentists from adequately recommending a dentifrice to patients. This study investigated these indices and contacted all toothpaste companies. The only data found were the RDAs of Colgate Total 12 (RDA 70) and OnGuard – doTerra (RDA 120) (Ganss et al. 2011; Giles et al. 2009).

In the control group, Colgate Total 12 showed an RDA of 70, which is low. This study verified that this product increased enamel roughness in the long term and did not produce significant tooth color change (Ganss et al. 2011; Giles et al. 2009). Colgate Total 12 has low abrasiveness and comprises fluoride; therefore, it is clinically estimated that releasing fluorides incorporated by saliva in the dental remineralization process would control enamel roughness. This product is inexpensive and viable and presents excellent cost-effectiveness considering its characteristics. This dentifrice does not show relevant color changes according to use but does not promise a whitening effect.

The Restore dentifrice (Jeunesse) showed tooth color changes after six months, slightly above the acceptability threshold. However, L* and b* remained very close to the initial value, and color change does not necessarily mean that the tooth has been whitened, as it occurs with specific products. This dentifrice promises a positive effect of Melaleuca oil (tea tree) in controlling oral microbiota and gingival diseases (Piekarz et al. 2017). The presence of hydroxyapatite microparticles also indicates a positive remineralizing impact. However, the literature does not demonstrate the benefits of hydroxyapatite microparticles in dentifrices. Saliva is typically used for remineralization because it is highly saturated in calcium and phosphate compared to the enamel solubility product, replacing naturally lost minerals and requiring only fluoride as an activator (Cury et al. 2014). Although the Restore dentifrice does not include fluoride, it did not clinically alter surface roughness, suggesting adequate abrasiveness. However, despite being expensive, this dentifrice does not whiten teeth as claimed. Considering the lack of fluorides, this product does not seem viable or offer any advantage over regular toothpaste.

The OnGuard dentifrice also showed color change above the acceptability threshold, mainly reflected by a higher b* value, suggesting that teeth brushed with this toothpaste tend to be yellower, unlike the suggested whitening effect. This dentifrice has silica and calcium carbonate as abrasives and increased surface roughness over time, similar to the control group. That is because of an RDA of 120, considered low, in the same category as the RDA of the control dentifrice. It contains several natural oils, such as rosemary (Rosemary Leaf Oil), which present scientific evidence to reduce gingival bleeding and bacterial plaque (Valones et al. 2019). However, this product is expensive and presents low benefits regarding tooth color changes and enamel protection.

The OzonCare dentifrice did not deliver the promised whitening effect and increased surface roughness, indicating inadequate abrasiveness. Ozone is promising for treating periodontal diseases and may be combined with a whitening effect because of hydrogen peroxide (Dietrich et al. 2021).

The Natural activated charcoal toothpaste did not show significant color changes over time, only altering b* values and showing a tendency to yellow teeth. The increased enamel surface roughness exceeded the clinically acceptable threshold in just 30 days of simulated use, generating a rapid risk of altering enamel morphology and the consequent possibility of dental biofilm accumulation, which may contribute to caries formation and periodontal diseases. The literature shows that activated charcoal toothpastes are highly abrasive to tooth enamel, falsely generating a whitening effect by removing the surface layer of enamel (Brooks et al. 2017).

This study has limitations because it is impossible to simulate the oral environment reliably. Toothbrushing strength and frequency, saliva pH, lifestyle, diet habits, medication use, and systemic health are difficult to measure in clinical and laboratory studies, potentially impacting the results. The slight roughness increase *in vivo* could be minimized by saliva, which, combined with fluoride, would contribute to proper tooth remineralization and roughness control (Roselino et al. 2015).

Therefore, natural toothpastes are oral hygiene options that must be used and indicated with caution, as they do not promote a whitening effect on teeth, do not contain fluoride to strengthen the dental structure, and are more expensive. Notably, natural toothpastes do not positively change tooth color, often yellowing them, which could aesthetically harm patients.

5. Conclusions

The research data and their analysis showed that toothbrushing with natural dentifrices for up to two years produces similar effects of enamel color change and roughness compared to traditional toothpaste.

Authors' Contributions: CAMPOLINA, M.G.: conception and design, acquisition of data, analysis and interpretation of data, and drafting the article; DIETRICH, L.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article, and critical review of important intellectual content; GALVÃO, A.M.: acquisition of data; CARLO, H.L.: acquisition of data and analysis and interpretation of data; OLIVEIRA, M.A.V.C.: acquisition of data and analysis and interpretation of data; SILVA, G.R.: conception and design, analysis and interpretation of data, drafting the article, and critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

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Ethics Approval: Not applicable.

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