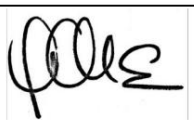
1. General information page

Study name	Clinical study to demonstrate benefits from the daily use of AlignerFresh (Aligners) and OrthoFoam (Brackets) as a whitening product during orthodontic treatments: A 4-week clinical study ALIGNERFRESH-
protocol identification	ORIGINAL-001-2022 Version 02.,
version and date	December 1, 2022 Foam with
research product	content of Hydrogen Peroxide (PH)
Authors	Mauricio Vargas Malagón OD, MSc. Camilo Triana ViBo.
study phase	Phase IV Equivalent, Post-Marketing Study
Sponsor	EVERBRANDS INC. 11791 Monarch St Garden Grove, CA 92841, Garden Grove. CA USA. camilo@everbrands.com
Authorization of the protocol and its modifications	EVERBRANDS INC. 11791 Monarch St Garden Grove, CA 92841, Garden Grove. CA USA. camilo@everbrands.com Independent ethics committee. Clinical Research Ethics Committee (CEIC) of the University of La Sabana.



Clara Marcela Collazos Encinales OD., Orthodontist // Invisalign Certified

• Responsibilities: Clinical phase of the study. •

Affiliation: Independent Invisalign Orthodontist

Mauricio Vargas Malagón OD., MSc •

Responsibilities: Author of the Report •

Affiliation: Independent Consultant

2. Table of Contents

3. Tables, figures and abbreviations

3.1 List of tables

3.2 List of Figures •

3.3 List of abbreviations

• L	Brightness
• C	Intensity
• H	color, chromatic hue
• CIE	International Commission of Illumination
• PH	Hydrogen peroxide

Glossary of terms

Spectrophotometer A color measurement device that measures the spectral reflection of light and converts it into color values (tristimulus values) or an internationally recognized numerical value.

VITA SYSTEM 3D-MASTER shades Refers to the 29 shade samples controls, including the 3 lightened shades of lightness group 0 and the 52 intermediate shades of VITA SYSTEM 3D-MASTER.
VITA classical A1–D4 shades Refers to the 16 original VITA classical A1–D4 shades from the VITA classical A1–D4 shade guide.
Lightness (L) The lightness of a color. The lightness or darkness of a color relative to a series of shades of gray in the range from white (L = 100) to black (L = 0).
Chroma (C) The intensity (purity) of a color. The difference between color and a shade of gray with the same lightness, measured as distance from the neutral axis. In some cases it is also called color purity.

Chromatic hue (h) What we commonly know as color (red, yellow, green, blue, or other colors). It corresponds to the wavelength of light. In the L*C*h* system it is represented as an angle that goes from 0° to 360°. Angles from 0° to 90° are shades of red, orange, and yellow; angles from 90° to 180° are shades of yellow, yellow-green, and green; angles from 180° to 270° are shades of green and blue; the angles from 270° to 360° are shades of blue, purple and magenta, which change back to red when 360° is reached (as in 0°).

Intermediate shades Mixing of two or more ceramic powder shades from VITA SYSTEM 3D MASTER to obtain an intermediate shade. So e.g. For example, 2M2 can be mixed with 2M3 to obtain the color 2M2.5.

CIEL*a*b* Three-coordinate (tristimulus) representation of color in color space, as defined by the Commission Internationale d'Éclairage (Commission Internationale d'Éclairage) or CIE. For more information on color theory, see our website: www.vita-zahnfabrik.com.

4. Summary

Title	Clinical study to demonstrate benefits from the daily use of AlignerFresh (Aligners) or OrthoFoam (Brackets) as a whitening product during orthodontic treatments: A 4-week clinical study Generic Name: Cleaning and whitening foam with Hydrogen
research product	Peroxide (PH) content Common name: Hydrogen Peroxide (PH)
Investigator Principal	Clara Marcela Collazos Encinales Dentist., Orthodontist. Certified Invisalign • Responsibilities: co-author of the protocol • Affiliation: Independent Invisalign Orthodontist • Cr19C N 86-30 Cons 306 • Tel +57 1 610 8855 Cell +57 312 5063860 • Email: mcollazose@gmail.com
Technical manager for the sponsor	Camilo Triana • Responsibilities: Study protocol approval • Affiliation: Legal representative of Everbrands Inc. • 11791 Monarch St Garden Grove, CA 92841, Garden Grove. CAUSE. • camilo@everbrands.com
Activities developed in the study	See table below
Ethics Committee of Investigation Clinic	Full name: Clinical Research Ethics Committee (CEIC) of the University of La Sabana. Address: Km 7 Autopista Norte de Chía-Cundinamarca, Building H office 205B Postal code: 140013.

Activity	**Date Realization**	**Final date**
protocol writing	3 nov 2022	1 Dec 2022
Approval by the ethics committee	10 Dec 2022	30 Jan 2023
Study drug remission	3 Feb 2023	N/A
volunteer recruitment	27 Feb 2023	18 Apr 2023
instrumental analysis	27 Feb 2023	18 Apr 2023
Preparation and delivery of the statistical report	20 Apr 2023	19 May 2023
Delivery and presentation of the final report	23 May 2023	29 May 2023

	24-hour telephone: 57-1-8615555 Fax: 57-1-8615555 Contact email: www.unisabana.edu.co
Trial Drugs	AlignerFresh Original Clean and its derivatives such as WhiteFoam® or OrthoFoam®, are products that contain the same ingredients and it is only differentiated by trade names AlignerFresh focused on aligners and OrthoFoam focused on brackets and are registered with Invima as a cosmetic product under the identification code of the Mandatory Sanitary Notification (NSO) NSOC12166-22CO assigned file number: 20222349 filed: 20221028170 date: 03/2022/07 valid until 03/2029/16 and (NSO) NSOC12001-22CO assigned file number: 20222785 filed: 20221029927 date: 03/2022/11 valid until 03/2029/16
Background and rationale	Orthodontic patients not only seek better orthognathic, functional and physiological conditions but also seek better aesthetics and demand not only a healthy mouth but also a perfect smile. In fact, it has been reported that in the UK 28% of adults are dissatisfied with the appearance of their teeth and in the US 34% of the adult population are dissatisfied with the current color of their teeth. Different toothpastes, rinses, and peroxide treatments have been used to improve tooth color, which is compromised by treatment with braces and orthodontic appliances. Dental applications of peroxide date back over a century, including prominent early use in antisepsis for wound healing or treatment of periodontal infections, many techniques have been developed for in-office and at-home treatment. , which involve short-term repeated application of a peroxide-based gel. AlignerFresh and OrthoFoam correspond to a rinse with a low concentration of hydrogen peroxide (3.79%) that, in addition to eliminating 99.9% of microorganisms, is expected to generate dental whitening during orthodontic treatment with aligners or brackets.
Goals	Objective To quantify the amplitude and magnitude of the lightening effect after 3 times daily use of the investigational product applied for 4 weeks of treatment. Secondary Objectives To evaluate the use of the investigational product (ie, AlignerFresh) for use in aligners and (ie, OrthoFoam) for use in Brackets. •Evaluate organoleptic satisfaction when using the research product. • Evaluate the sensation of cleanliness when using the research product. • Assess dental sensitivity from the use of the investigational product. • Evaluate the sensation of oral irritation when using the investigational product.
Study design	Open study of two treatment groups (use of aligners or use of brackets), controlled (ie, active control), and a single center. The study has a 4-week follow-up (ie, 30 days) and compares the initial color of 3 healthy teeth from the arch.

	upper surface and color changes during the 4 weeks following the use of the investigational product (ie, AlignerFresh) for use in aligners and (ie, OrthoFoam) for use in Brackets Inclusion Criteria
Selection criteria	1. Male or female patients 2. Older patients between 12 and 55 years old 3. Patients who use aligners or brackets during their orthodontic treatment 4. Patients who have signed the informed consent or the assent signed by one of the parents of the minor Exclusion Criteria 1. Patients with less than 75% healthy teeth 2. Patients with previously diagnosed periodontal disease and in treatment 3. Patients with active caries or being treated by a professional 4. Pregnant or lactating women. 5. Patients with physical disabilities in the upper limbs that prevent their oral hygiene. 6. Patients who smoke two or more cigarettes a day. 7. Patients who chronically use medications that can cause changes in tooth color. (tetracycline, doxycycline, chlorhexidine, antihistamines, antipsychotics or antidepressants and antihypertensives) 8. Patients with allergies to any of the components of the treatments studied. 9. Patients with terminal illnesses undergoing treatment. 10. Patients who drink coffee, tea or red wine more than 2 times a week and do not discontinue during the study. 11. Patients who do not comply with the proper use of hygiene elements and the established periodicity.
sample size	To determine the sample size, a confidence level of 95%, a power of 80% (ÿ = 20%) and a loss rate of 5% were considered. Using the formula to compare the means in the color variable (quantitative variable) using the VITA guide, bilateral contrast and finally detecting an expected effect of 30% in the average values of color by means of the equation, it is necessary to include a minimum of 40 subjects in 2 groups of 20 subjects for the aligners group and 20 subjects for the Brackets group.
Description of interventions	Eligible patients according to the eligibility criteria were invited to participate. The benefits and possible risks of the study were explained to the patients who met the eligibility criteria, and informed consent or assent authorized by the ethics committee was obtained. At the beginning of the study, the patients were divided into 2 groups according to the orthodontic treatment they were undergoing, orthodontic treatment with aligners or orthodontic treatment with brackets. The two groups received oral health recommendations and the proper use of oral hygiene items.

	(Toothbrush + Colgate toothpaste (Delivered by orthodontist) + assigned whitener) Instructions for patients. · Visit 0: (Day 0) Screening visit, informed consent or assent was taken, assigned to the corresponding orthodontic treatment group, oral health recommendations and proper use of oral hygiene items were given. 3 healthy teeth from the upper arch without restorations were selected (an incisor, a canine and a premolar) to which the color of the gingival area, middle area and incisal area was recorded, a photo of the 3 teeth was taken and they were delivered. oral hygiene items and registration forms. · Visit 1: (Day 10) Follow-up visit, where the professional recorded the color of the gingival area, the middle zone and the incisal area, a photo was taken of the 3 selected teeth of the patient's upper arch, after 10 to 12 days of additionally, the professional collected the forms for the use of oral hygiene items filled out by the patient and delivered the registration forms for the following week of treatment, the professional filled out the information electronically in the system set up for this study. · Visit 2: (Day 20) Follow-up visit, where the professional recorded the color of the gingival area, the middle zone and the incisal area, a photo was taken of the 3 selected teeth of the patient's upper arch, after 20 to 22 days of additionally, the professional collected the forms for the use of oral hygiene items filled out by the patient and delivered the registration forms for the following week of treatment, the professional filled out the information electronically in the system set up for this study. · Visit 3: (Day 30) Follow-up visit, where the professional recorded the color of the gingival area, the middle zone and the incisal area, a photo was taken of the 3 selected teeth of the patient's upper arch, after 30 to 32 days of additionally, the professional collected the forms for the use of oral hygiene elements filled out by the patient and closed the CRF of the study, the professional filled out the information electronically in the system enabled for this study.
conclusions	Based on the interpretation of the amplitude expressed as a percentage, which corresponds to the number of patients treated in whom positive color changes were evidenced, and the interpretation of the magnitude expressed as a number, which corresponds to the values of change towards tones less than those found as baseline data. It was concluded that the use 3 times a day for 4 weeks, both AlignerFresh and OrthoFoam resulted in positive changes, which lightened the teeth in different amplitudes and magnitudes. Likewise, positive but not quantified results were evidenced in the state of periodontal health, by decreasing the gingival inflammatory processes presented in some patients.
limitations	In this study, the "Hawthorne effect" was minimized by the presence of a spectrophotometer for color measurement, thus measuring the spectral reflection of light and converting it into chromatic values for color acquisition, as well as obtaining

	a baseline in order to evaluate the outcome of the treatment administered. The evaluation of the treatments with the different mouth rinses helps in making future clinical decisions and in the development of clearance evidence provided by the rinses under study.

5. Introduction

5.1 Background and rationale

It has been said that a smile is one of a person's most important interactive communication skills. (1) The ultimate goal of esthetic dentistry is to create a beautiful smile, with teeth of inherently pleasing proportions to one another and a pleasing dental arrangement in harmony with the patient's gingiva, lips, and face. In addition, the aesthetics of any restoration must consider the parameters of superficial shape, translucency, and color (2) (3). Now, patients and consumers demand not only a healthy mouth but also a perfect smile. In fact, it has been reported that in the UK 28% of adults are dissatisfied with the appearance of their teeth (4) and in the US that 34% of the adult population is dissatisfied with the color of their teeth. your current teeth. (2)

5.1.1 The phenomenon of tooth color

The color of natural teeth is the result of the combination of light reflected from the enamel surface and light scattered and reflected from the enamel and dentin (2) (3). The dentin is the main source of color and is modified by the translucency and thickness of the enamel (4).

Visual color assessment depends on the observer's physiological and psychological responses to stimulation from reflected radiant energy. Some variables will affect color perception, such as external light condition, previous eye exposure, position of the illuminating object, and metamerism. Tooth color is determined by the combined effects of intrinsic and extrinsic colorations (5). Intrinsic tooth color is associated with the light-scattering and light-absorbing properties of enamel and dentin. (6) Extrinsic color is associated with the absorption of materials (eg, tea, red wine, coffee, chlorhexidine, iron salts) into the enamel surface and, in particular, into the pellicle coating, ultimately causing extrinsic stain.(7) Other uncontrolled factors, such as fatigue, aging and emotions, influence the interpretation of the color stimulus by the observer (1) (2) (3). Commercially manufactured shade guides are commonly used as the shade standard against which tooth color is matched (1)(2). Matching colors with available shade guides can often cause viewer frustration and lead to errors due to varying viewer interpretation and environmental influences. In addition, the shade guides do not represent the full range of natural tooth colors and, in general, the oldest available shade guides are not distributed systematically (4) (5) (6). Matching colors with available shade guides can often cause viewer frustration and lead to errors due to varying viewer interpretation and environmental influences.

Spectrophotometers and colorimeters have been used in an attempt to overcome visual matching problems in dentistry. Instrumental measurements can quantify color and allow communication to be more consistent and accurate. In addition, instrumental readings are objective and are obtained more quickly (5) (8) (11). Such devices provide measurements in CIE LAB units (Commission Internationale du'Eclairage color system L) which, when analyzed mathematically, you can compare the color parameters of different objects.

The phenomenon of color is a psychophysical response to the physical interaction of light energy with an object and the subjective experience of an individual observer. (8) Three factors can influence color perception,

namely, the light source, the object being viewed, and the observer viewing the object. The light source can emit radiant energy of a range of wavelengths and this is characterized by the relative amount of energy emitted at each wavelength in the visible spectrum. The light source illuminating an object affects color perception, as individual sources contain varying amounts of each of the visible wavelengths of light. The spectral reflectance or transmittance of an object characterizes the color composition of that object. The object's spectral reflection or transmission curve represents it graphically and provides a way to quantify color numerically. As objects vary in color, so do graphs representing absorbed or reflected energy. For example, a red object appears red primarily because it reflects red wavelengths more than green and blue. The visual system of the observer's eye and brain ultimately affects the overall perception of color. (9) (10)

5.1.2 Conditions of use of the equipment to take the shade In order to standardize the taking of the shade avoiding biases, it was decided to select the *VITA Easyshade V* equipment , which is a digital spectrophotometer for the precise, fast and reliable determination of dental shade in teeth. natural.

The correct use of the equipment is obtained from the Instruction Manual of the VITA Easyshade® V spectrophotometer and can be consulted in the protocol of this study, the characteristics of use of the equipment in this study are summarized.

- The equipment was adjusted in the function *Determination of the color of dental areas.*
  - This measurement made it possible to determine the color of the gingival, middle and incisal areas of a natural tooth. To do this, the icon for determining the shade of the dental zones was pressed in the main menu.The order of measurement was: gingival, middle, incisal.
- First fig. 3 (top left empty circle tooth icon), the measuring tip was placed in the gingival area of the tooth and the measuring button was pressed. If the measurement process was successful, two tones sound in succession and a filled circle appears on the screen.

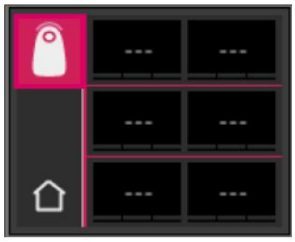


Figure 3. Function *Determination of the color of dental areas*

- Next, the middle and incisal areas of the selected tooth were measured.
- If an error occurs during the measurement, the device emits a long acoustic signal and an “X” appears over the corresponding tooth area on the screen. Therefore, the measurement of the area had to be repeated until it was correct.

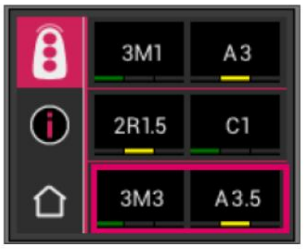


Figure 4. Measurement result, once all the areas have been correctly measured

- The result of the measurement, once all the zones have been correctly measured, shows the values in theVITA SYSTEM 3D-MASTER and VITA classical A1–D4 shade systems. Fig.4
- For the numerical quantification of the VITA classical A1–D4 color scale, the classification validated by Renata Pedrosa Guimarães in her article Vital Teeth Whitening (7) and used by several authors who also use it, was used. (8) (9) (10) which is presented below in figure 5.

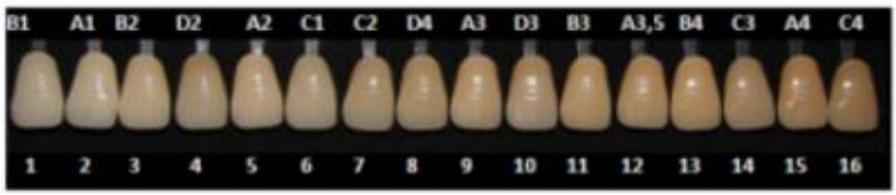


Figure 5: Numerical conversion of the Classic Vita™ Scale.

The values obtained with the spectrophotometer, based on the Classic Vita™ Scale, were converted into a numerical scale (8) (9) (10) (Figure 5) in order to facilitate statistical analysis of the data.

For the inferential statistical analysis, the initial records were considered and compared with those taken after each week of treatment, in order to assess the amplitude and magnitude of the clearance.

For the analysis of the amplitude of the whitening effect, the averages of the individual differences per tooth (incisor, canine and premolar), per area evaluated (gingival, middle and incisal), for each patient undergoing treatment were considered. For the magnitude, the values of change towards shades lower than those found as basal data before treatment, quantified per tooth, per area evaluated and for each patient undergoing treatment, were used.

The interpretation of the amplitude expressed as a percentage corresponds to the number of patients treated in whom positive color changes were evidenced.The interpretation of the magnitude expressed in number corresponds to the values of change towards lower tones than those found as basal data.

5.1.3 Rinses for dental whitening

Although the mechanism of action of tooth whitening remains questionable, it begins when hydrogen peroxide gel comes into contact with tooth structure. After that, a rearrangement occurs.

molecular structure of the tooth by a redox reaction. The free radicals in the gel break long carbon chains of double bonds into short chains of single bonds. Thus, the chains, which previously had a great capacity to absorb light, are now reflective and make the tooth visually lighter (11) (12) (13)

Dental applications of peroxide date back more than a century, including prominent early use in antisepsis for wound healing or treatment of periodontal infections. (14) Since the 1980s, the chemistry of oxidative peroxide has been increasingly used for its potential to lighten teeth. Many techniques have been developed for in-office and at-home treatment that involve short-term, repeated application of a peroxide-based gel. (15) In general, a strip is used to extend the contact of the peroxide with the dental surfaces, due to the relationship between the peroxide concentration, the treatment time and the clinical response. (16)

For whitening, a meta-analysis (17), involving 25 clinical trials with prespecified treatments, endpoints, and times, reported that home whitening was safe and effective, with major adverse events limited to tooth sensitivity and irritation. oral.

5.1.3.1 Antibacterial properties of rinse rinses

The antibacterial properties of peroxides are well documented. Bacterial growth is markedly inhibited under peroxide stress (18) through hydroxyl radicals reacting with bacterial membrane lipids and DNA, resulting in bacterial cell death. This has been demonstrated with both hydrogen peroxide-based and carbamide peroxide-based lightening agents; in one investigation, increasing the size of the zone of inhibition of bacterial growth tended to be associated with increasing hydrogen peroxide concentrations of the whitening agents tested (19). Other authors have shown, using the zone of inhibition test, that three lightening agents had a greater antibacterial effect than that of chlorhexidine (18). Furthermore, in another study, artificially demineralized fissures (simulating carious lesions) inoculated with lactobacillus and then treated with peroxides for two hours did not show further lactobacillus growth when plated. The authors of this study concluded that the peroxide penetrated the carious fissures and killed the lactobacillus. (20). The ammonia resulting from peroxide degradation plays an important role in modifying salivary and plaque pH. In the 1960s, researchers demonstrated that applying urea solutions to plaque caused an initial rapid rise in pH followed by a slow fall (21). The increase in the pH of the plate was related to the concentration of urea. More recently, peroxide applied using a custom tray resulted in a significant increase in salivary pH after five minutes of use, despite the fact that the peroxide products tested had an acidic pH (4.8 to 5.2) (22). Salivary pH remained elevated above baseline during the two hours of cuvette use. The buffering effect of peroxide in custom cuvettes extends to the pH of the plate; Measurements of plate pH during two hours of peroxide application by means of a custom cuvette showed that final mean plate pH was significantly higher than reference plate pH levels (23).

The use of mouth rinses at a concentration of 1% or nasal rinses at 0.5% hydrogen peroxide demonstrated significant clinical evidence of the protective effect of hydrogen peroxide against COVID-19, and considering

which is also quite safe, cheap and easily available, makes it a valuable prophylaxis recommended for the protection of health workers, hospitalized patients and all vulnerable groups against COVID-19. (24)

5.1.3.2 Effects of whitening rinses on plaque, caries, and gingival health

Pure hydrogen peroxide is a bright blue liquid that becomes colorless in solution with water. This solution is odorless and tasteless and its use in a 3% concentration is suggested for its antiseptic properties as well as its favorable effects on hemostasis (25). Hydrogen peroxide irrigation has also been shown to be effective in shortening the healing time of surgically manipulated tissues (25). The results of the comparative test of two groups controlled with 3% hydrogen peroxide carried out by Dr. Maghsoudipour's group (26) showed that edema and inflammation were significantly less when irrigation was performed with hydrogen peroxide.

5.1.3.3 Safety and side effects of whitening rinses

Studies of the efficacy and safety of 6% hydrogen peroxide whitening strips showed that oral irritation and tooth sensitivity were the most common adverse events, reporting that the Oral irritation was reported by 22% on at least one occasion during the 14-day treatment period with a 95% confidence interval of (15.3%, 29.1%). Tooth sensitivity was nearly as common, reported by 20% of subjects with a 95% confidence interval of (14.1%, 27.7%). The occurrence per study was reasonably consistent, ranging from 10% to 28% for tooth sensitivity and 4% to 31% for oral irritation. Adverse events were mainly symptomatic in nature without presenting clinical signs and of mild severity (84%). There were no severe or serious adverse events among any of the subjects assigned to the 6% hydrogen peroxide strips. In virtually all circumstances, the adverse events were transient in duration. Onset was typically early and resolved during treatment without affecting use of treatment. The clinical examination was normal and other side effects were infrequent (27).

A recent review of 25 clinical trials that included the use of peroxides for 14 days to whiten teeth found that tooth sensitivity and oral irritation represent the most common adverse events associated with treatment (28). Such events are reported to generally resolve during or shortly after treatment, often without any special intervention.

Lightening agents that use or generate high levels of hydrogen peroxide or organic peroxides can cause localized oral toxicity after sustained exposure if mishandled. Potential health concerns related to long-term use of hydrogen peroxide have been raised, based on animal studies. From a single study using the hamster cheek pouch model, 30% hydrogen peroxide was reported as a cocarcinogen in the oral mucosa. (29). This study and subsequent studies have shown that at 3% or less, no cocarcinogenic activity or adverse effects were observed in the hamster cheek pouch after prolonged exposure to hydrogen peroxide (30) (31) (32) (33). In patients, prolonged use of hydrogen peroxide decreased plaque and gingivitis rates. However, the therapeutic delivery of hydrogen peroxide to prevent periodontal disease required mechanical access to subgingival pockets. Furthermore, wound healing after gingival surgery was improved due to the antimicrobial effects of the administered hydrogen peroxide.

topically. For most subjects, beneficial effects were observed with hydrogen peroxide levels greater than 1%. (21) Most safety reports, even for tooth sensitivity and oral irritation, are limited to single occurrence rates, sometimes using indirect comparisons. To date, there are few rigorous statistical comparisons, or placebo-controlled trials, in which causation can be directly inferred. (3. 4). Results from a 12-month study demonstrated that the peroxide strip was remarkably well-tolerated. Adverse event occurrence rates were low in absolute terms in the peroxide strips group. Tooth sensitivity represented the most common finding, with 10% of subjects in the peroxide strip group reporting at least one event over 12 months. There were no reports of oral irritation in the peroxide strips group at any time during the study. In the placebo strip group, 5% of subjects reported tooth sensitivity and 3% reported oral irritation. Similar results were reported for both groups with regard to examiner-observed oral irritation. The groups did not differ significantly ($p > 0.67$) in terms of the occurrence of tooth sensitivity or oral irritation. (34)

The results of this 12-month continuous use study are consistent with previous clinical trials with 6% hydrogen peroxide strips. In those studies, in which 6% hydrogen peroxide whitening strips were used daily for a few weeks, safety results were limited primarily to mild and transient tooth sensitivity and oral irritation, which generally resolved during treatment (35) (36). Post-treatment follow-up for periods of up to 18 months has not shown any latent or persistent adverse events after complete use of the strip (37).

5.1.3.4 Color changes with the use of lightening rinses

No significant difference in color change was found for hydrogen peroxide at high concentrations versus medium concentrations, however, a significant difference was found between hydrogen peroxide at low vs high concentration, although this can be seen as a controversial finding, it can be taken into account that color assessment is more sensitive for detecting subtle and small changes (38) (39) than subjective color instruments such as color guides. colors. Color evaluation with spectrophotometry or chromameter is more accurate (40) (41) .

Tooth color varied in the age-matched sample significantly ($p < 0.0001$) relative to baseline b and L tooth color. This relationship was direct for and indirect for L*, meaning that at baseline, older subjects had less bright and yellower tooth color as measured by annualized digital image analysis, this represented approximately +0.05 by ÿ0.11 L of change per year.(27). This clearance was evident after 1 week of use, and response improved significantly over 1 to 2 * weeks. This absolute efficacy is perhaps one of the less remarkable findings, as several other studies at independent sites have shown similar clearance. (42) (43) (44)

5.2 Study Drug

5.2.1 Structure and use of the product

Both AlignerFresh Original Clean and its derivatives, such as OrthoFoam, are products that contain the same ingredients: Water, hydrogen peroxide, glycerin, PVP, PEG, sodium lauryl sulfate, sucralose, sodium citrate, sodium benzoate, etidronic acid, mint arvensis oil. (45) and that only differs by

trade names AlignerFresh focused on aligners and OrthoFoam focused on brackets and are registered with Invima as a cosmetic product under the identification code of the Sanitary Notification Mandatory (NSO) NSOC12166-22CO assigned file number: 20222349 filed: 20221028170 date: 03/2022/07 valid until 03/2029/16 and (NSO) NSOC12001-22CO assigned file number: 20222785 filed: 20221029927 date : 2022/ 03/11 valid until 2029/03/16

Ingrediente activo

Nombre químico	Número CAS	Concentración
Peróxido de hidrógeno	7722-84-1	3.7875%

Everbrands AlignerFresh is an oral care breakthrough for cleaning clear aligners and clear retainers. AlignerFresh is worn throughout the day and works by using hydrogen peroxide to not only clean and kill odor-causing bacteria, but also to brighten teeth under and over the appliance. It is ideal for patients with alignment problems due to wearing aligners 22 hours a day who also want whiter teeth. Patients who undergo orthodontic treatment with clear aligners change their trays every one to two weeks. As soon as you start wearing your new aligners, bacteria begin to thrive in the low saliva environment of your aligners, producing odors and potentially causing cavities. These bacteria also cloud the aligners and make them appear yellow and dirty. Also, aligner attachments or buttons can stain over time, making your teeth look yellow and stained. The above applies not only to aligners but also to clear retainers. And since retainers are typically replaced every six months, the amount of bacteria or pigmentation on them can become overwhelming. AlignerFresh's proprietary formula - low dose hydrogen peroxide kills the bacteria that cause bad breath, cavities and brightens teeth in the process. Tests based on the international test method ASTM E1153 demonstrate the effectiveness of the formula in killing 99.9% of test microorganisms, including bacteria from the oral cavity *Staphylococcus aureus 6538, Escherichia coli *8739 , Streptococcus* pneumoniae *49619 , Streptococcus mutans* 25175. Because a low dose of hydrogen peroxide is used, there are virtually no problems with tooth sensitivity or oral irritation and it is recommended to wear comfortably throughout the day. Recommended use is to apply 1/2 to a full dose of Everbrands AlignerFresh to the upper and lower aligners and spread evenly with your finger to cover all aligner areas. Replace the aligners in the mouth and spit out the excess foam. Use as directed by your dentist or orthodontist.

Everbrands® OrthoFoam is a patent-pending cleaning formula designed to clean and brighten the teeth of adults and children undergoing orthodontic treatment with metal and ceramic appliances.
With a simple brushing formula, you can be sure that your bracket appliances will be clean on all sides, over, around and even under metal and ceramic brackets. This whitener is safe for braces and can be used as an extra brushing booster, foaming rinse, or teeth cleaner and whitener. OrthoFoam's bubbles reach rings and brackets where toothbrush bristles can't. After a week of consistent use, you can see a reduction in plaque buildup and better gum health, as the peroxide-based formula of hydrogen clears under brackets so tooth whitening can begin during orthodontic treatment, the use of OrthoFoam kills 99.999% of bacteria, which are killed within 60 seconds, preventing

decalcification lesions and white spots. OrthoFoam is safe and works on all forms of brackets, wires and accessories without damaging them. However, if you have colored elastics, they may suffer some discoloration.

5.2.2 Warnings, contraindications and precautions for use

If irritation, redness, swelling or pain of the gums or mouth occurs, discontinue use and consult your dentist. Adult supervision is required if the product was used by children under 12 years of age. Avoid contact with eyes, skin or hair. If contact occurs, rinse area with water. Avoid ingesting large amounts of the product and if you do, it is not necessary to induce vomiting, hydrogen peroxide concentrations are low. Do not apply directly to the gums or under the tongue. Do not use if you are lactating or pregnant. Keep the product away from sunlight and heat as its contents could become pressurized. Prolonged use may require supervision by a dentist.

5.3 Objective

To quantify the amplitude and magnitude of the lightening effect after 3 times daily use of the investigational product applied for 4 weeks of treatment.

5.3.1 Secondary objectives

To evaluate the use of the investigational product (ie, AlignerFresh) for use in aligners and (ie, OrthoFoam) for use in Brackets.

- •Evaluate organoleptic satisfaction when using the research product.
- •Ealuate the sensation of cleanliness when using the research product
- • Assess dental sensitivity from the use of the investigational product.
- • Evaluate the sensation of oral irritation when using the investigational product.

6. Treatments Administered and Study Design.

The medication administered to the patients was listed and stored in office 306 according to the Instructions: Management of Medications in the Dental Office (Table 1.)

Laboratory	Formulation	Form pharmaceutical	Batch	Expiration
EverSmile	WhiteFoam® Equals AlignerFresh®.	foam dispenser bottle	23011601	2025-01-1
EverSmile	OrthoFoam®	foam dispenser bottle	23011601	2025-01-1

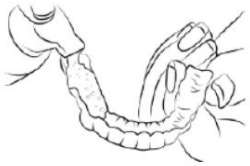
Table 1. Study drugs.

WhiteFoam® o **AlignerFresh®**

• **Figure 1 Bottle of WhiteFoam® Same as AlignerFresh® for Use in Aligners or Retainers**

Use of the drug in patients undergoing orthodontic treatment with aligners

- Patients undergoing orthodontic treatment with aligners wore the aligners for at least 22 Daily hours.
- The frequency of brushing was 3 times a day and the duration was 1 minute of brushing as Minimum.
- During each brushing they used Colgate toothpaste (Provided by the orthodontist) only in the mouth and in no way on the aligner.
- The assigned lightener was AlignerFresh which was used in 3 steps as follows:
  - Step 1: 1 pump-filled foam was dispensed into the upper aligner,
  - Step 2: Spread evenly with finger or mouthpiece of the bottle
  - Step 3: The aligner was placed in the mouth and excess foam was spit out . The foam dispersed with the natural flow of saliva, it was not necessary to rinse the mouth.



Step 1.

Step 2

Step 3

- The use of these elements was recorded in the form designed to monitor the use of the treatment rinse.

OrthoFoam®

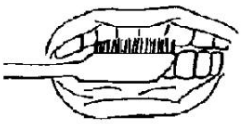
- **Figure 2 OrthoFoam® Bottle for Orthodontic Use with Brackets**

Use of the drug in patients undergoing orthodontic treatment with brackets

- The frequency of brushing was 3 times a day.

- During each brushing they used Colgate toothpaste (Provided by orthodontist) in their mouth for 30 seconds.
- The whitener assigned was OrthoFoam which was used in 2 steps as follows:
  - Step 1: 1 pump full of foam was dispensed onto the toothbrush,
  - Step 2: The upper arch was brushed normally for 1 minute. the foam dispersed with the natural flow of saliva, it was not necessary to rinse the mouth.

Step 1.



Step 2

- Record the use of these elements in the form designed to track the use of the treatment.

Compliance monitoring

Since the patient was responsible for following the indications for the use of the hygiene elements, the patient was required to fill out the use forms, in order to verify the correct use of the study product, additionally the patient had to attend appointments to evaluate their evolution and deliver the completed forms and the information was recorded in the system enabled for this study.

6.1 Study Patients.

The study was carried out in Bogotá, Cundinamarca (Colombia) with the population of the capital district of Bogotá and neighboring communities, in the private office of Dr. Clara Marcela Collazos, who has specially conditioned areas for carrying out orthodontic treatments with aligners (Invisalign certification) or with brackets for the patients in his private practice, a *VITA Easyshade V* digital spectrophotometer was used for standardized color measurement, as well as conditions for documenting the results.

The patients who participated in the study signed an informed consent or informed assent form (Annex 2), clarifying that they were aware of the characteristics of the study product, its use, and its possible side effects; the patients have not participated in similar studies within the previous four months and have not received any medical treatment during the 15 days prior to the study.

The clinical phase of the study began on February 27, 2023 with the first patient and closed on April 18 with the closing of the clinical history of the last patient who completed the study.

6.2 Design of the trial

Open study of two treatment groups (use of aligners or use of brackets), controlled (ie, active control), and a single center. The study had a 4-week follow-up (ie, 30 days) and compared the initial color of 3 healthy upper arch teeth and the color changes during the 4 weeks following use.

of the research product (ie, AlignerFresh) for use in aligners and (ie, OrthoFoam) for use in Brackets

6.3 Interventions

Eligible patients according to the inclusion and exclusion criteria were invited to participate. Inclusion criteria

1. Male or female patients
2. Patients between the ages of 12 and 55
3. Patientswho use aligners or brackets during their orthodontic treatment
4. Patients who have signed the informed consent or assent signed by one of the parents of the minor

Exclusion criteria

1. Patients with less than 75% healthy teeth
2. Patients with previously diagnosed periodontal disease and under treatment
3. Patients with active caries or under treatment by a professional
4. Pregnant or lactating women.
5. Patients with physical disabilities in the upper limbs that impede their oral hygiene.
6. Patients who smoke two or more cigarettes a day.
7. Patients who chronically use medications that can cause changes in tooth color. (tetracycline, doxycycline, chlorhexidine, antihistamines, antipsychotics or antidepressants and antihypertensives)
8. Patients with allergies to any of the components of the treatments studied.
9. Patients with terminal illnesses undergoing treatment.
10. Patients who drink coffee, tea or red wine more than 2 times a week and do not stop during the study.
11. Patients who do not comply with the proper use of hygiene elements and the established periodicity

The benefits and possible risks of the study were explained to the patient who met the eligibility criteria, and informed consent or assent authorized by the ethics committee was obtained.

At the beginning of the study, the patients were divided into 2 groups according to the orthodontic treatment they were undergoing, orthodontic treatment with aligners or orthodontic treatment with brackets. The two groups received oral health recommendations and proper use of oral hygiene items (Toothbrush + Colgate toothpaste (Delivered by the orthodontist) + assigned whitener) Instructions for patients.

Visit 0: (Day 0) Screening visit, informed consent or assent was taken, assigned to the corresponding orthodontic treatment group, oral health recommendations and proper use of oral hygiene items were given. 3 healthy teeth from the upper arch without restorations (an incisor, a canine, and a premolar) were selected, to which the color of the gingival area, middle area, and incisal area were recorded, a photo was taken, and hygiene items were delivered. oral and registration forms.

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Visit 1: (Day 10) Follow-up visit, where the professional recorded the color of the gingival area, the middle zone and the incisal area, a photo was taken of the 3 selected teeth of the patient's upper arch, after 10 to 12 days of additionally, the professional collected the forms for the use of oral hygiene items filled out by the patient and delivered the registration forms for the following week of treatment, the professional filled out the information electronically in the system set up for this study.

Visit 2: (Day 20) Follow-up visit, where the professional recorded the color of the gingival area, the middle zone and the incisal area, a photo was taken of the 3 selected teeth of the patient's upper arch, after 20 to 22 days of additionally, the professional collected the forms for the use of oral hygiene items filled out by the patient and delivered the registration forms for the following week of treatment, the professional filled out the information electronically in the system set up for this study.

Visit 3: (Day 30) Follow-up visit, where the professional recorded the color of the gingival area, the middle zone and the incisal area, a photo was taken of the 3 selected teeth of the patient's upper arch, after 30 to 32 days of additionally, the professional collected the forms for the use of oral hygiene items filled out by the patient and through the form evaluated the patient's perception of the assigned product and closed the CRF of the study, the professional filled out the information electronically in the system enabled for this study.

6.4 Determination of the color of dental areas.

Before taking a measurement:

• Before starting the shade measurement procedure, the tooth was cleaned of any adhesion, plaque, pigmentation or calculus that could hinder the appreciation of the shade, using prophylactic paste and a rotating brush for 10 seconds.

• The items that could cause bias due to their intense color were removed, such as lipstick on women, and if applicable, abundant and dark mustaches on men.

•The surface of the tooth to be measured was dried with a wad of cotton to prevent the measuring tip from will slip

•It was ensured that it was really a natural tooth, the filling and restoration materials would generate changes in the measurement result.

• The color was taken according to the conditions of use of the equipment.

The office conditions were the same for all patients and were as follows:

• The office lights were fully on.

• The dental unit lamp was not used.

• The blinds on the windows were completely closed.

• The wall behind the patient's head was white.

• The patient sat in the dental chair which is at an angle of 45 degrees supporting the head to keep it in a stable position.

• It was ensured that the measuring tip was in contact with the tooth surface.

• With the measuring tip resting on the tooth, the measuring button was pressed.

•The measuring tip was kept resting against the tooth until two consecutive tones sounded, indicating the completion of the measurement process.

• The information from the measurements made was transferred to the patient's CRF for statistical evaluation.

The patients underwent a complete clinical evaluation of their state of health, which included the medical history.

7. Outcomes

7.1 Primary

•In order to standardize the color measurement avoiding biases, the *VITA* Easyshade® *V* digital spectrophotometer was used , which was adjusted in the function Determination of the color of dental areas, this measurement allowed to determine the color of the areas gingival, middle and incisal area of a natural tooth, the measurement result shows the values in the VITA SYSTEM 3D-MASTER and VITA classical A1–D4 shade systems.

• The values obtained with the *VITA* Easyshade® V digital spectrophotometer, based on the Classic Vita™ Scale, were converted into a numerical scale (8) (9) (10) (Figure 5) in order to facilitate the statistical analysis of the results. data.

•For the numerical conversion of the VITA classical A1–D4 color scale, the classification validated by Renata Pedrosa Guimarães in her article vital teeth whitening (7) also used by several authors, was used. (8) (9) (10) which is presented below in figure 3.

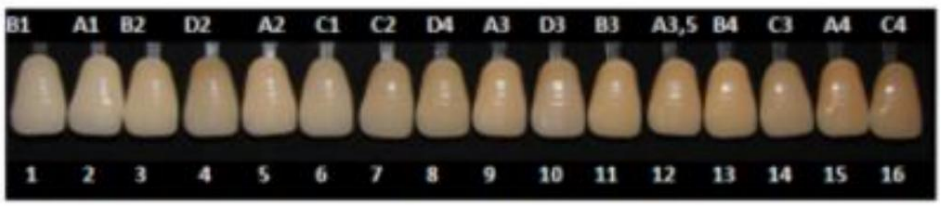


Figure 3: Numerical conversion of the Classic Vita™ Scale.

For the inferential statistical analysis, the initial records were considered and compared with those taken after each week of treatment, in order to assess the amplitude and magnitude of the clearance.

For the analysis of the amplitude of the whitening effect, the averages of the individual differences per tooth (incisor, canine and premolar), per area evaluated (gingival, middle and incisal), for each patient undergoing treatment were considered. For the magnitude, the values of change towards values lower than those found as baseline data before treatment, quantified per tooth, per area evaluated and for each patient undergoing treatment, were used.

7.2 Secondary

• Organoleptic satisfaction when using the research product.

•The clean feeling when using the research product.

• Dental sensitivity from the use of the research product. • The feeling of oral irritation when using the investigational product.

8. Monitoring

8.1 Data monitoring

The development of this study implies the use of non-invasive rinses whose information was completed in physical CRFs and digitized in databases, the data management was carried out by the main researcher, orthodontist and his auxiliary office team, therefore it was not established a data monitoring committee, collection and digitization, guaranteed full independence from the sponsor.

The data was treated confidentially and their identity in this study was suppressed in compliance with Colombian Habeas Data regulations, as determined by Law 1581 of October 17, 2012 (47) and Decree 1377 of June 27, 2013. , (48) and the regulations that modify or replace them.

Data management was carried out through an electronic platform for data capture and the forms filled out by the patients were entered by the orthodontist.

All information collected in the database was analyzed to ensure its integrity and consistency. Contingency tables were considered when describing clinical characteristics related to demographic variables.

8.2 Adverse events

The procedures performed are non-invasive treatment interventions with a good safety profile, as well as routinely applied in regular dental practice and considered in daily oral health practices. Because of this, no additional or superior risks to the discomfort associated with these tests during usual oral hygiene were anticipated.

Research staff inquired for possible local reactions and inquired about any systemic symptoms. The information was recorded by the orthodontist who took the necessary measures to guarantee the patient's safety in accordance with his expert criteria and initiated the report of adverse events in accordance with current regulations.

All adverse reactions, incidents or adverse events, serious or not serious, were reported in the terms established in Resolution 4816 of 2008. (46).

The reports of adverse events and incidents were made virtually from the web application of the INVIMA page with the username and password registered by the study orthodontist. (https://farmacoweb.invima.gov.co/TecnoVigilancia/faces/index.xhtml).

9. Allocation of interventions

The allocation was made as follows:

- Orthodontic treatment with aligners, whitening research product assigned AlignerFresh
- Orthodontic treatment with brackets, whitening research product assigned OrthoFoam

All the information collected was analyzed to assess the amplitude and magnitude of whitening achieved during treatment with the assigned product according to their orthodontic treatment.

Additionally, the perception of the patients was evaluated, quantifying their organoleptic satisfaction, the sensation of cleanliness, whether dental sensitivity or irritation of the mucous membranes occurred during the treatment of the patient with the use of the research product.

9.1 Sequence generation assignment Not applicable.

9.2 Mechanism to hide the assignment Not applicable.

9.3 Masking or blinding Not applicable.

10. Sample size

To determine the sample size, a confidence level of 95%, a power of 80% (ÿ = 20%) and a loss rate of 5% were considered. Using the formula to compare the means in the color variable (quantitative variable) using the Vita™ Classic Scale guide, bilateral contrast and finally detect an expected effect of 30% in the average color values, by means of the equation it is needed include a minimum of 40 subjects in 2 groups of 20 subjects for the aligners group and 20 subjects for the Brackets group.

11. Quantitative variables

11.1 Primary variables:

Color change using the VITA classical A1–D4 guide. In terms of breadth and magnitude.

11.2 Secondary variables:

The organoleptic perception, cleanliness, dental sensitivity (pain), the sensation of irritation, and the presence of adverse events are quantified.

11.3Factor variables:

Reported and observed oral irritation

12. Statistical methods:

The descriptive data were analyzed by gender (female, male), by age groups (12-19, 20-29, 30-39, 40.49 and over 50 years) in the 3 selected teeth of the upper arch. (Incisor, canine and premolar) and by the 3 areas of each tooth (gingival, medial and incisal areas) of patients being treated with aligners and in patients being treated with brackets.

For descriptive statistical analysis, quantitative variables were summarized using measures of mean trend and dispersion (means, standard deviation, ranges).

The qualitative variables, both nominal and ordinal, were described through absolute and relative frequencies and in a total cumulative manner. The collected data will be summarized in tables and figures for descriptive and inferential analysis, as appropriate.

Baseline data and through time will be evaluated for differences in ÿE before and after the assigned treatment, in addition to the evaluation of the effectiveness of the clearance, as well as variability analysis and average data of the changes registered through the treatment time, for each one of the two treatments studied according to the orthodontic treatment in the study.

In addition, the effects of age and gender as a factor for 'tooth' were analyzed to account for multiple observations on each tooth to determine the amplitude and magnitude of whitening at 10, 20 and 30 days of treatment.

The evaluation of oral sensitivity and irritation was analyzed by the number and percentage of subjects with adverse effects (oral irritation and dental sensitivity) and the degree of response they registered.

The inclusion of patients was presented according to a CONSORT diagram.

12.1 Audits/ Inspections

The national or international health authorities, the Ethics Committee, the sponsor or its delegate may audit/inspect the study at any time during its execution to guarantee compliance with the protocol, processes, Good Clinical Practices and the accuracy of the data recorded in the CRFs. and ethics during its development.

Additionally, a data monitor will be appointed in order to audit the signing of the informed consents, the correct selection of the subjects, the correct transcription of the data, the correct development of the study schedule, the collection of adverse events and their adequate reporting. among other activities of the monitor.

13. Ethics and dissemination

13.1 Ethical approval of the research

This was a study with higher than minimum risk for volunteers as defined by Resolution No. 008430 of 1993 (47) and the risks associated with the use of oral rinses are described in section 7.1.5.1 and 7.4.2 of the protocol.

This study was carried out following the Good Clinical Practice (GCP) guidelines, the principles of the Declaration of Helsinki and national regulations. The protocol, consents and clinical settlements were reviewed and approved by the Clinical Research Ethics Committee (CEIC) of the Universidad de la Sabana before beginning the study. For the admission of patients to this study, the informed consent or assent of the patients was required.

Authorship of any publication resulting from this study will be determined based on the recommendations of the International Committee of Medical Journal Editors for the Conduct, Information, Editing, and Publication of Scholarly Work in Medical Journals.

13.2 Confidentiality

By signing the protocol, the researchers agreed to keep all information collected for this study strictly confidential and to request the same confidentiality from their staff and the independent Research Ethics Committee.

All study-related information will be stored securely at the study site. All data that is transferred to the sponsor or sample processing personnel is identified with a coded number to maintain the confidentiality of the participants. All local databases have access keys, which restricts the manipulation of the data. Only the study staff of the office or regulatory bodies when so required, may have access to the patient's identification data.

13.3 Access to data

The sponsor Everbrands is the owner of the information that was collected in this research study, the publications that arise from this study must be previously approved by the sponsor. The researcher has a confidentiality agreement with the sponsor in accordance with applicable laws and regulations, including but not limited to data privacy laws and regulations

14. Results

Verification of the following aspects was carried out:

- 100% of the duly signed informed consents or assents are found.
- The selection of the subjects meetst he inclusion criteria and there are no exclusion criteria that disable any of them.
- The data were transcribed from the primary source documents correctly.
- The protocol execution schedules were met as required.
- The follow-up, the collection of adverse events and their proper reporting were carried out in accordance with the protocol requirements.

The descriptive data were analyzed by gender (female, male), by age groups (12-19, 20-29, 30-39, 40.49 and over 50 years) in the 3 selected teeth of the upper arch (Incisor, canine and premolar) and by the 3 zones of each tooth (gingival, middle and incisal zones) of the patients treated with aligners and in patients treated with brackets.

There were no missing data during the study, as patients who withdrew were replaced until the study sample size was achieved.

43 patients who started the use of the research products were included, of which 3 patients did not complete the 30 days of treatment, 43 patients of which 22 patients correspond to the treatment group with aligners and 21 patients to the treatment group with Brackets, withdrawn patients are not included in the data analysis.

In the treatment group with aligners, 20 patients finished, since 2 patients in this group withdrew, 1 for reasons of time (He could not attend control every 10 days) and the other for an adverse reaction of sensitivity to the study product which in light of the Naranjo Algorithm to assess the causality of an adverse drug reaction and it was classified as probable, which is why the treatment was suspended.

In the treatment group with brackets, 20 patients finished, since 1 patient withdrew due to liver inflammation diagnosed by cancer control, the adverse reaction of sensitivity to the study product was evaluated in light of the Naranjo Algorithm to assess the causality of an adverse reaction. medication and was classified as doubtful, however the patient withdrew his consent, which is why the treatment was suspended.

In total, 40 volunteers finished.

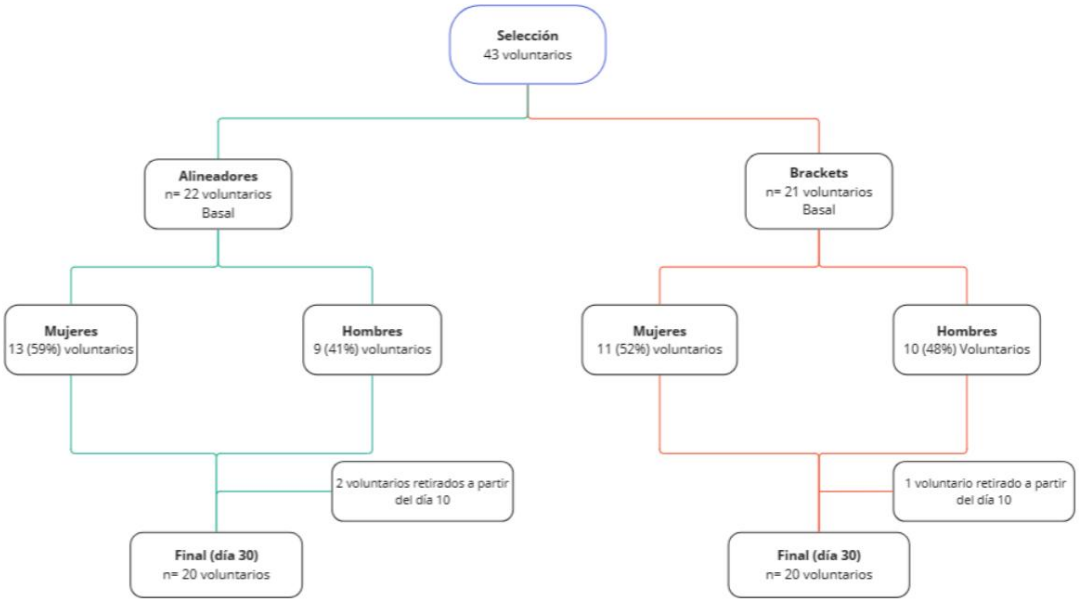


Figure 4. Diagram of the disposition of the volunteers throughout the study

The average age for the aligner group was older, 36 years, while for the bracket treatment group it was 27 years, which leads us to believe that aligner treatments, which are more aesthetic and more expensive, are paid for by young adults with purchasing power.

The aligners are more frequently used in adults (90%) of the patients studied and the use of orthodontics with brackets is more used by minors (<18 years) 40% of the patients in this study.

In the gender variable, the group of aligners 60% were women and 40% men and for the group of brackets 45% were women and 55% were men, which shows that there are no differences between the treatment groups. .

The classic demographic measurements of weight (Kg), height (Cm) and BMI (Kg/m2) are also presented, finding average values corresponding to the Colombian population, weight of 63 kg, height of 165 cm and BMI of 23 Kg/m2 as shown and extensive information in table 1 Demographic data and physical conditions of the volunteers

Characteristics	**groups**	
	Aligners n= 20 volunteers	**Brackets n= 20 volunteers**
Age Mean ± SD (Min MAX)	36.8 ± 13.2 (12-55)	27.3 ± 12.4 (12-55)
By age		

Under 18 years Over 18 years **n** (%)	2 (10%) 18 (90%)	8 (40%) 12 (60%)
Gender **Male n (%)** Female **n (%)**	8 (40%) 12 60%)	11 (55%) 9 (45%)
Weight (kg) Mean ± DE (Minimum-Maximum)	63.3 ± 11.8 (58-88)	64.7 ±14.1 (64.0-88.0)
Size (cm) Mean ± DE (Minimum-Maximum)	165.0 ± 7.6 (169-185)	167.2 ± 7.1 (178-181)
IMC (kg/m2) Mean ± DE] (Minimum-Maximum)	23.1 ± 2.8 (20-28)	23.1 ± 5.0 (20-34)

Table 2. Demographic data and physical conditions of the volunteers.

14.1 Orthodontic treatment with aligners, research product AlignerFresh 14.1.1 Analysis by gender AlignerFresh.

The measurements made allowed us to identify 3 teeth (incisors, canine and premolar) and additionally in each tooth 3 areas were measured (Gingival, middle area and incisal) for this reason a total of 9 measurements were taken in each patient, we followed up at 10 , 20 and 30 days in each patient where changes in tooth color can be identified.

By gender	**categories**	**Lightening results using AlignerFresh comparing men and women (Zones of the tooth)**						**Total**	
		Gingival		**Half**		**Incisal**			
		n	%	**n**	**%**	n	%	n	**%**
Women N=12 patients	No Change	14.0	38.9	2.0	5.6	5.0	13.9	21.0	19.4
	changes	22.0	61.1	34.0	94.4	31.0	86.1	87.0	80.6
Men N= 8 patients	No Change	7.0	29.2	6.0	25.0	0.0	0.0	13.0	18.1
	Changes	17.0	70 .8	18.0	75.0	24.0	100.0	59.0	81.9

Table 3. Whitening results using AlignerFresh comparing men and women (Zones of the tooth)

In women, a percentage of dental whitening of 61% in the gingival areas, 94% in the middle areas and 86% in the incisal edges was evidenced. The smallest changes were found in the gingival areas with 38%.

In men, the percentage of whitening was 70% in the gingival areas, 75% in the middle areas and 100% for the incisals, Figure 5, which shows these results, is presented below.

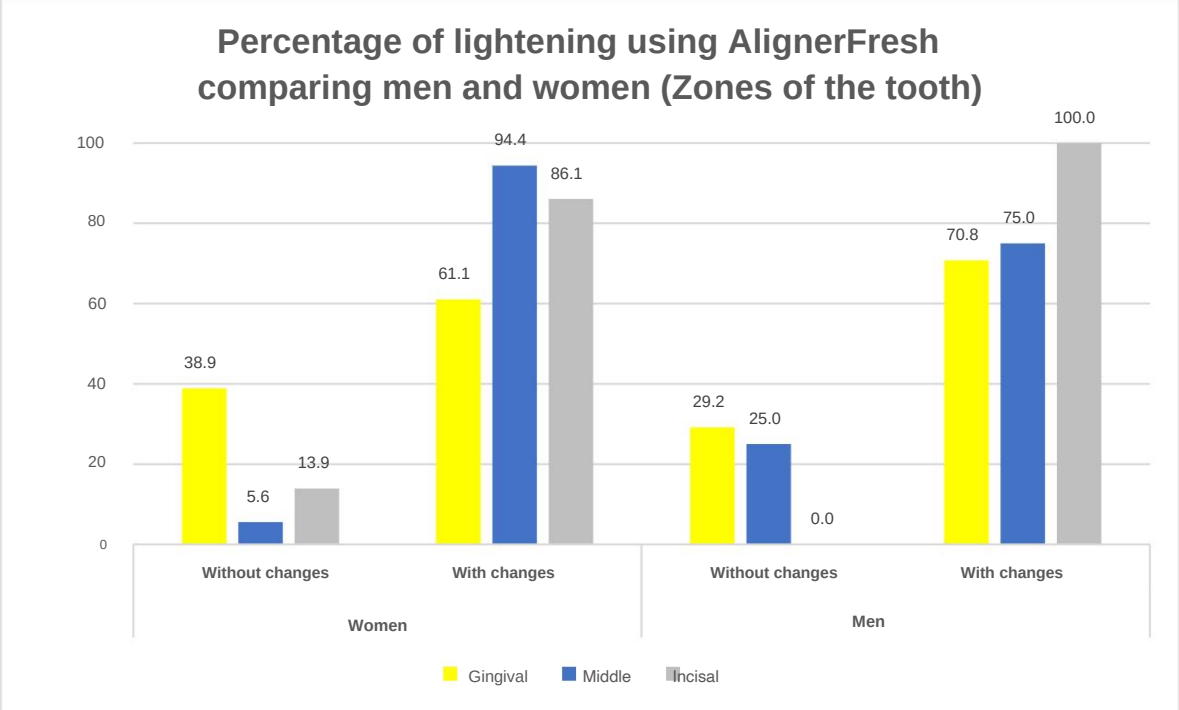


Figure 5. Percentage of lightening using AlignerFresh comparing men and women (Zones of the tooth)

The gingival areas in both women (61%) and men (70%) are the areas that show the least whitening, unlike the middle areas and incisal edges, which, as evidenced, show the best results in both genders. greater than 75%.

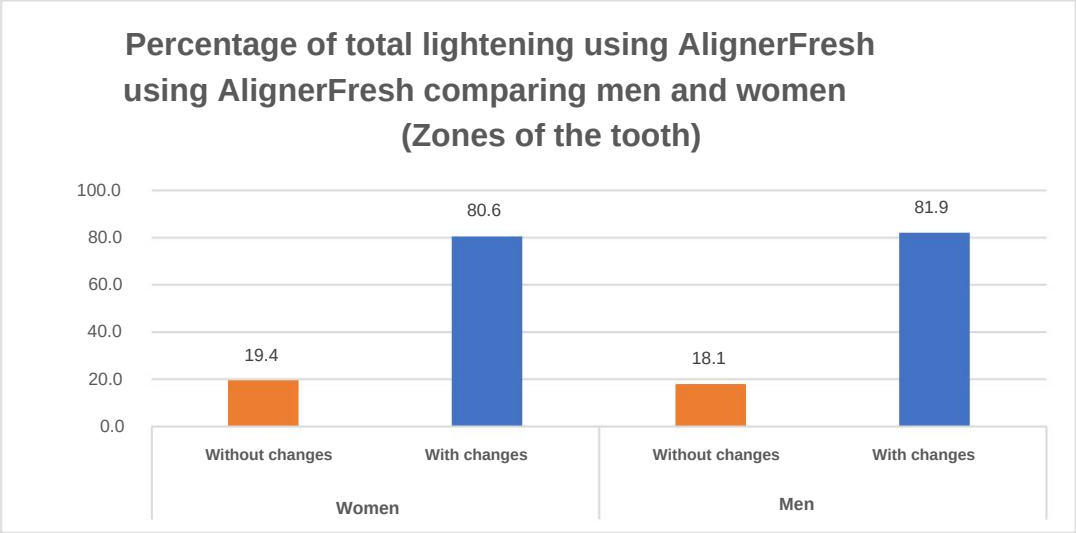


Figure 6. Percentage of total whitening using AlignerFresh comparing men and women (Zones of the tooth)

The percentage of dental whitening shown with AlignerFresh is totaled in figure 6, evidencing 80% for women and 81% for men, this is how AlignerFresh showed good results lightening the color after 4 weeks of treatment.

The magnitude of the changes is presented in Table 4 below.

Classification	n	Magnitude of clearance	% improvement
Women	12	3.5	51.8 (56/108)
Men	8	3.5	58.3 (42/72)

Table 4 Magnitude of lightening using AlignerFresh comparing gender.

By totaling the magnitude of the change, we can predict that the color change we expect after 4 weeks of treatment with the use of AlignerFresh is 3.5, the same for women as for men, that is, if we start with a color #10 or D3 scale. Vita™ Classic, after 4 weeks of treatment we would expect to end up with a color between #6 or C1 Vita™ Classic scale or #7 or C2 Vita™ Classic scale which appears to be a significant change for such a short period of treatment.

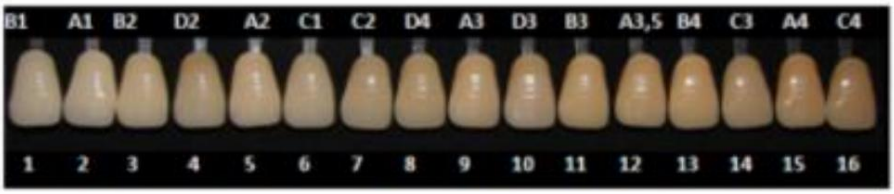


Figure 3: Numeric Conversion of the Classic Vita™ Scale

14.1.2 AlignerFresh Age Analysis.

The measurements made allowed us to identify the percentages of whitening in all ages, showing that the age groups between (12-19) improved by 88% and the groups between (20-29) improved by 87%.

by ages	categories	Lightening results using AlignerFresh comparing ages (Zones of the tooth)						Total	
		Gingival		Half		Incisal			
		n	%	n	%	n	%	n	%
12-19 years N=2 Patients	Without changes	1.0	16.7	1.0	16.7	0.0	0.0	2.0	11.1
	With changes	5.0	83.3	5.0	83.3	6.0	100.0	16.0	88.9
20-29 years No.=6 Patients	Without changes	5.0	27.8	2.0	11.1	0.0	0.0	7.0	13.0
	With changes	13.0	72.2	16.0	88.9	18.0	100.0	47.0	87.0
30-39 years No.=3 Patients	Without changes	2.0	22.2	2.0	22.2	2.0	22.2	6.0	22.2
	With changes	7.0	77.8	7.0	77.8	7.0	77.8	21.0	77.8
40-49 years No.=4 Patients	Without changes	7.0	58.3	1.0	8.3	0.0	0.0	8.0	22.2
	With changes	5.0	41.7	11.0	91.7	12.0	100.0	28.0	77.8
50-59 years No.=5 Patients	Without changes	6.0	40.0	5.0	33.3	0.0	0.0	11.0	24.4
	With changes	9.0	60.0	10.0	66.7	15.0	100.0	34.0	75.6

Table 5 Whitening results using AlignerFresh comparing ages (Zones of the tooth)

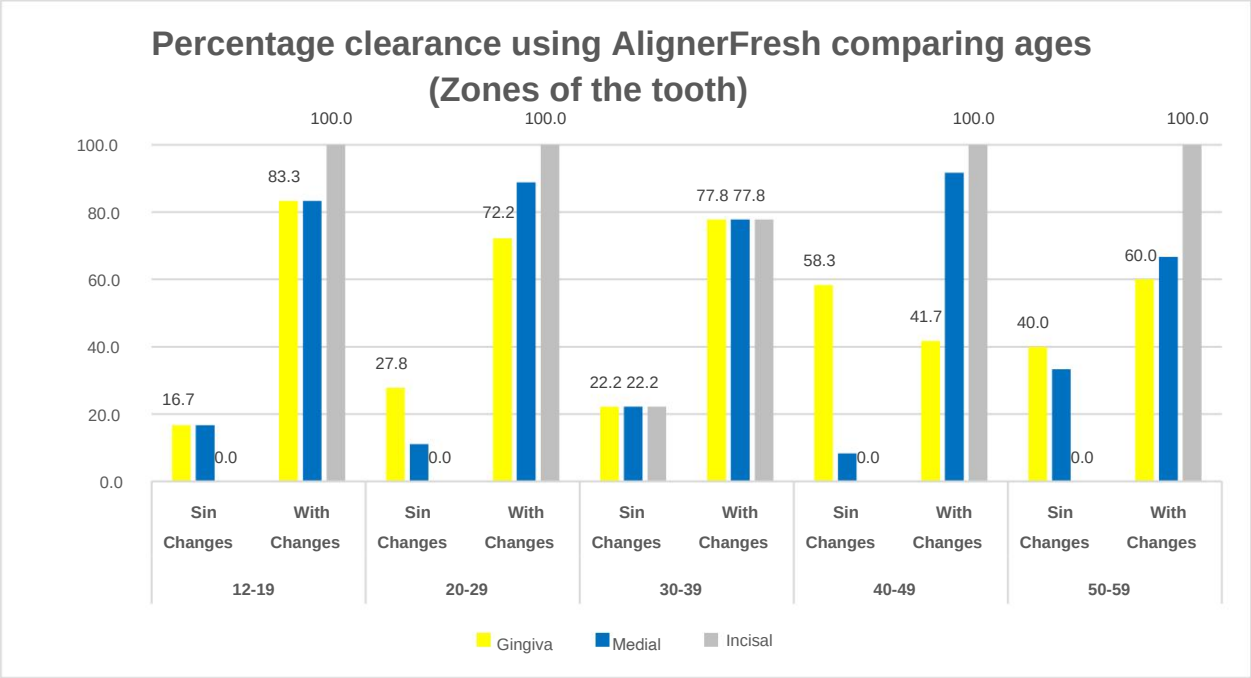


Figure 7 Percentage clearance using AlignerFresh comparing ages (Zones of the tooth)

The analysis of the different age groups showed that the greatest changes were obtained in the incisal edges of all age groups and again the lowest percentage of whitening was seen in the gingival areas with results of 41% in the 40-49 age group. years, however more than 60% of patients in all other age groups treated with AlignerFresh were shown to have positive color changes.

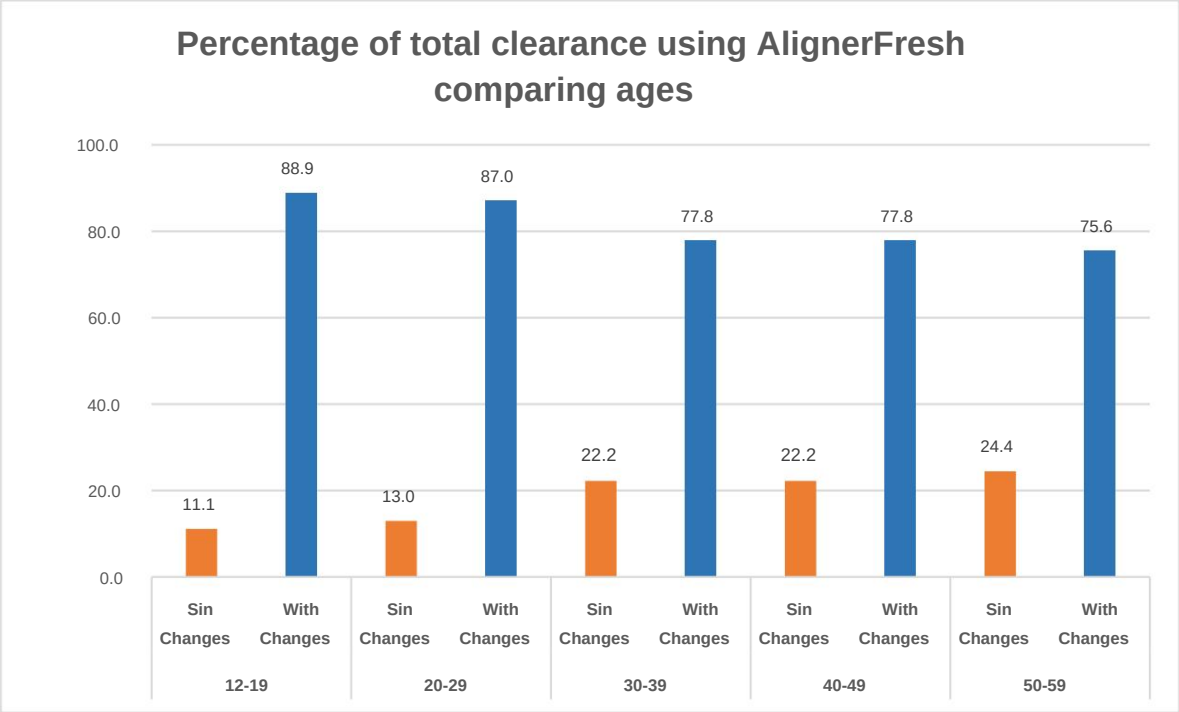


Figure 8. Percentage of total clearance using AlignerFresh comparing ages

The percentage of dental whitening shown with AlignerFresh is totaled in the previous figure, evidencing whitening greater than 87% in patients aged 12-19 and 20-29, the other age groups showed whitening greater than 75%, thus it was demonstrated that AlignerFresh showed good results lightening the color after 4 weeks of treatment.

The magnitude of the changes is presented in the following table.

By age	Magnitude of lightening using AlignerFresh comparing age groups.		
	N of patients	Average lightening	Percentage of possible whitening in studied areas
12-19 years	2	5.2	58.3% (42//72)
20-29 years	6	2.8	48.1% (26/54)
30-39 years	3	4.3	48.1% (13/27)
40-49 years	4	3.0	58.3% (21/36)
50-59 years	5	3.2	51.1% (23/45)
Total	20	3.7	52.8%

Table 6. Magnitude of lightening using AlignerFresh comparing age groups

By totaling the magnitude of the change, we can predict that the color change we expect after 4 weeks of treatment with the use of AlignerFresh is 3.7 on average for all ages, the magnitude varied between 2.8 and 5.2, that is, if we start with a shade #10 or D3 Vita™ scale, after 4 weeks of treatment we would expect to end up with shade #6 or C1 Vita™ scale which appears to be a significant change for such a short period of treatment. The greatest change was seen in the youngest group of 12-19 years old where the expected change if we start with a #10 or D3 Vita™ scale color, would end with a #5 or A2 Vita™ scale color.

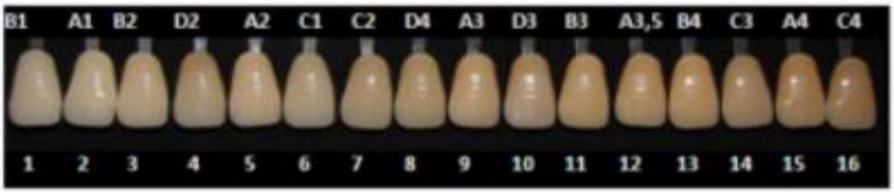


Figure 3: Numeric Conversion of the Classic Vita™ Scale

14.1.3 Analysis per tooth AlignerFresh.

The measurements made allowed us to identify that the use of AlignerFresh resulted in whitening in all the teeth studied, showing that the best results in the incisors were 91% and the teeth that presented the least change were the canines in 73% of the treated patients.

Consolidated 3 teeth per area	categories	Percentage clearance using AlignerFresh comparing 3 teeth and Tooth Zones						
		Gingival		Half		Incisal		TOTAL
		n	%	n	%	n	%	Average
incisors	Without changes	5	25	0	0	0	0	8.3
	With changes	15	75	20	100	20	100	**91.7**
Canine	Without changes	8	40	7	35	1	5	26.7
	With changes	12	60	13	65	19	95	**73.3**
premolars	Without changes	8	40	4	20	1	5	21.7
	With changes	12	60	16	80	19	95	**78.3**

Total	Without changes	21	35	11	18.3	2	3.3	**18.9**
	With changes	39	**65**	49	**81.7**	58	**96.7**	**81.1**

Table 7. Percentage clearance using AlignerFresh comparing 3 teeth and Tooth Zones

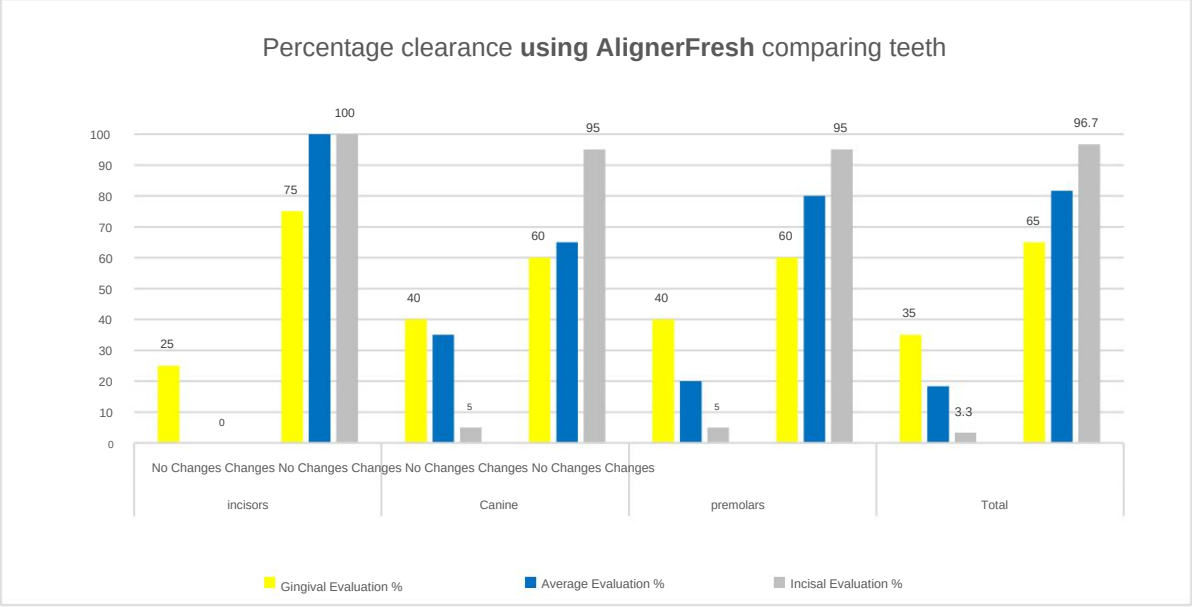


Figure 9. Percentage clearance using AlignerFresh comparing teeth

When analyzing Figure 9, we can conclude that the incisor teeth had the highest percentage of change, evidencing lightening in the middle and incisal areas in all the cases studied, as in the gender and age analyzes presented above, it was evidenced that the area gingival is the one that presents the least changes 75%, additionally the changes of each tooth were analyzed by zones finding that the zone that presented the highest percentage of clearance was the incisal edges 96% and the one with the lowest percentage of change was the gingival zone 65% of the treated patients.

The magnitude of the changes obtained per tooth was as follows

Consolidated 3 teeth per area	Magnitude of whitening using AlignerFresh comparing 3 teeth and Tooth Zones						
	Gingival		Half		Incisal		
	Magnitude of clearance	% of possible lightening	Magnitude of clearance	% of possible lightening	Magnitude of clearance	% of possible lightening	Average magnitude per tooth
incisors	3.3	50.00%	3.3	20.80%	2.4	14.80%	**3.0**
canines	2.9	18.10%	2.5	15.60%	3.7	22.90%	**3.0**
premolars	4.3	26.90%	3.7	22.90%	3.9	24.60%	**4.0**
Total	**3.5**	20.90%	**3.5**	19.60%	**3.5**	23.50%	**3.5**

Table 8. Magnitude of whitening using AlignerFresh on front teeth

By totaling the magnitude of the change, we can predict that the color change we expect after 4 weeks of treatment with the use of AlignerFresh is 3.5 on average for all teeth, the magnitude varied between 3.0 and 4.0, that is, if we start with a shade #10 or D3 Vita™ scale, after 4 weeks of treatment we would expect to end up with shade #6 or C1 Vita™ scale which appears to be a significant change for such a short period of treatment.

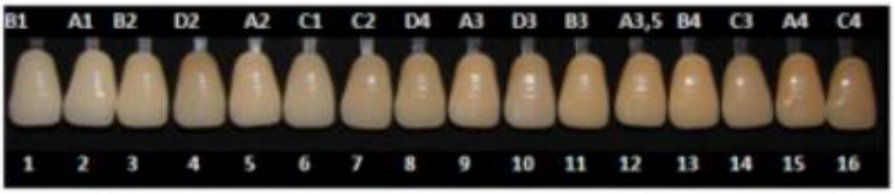


Figure 3: Numeric Conversion of the Classic Vita™ Scale

14.2 Orthodontic treatment with brackets, research product OrthoFoam

14.2.1 Analysis by gender OrthoFoam.

The measurements made allowed us to identify 3 teeth (incisors, canine and premolar) and additionally in each tooth 3 areas were measured (Gingival, middle area and incisal) for this reason a total of 9 measurements were taken in each patient, we followed up at 10 , 20 and 30 days in each patient where changes in tooth color can be identified.

By gender	categories	Lightening results using OrthoFoam comparing men and women (Zones of the tooth)						Total	
		Gingival		Half		Incisal			
		n	%	n	%	n	%	n	%
Women No.=11 patients	Without changes	10.0	30.3	8.0	24.2	6.0	18.2	24.0	24.2
	With changes	23.0	69.7	25.0	75.8	27.0	81.8	75.0	75.8
Men N=9 patients	Without changes	6.0	22.2	6.0	22.2	7.0	25.9	19.0	23.5
	With changes	21.0	77.8	21.0	77.8	20.0	74.1	62.0	76.5

Table 9. Whitening results using OrthoFoam comparing men and women (Zones of the tooth)

In women, a percentage of dental whitening of 69% was evidenced in the gingival areas, 75% in the middle areas and 81% in the incisal edges. The smallest changes were found in the gingival areas with 30%.

In men, the percentage of whitening was 77% in the gingival areas and in the middle areas and 74% for the incisal edges, Figure 10, which shows these results, is presented below.

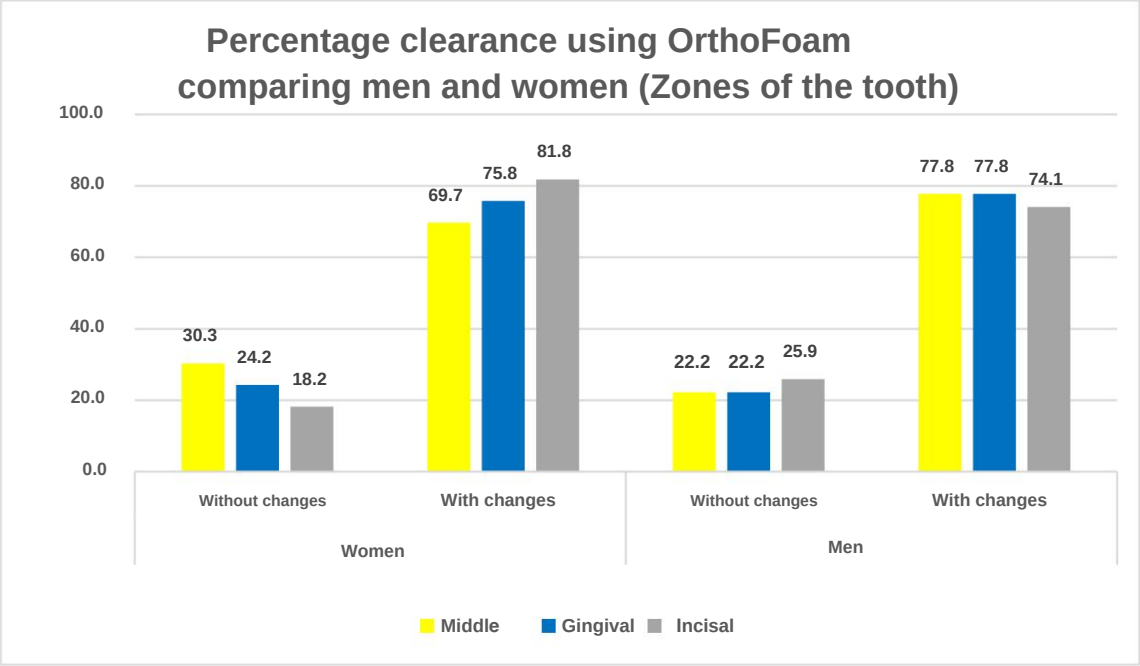


Figure 10. Percentage clearance using OrthoFoam comparing men and women (Zones of the tooth)

The gingival areas in women are the areas that show the least whitening (69%) and in men it was the incisal area (74%) without showing great differences when compared with the gingival area (77%) and the middle area (77%).).

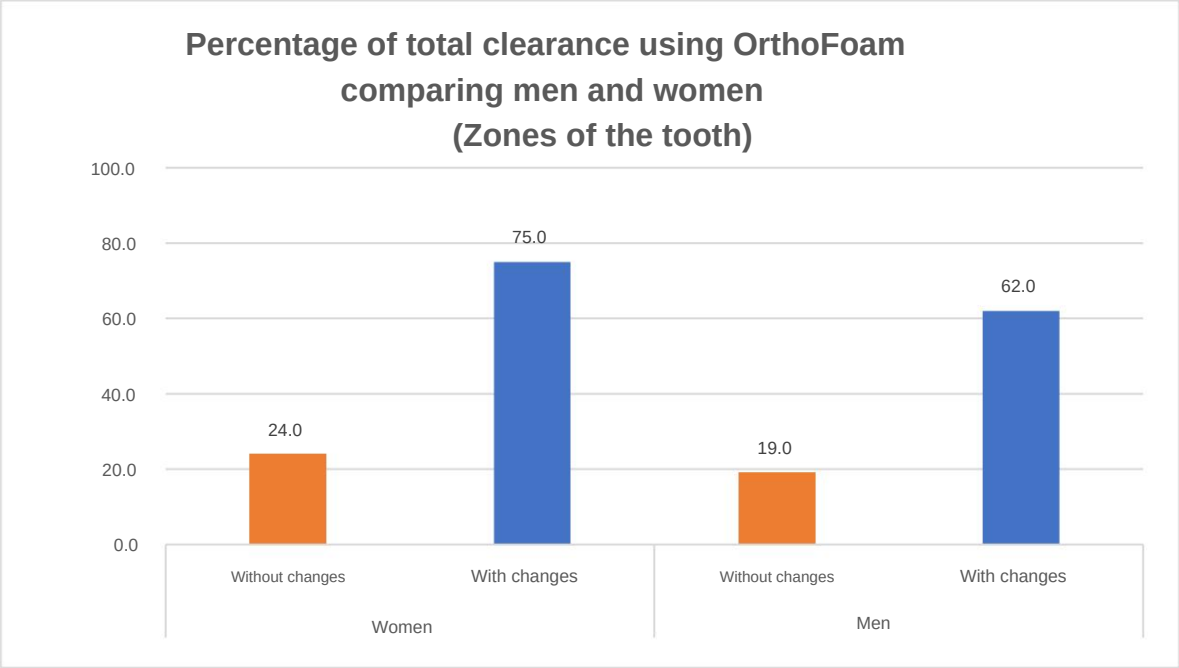


Figure 11. Percentage of total whitening using OrthoFoam comparing men and women (Zones of the tooth)

The percentage of dental whitening shown with OrthoFoam is totaled in figure 11, evidencing 75% for women and 62% for men, this is how OrthoFoam showed good results lightening the color after 4 weeks of treatment.

The magnitude of the changes is presented in Table 10 below.

Classification	n	Magnitude of clearance	% improvement
Women	11	3.9	64.6 (64/99)
Men	9	3.8	53.5 (53/81)

Table 10. Magnitude of clearance using OrthoFoam comparing gender

By totaling the magnitude of the change, we can predict that the color change we expect after 4 weeks of treatment with the use of OrthoFoam is 3.9 for women and 3.8 for men, that is, if

we started with a shade #10 or D3 Vita™ Classic scale, after 4 weeks of treatment we would expect to finish with a shade #6 or C1 Vita™ scale, which appears to be a significant change for such a short period of treatment.

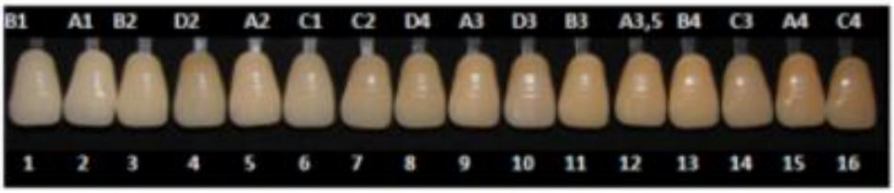


Figure 3: Numeric Conversion of the Classic Vita™ Scale

14.2.2 Analysis by age OrthoFoam.

The measurements made allowed us to identify the percentages of whitening in all ages, showing that the age groups between (20-29) improved by 82% and the groups between (30-39) improved by 75%.

By gender	categories	Whitening results using OrthoFoam comparing ages (Zones of the tooth)						Total	
		Gingival		Half		Incisal			
		n	%	N	%	n	%	n	%
12-19 years N=3 zones	Without changes	1.0	33.3	1.0	33.3	1.0	33.3	3.0	33.3
	With changes	2.0	66.7	2.0	66.7	2.0	66.7	6.0	66.7
20-29 years N= 15 zones	Without changes	3.0	20.0	3.0	20.0	2.0	13.3	8.0	17.8
	With changes	12.0	80.0	12.0	80.0	13.0	86.7	37.0	82.2
30-39 years N= 12 zones	Without changes	1.0	8.3	5.0	41.7	3.0	25.0	9.0	25.0
	With changes	11.0	91.7	7.0	58.3	9.0	75.0	27.0	75.0
40-49 years N= 12 zones	Without changes	3.0	25.0	3.0	25.0	4	33.3	10.0	27.8
	With changes	9.0	75.0	9.0	75.0	8	66.7	26.0	72.2

50-59 years N= 18 zones	Without changes	8.0	44.4	2.0	11.1	3.0	16.7	13.0	24.1
	With changes	10.0	55.6	16.0	88.9	15.0	83.3	41.0	75.9

Table 11. 55.6 Whitening results using 88.9 OrthoFoam 15.0 comparing ages (Zones of the 41.0 tooth)

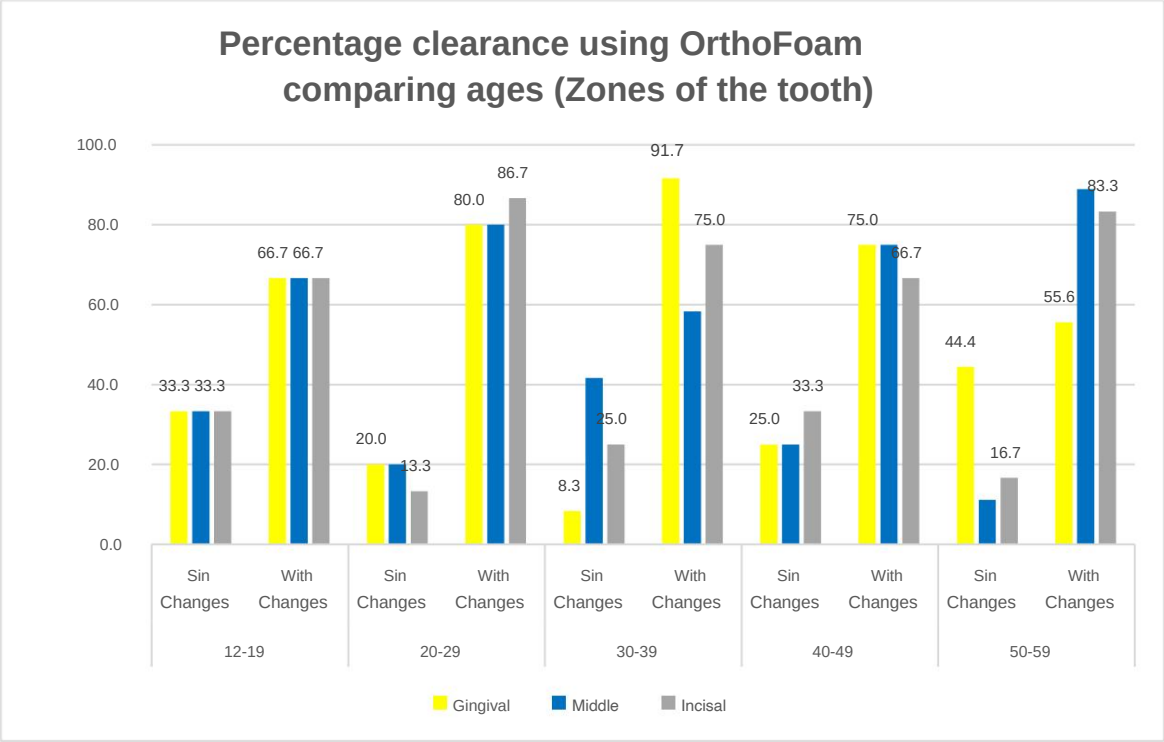


Figure 12. Percentage clearance using OrthoFoam comparing ages (Zones of the tooth)

The analysis of the different age groups showed that the greatest changes were obtained in the 20-29 age group, showing clearance percentages between 80% and 86%, in all areas of the tooth. The 50-59 age group showed the least changes between 55% and less than 83%, however it was shown that more than half of the patients treated with OrhoFoam had positive color changes.

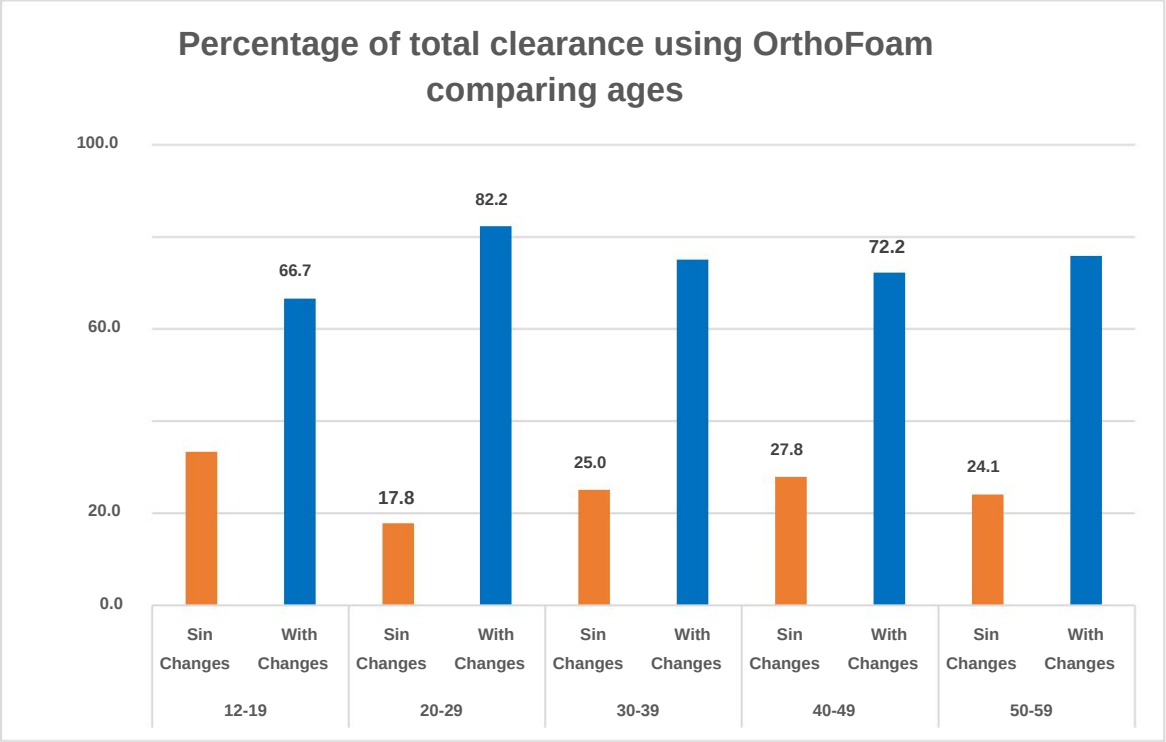


Figure 13. Percentage of total clearance using OrthoFoam comparing ages

The percentage of dental whitening shown with OrthoFoam is totaled in the previous figure, evidencing 82% whitening in patients aged 20-29, the other age groups showed whitening greater than 66%, thus demonstrating that OrthoFoam showed good results lightening the color after 4 weeks of treatment.

The magnitude of the changes is presented in the following table.

By age	Magnitude of clearance using OrthoFoam comparing age groups.		
	N of patients	Mean clearance	Percentage of possible whitening in studied areas
12-19 years	1	4.0	66.7% (6/9)
20-29 years	5	3.8	71.1% (32/45)
30-39 years	4	3.8	75.0% (27/36)
40-49 years	4	3.9	(55.3% (21/36)
50-59 years	6	4.1	57.4% (31/54)
Total	20	3.9	65.0%

Table 12. Magnitude of clearance using OrthoFoam comparing age groups

By totaling the magnitude of the change, we can predict that the color change we expect after 4 weeks of treatment with the use of OrthoFoam is 3.9 on average for all ages, the magnitude varied between 3.8 and 4.1, that is, if we start with a shade #10 or D3 Vita™ scale, after 4 weeks of treatment we would expect to end up with shade #6 or C1 Vita™ scale which appears to be a significant change for such a short period of treatment.

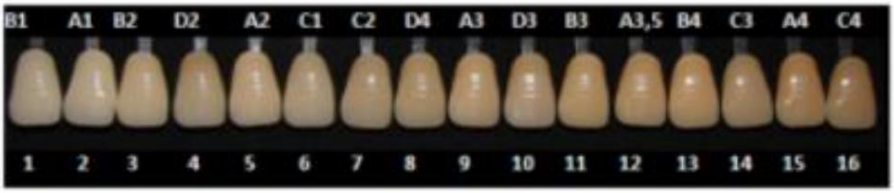


Figure 3: Numeric Conversion of the Classic Vita™ Scale

14.2.3 Analysis per OrthoFoam tooth.

The measurements made allowed us to identify that the use of OrthoFoam resulted in whitening in all the teeth studied, showing the best results in the incisors 83% and the teeth that presented the least change were the premolars 68% of the treated patients.

Consolidated 3 teeth per area	categories	Percentage clearance using OrthoFoam comparing 3 teeth and Zones of the tooth						
		Gingival		Half		Incisal		TOTAL
		n	%	n	%	n	%	Average
incisors	Without changes	4	20	3	15	3	15	16.7
	With changes	16	80	17	85	17	85	**83.3**
Canine	Without changes	4	20	5	25	5	25	23.3
	With changes	16	80	15	75	15	75	**76.7**
premolars	Without changes	8	40	6	30	5	25	31.7
	With changes	12	60	14	70	15	75	**68.3**

Total	Without changes	16	26.7	14	23.3	13	21.7	23.9
	With changes	44	**73.3**	46	**76.7**	47	**78.3**	**76.1**

Table 13. Percentage clearance using OrthoFoam comparing 3 teeth and Tooth Zones

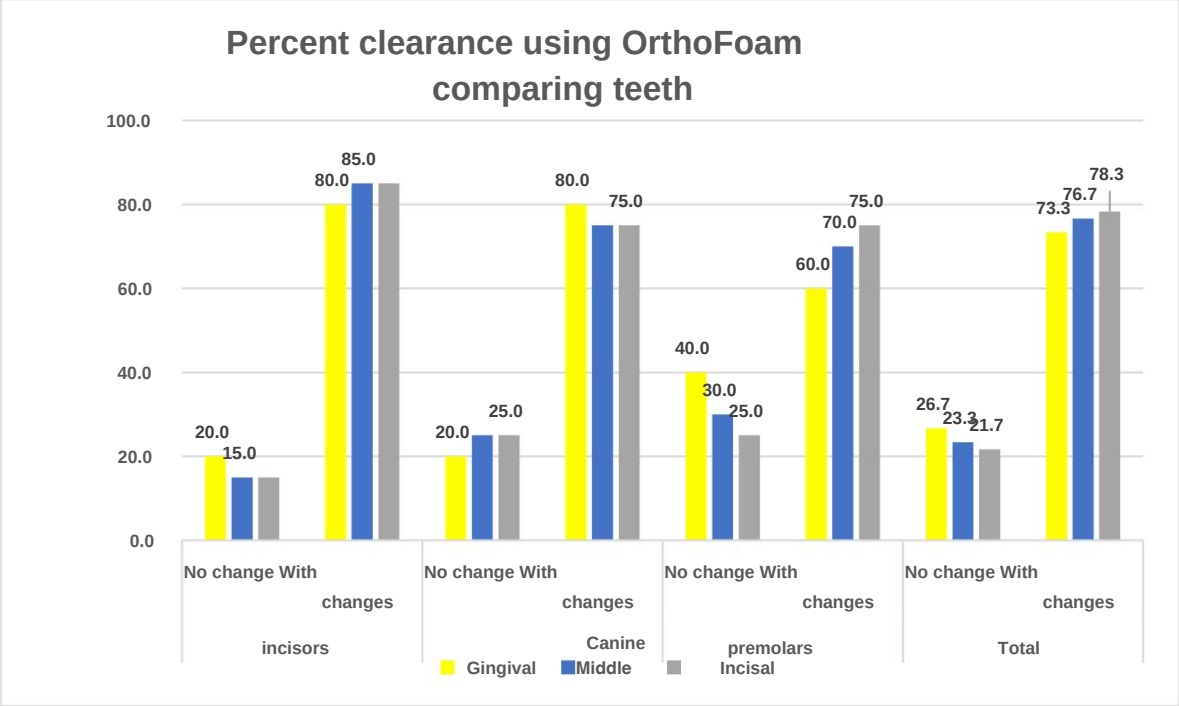


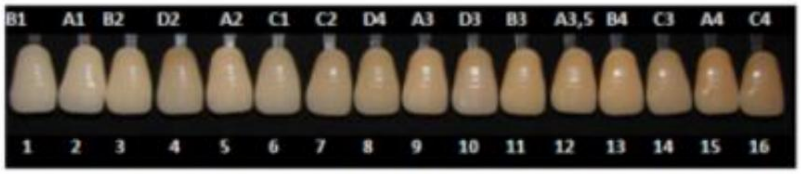
Figure 14. Percentage clearance using OrthoFoam comparing teeth

When analyzing figure 14, we can conclude that the incisor teeth had the highest percentage of change (85%), evidencing clearance in the 3 very similar areas. In addition, the changes of each tooth were analyzed by areas, finding that the area with the highest percentage of clearance 78% presented were the incisal edges and the one with the lowest percentage of change was the gingival area 73% of the treated patients.

The magnitude of the changes obtained per tooth was as follows

Consolidated or 3 teeth per area	Magnitude of clearance using OrthoFoam comparing 3 teeth and Tooth Zones						
	Gingival		Half		Incisal		
	average clearance o	% clearances possible	average clearance o	% clearances possible	average clearance o	% clearances possible	Average magnitude per tooth
incisors	3.9	80.00%	4.5	60.00%	3.4	60.00%	**3.9**
canines	3.3	80.00%	3.5	65.00%	2.6	55.00%	**3.1**
premolars	4.5	60.00%	5.4	60.00%	3.8	65.00%	**4.6**
Total	**3.8**	73.30%	**4.4**	61.70%	**3.3**	60.00%	**3.8**

• **Table 14. Magnitude of whitening using OrhoFoam comparing the treated teeth.** • By totaling the magnitude of the change, we can predict that the color change we expect after 4 weeks of treatment with the use of OrthoFoam is 3.8 on average for all teeth, the magnitude varied between 3.1 and 4.6, that is, if we start with a shade #10 or D3 Vita™ scale, after 4 weeks of treatment we would expect to end up with a shade #6 or C1 Vita™ scale or #5 or A2 Vita™ scale which appears to be a significant change for such a short treatment period .



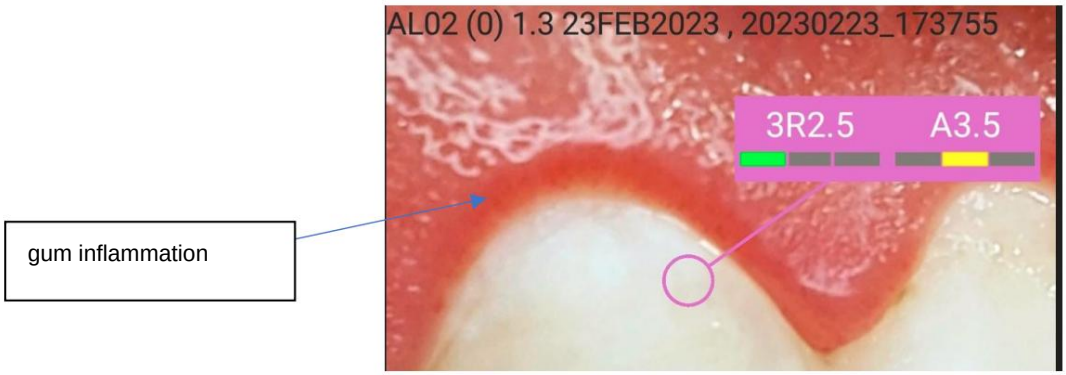
• Figure 3: Numerical conversion of the Classic Vita™ Scale

14.3 Additional study observations.

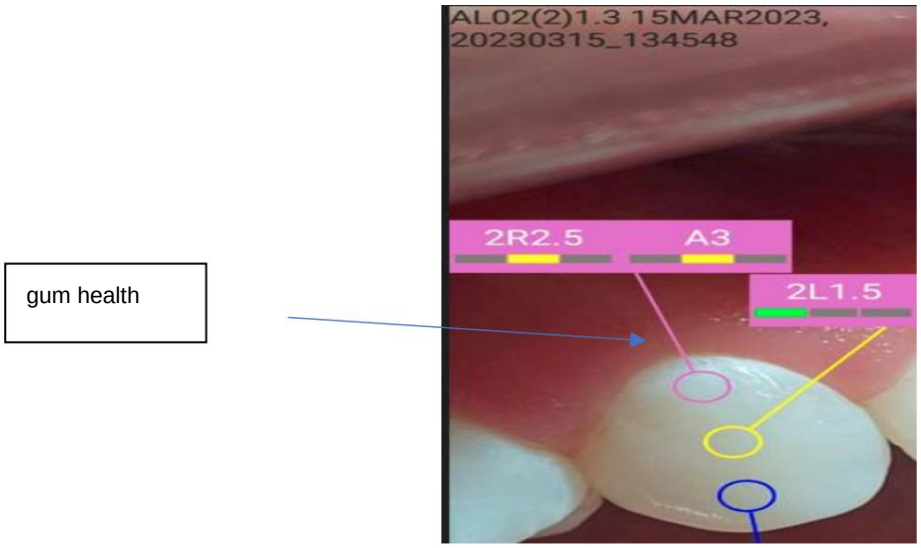
The sponsor has 100% of the photos and the values obtained in both the VITA SYSTEM 3D-MASTER scale and the VITA™ CLASSIC SCALE delivered so that the sponsor can verify the quality of the information and carry out the reanalyses that consider relevant to the data collected.

Below are some photos of the results obtained in this study.

Patient AL02 in the canine (1.3) presented clear signs of gingival inflammation when starting treatment with **AlignerFresh.**

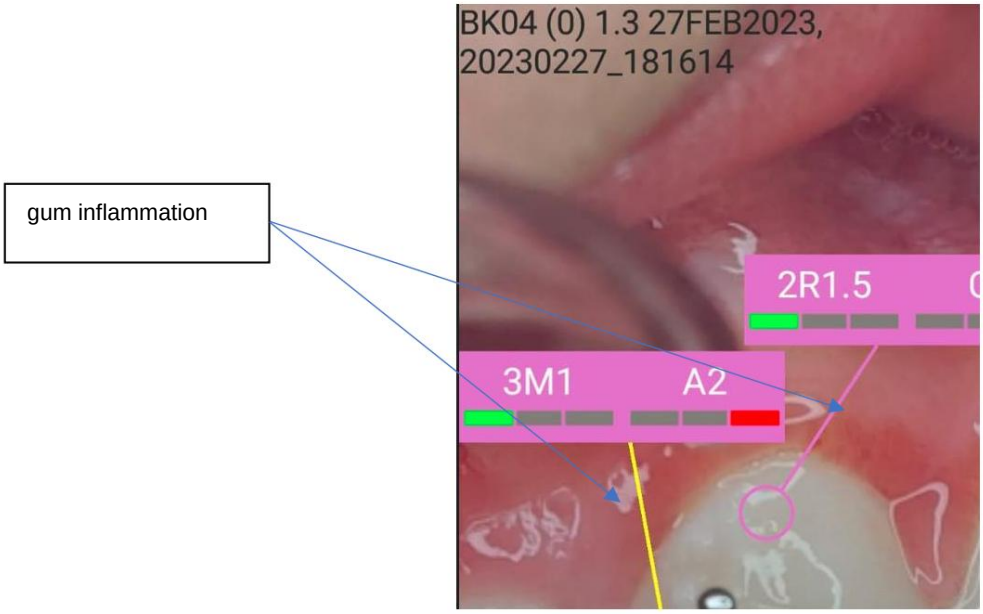


Patient AL02 in the canine (1.3) as a result of treatment with AlignerFresh, improved and recovered periodontal health in just 3 weeks of treatment.

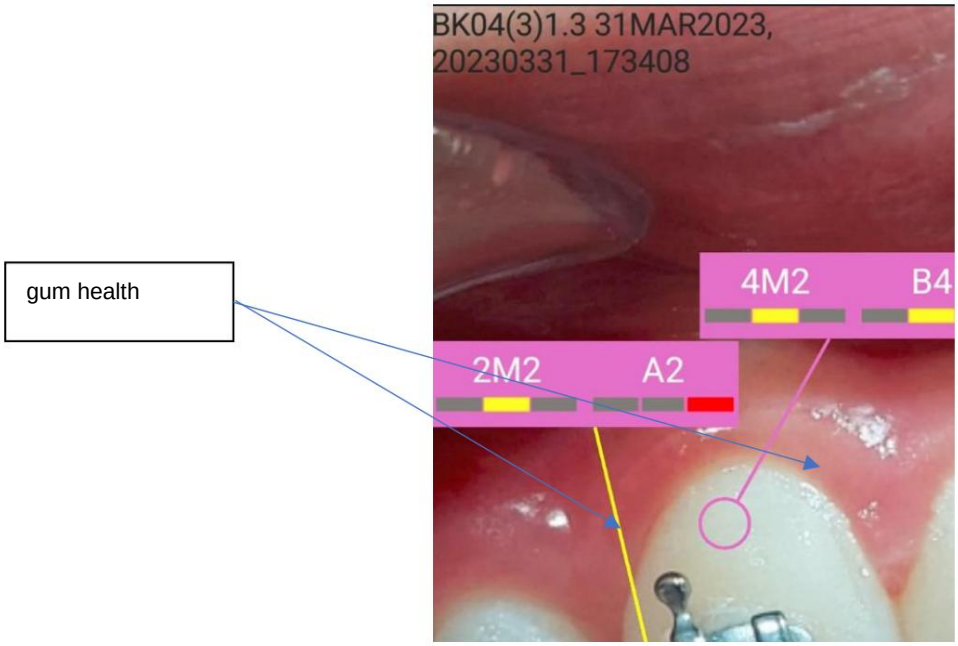


Below are some photos of the results obtained in this study.

The patient BK 04 in the canine (1.3) presented clear signs of gingival inflammation when starting treatment with OrthoFoam.

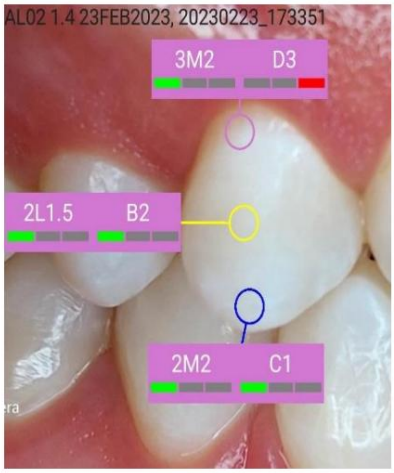


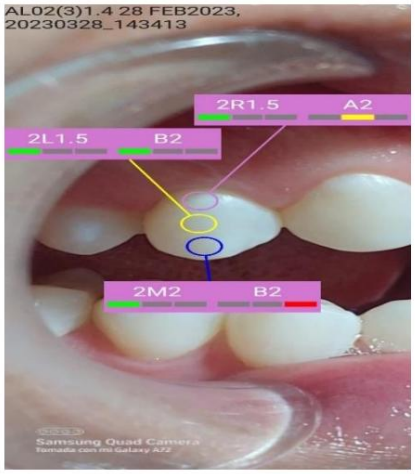
The patient BK 04 in the canine (1.3) as a result of treatment with OrthoFoam, the improvement and recovery of periodontal health was evidenced in just 4 weeks of treatment.



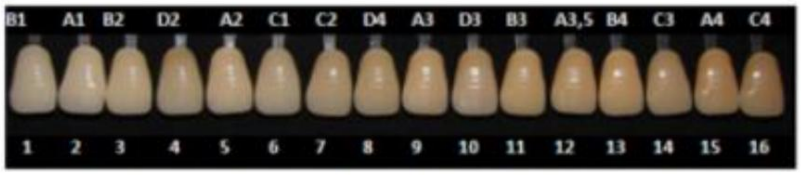
14.4 Some of the photographic evidence obtained during the study.

Results obtained with AlignerFresh.



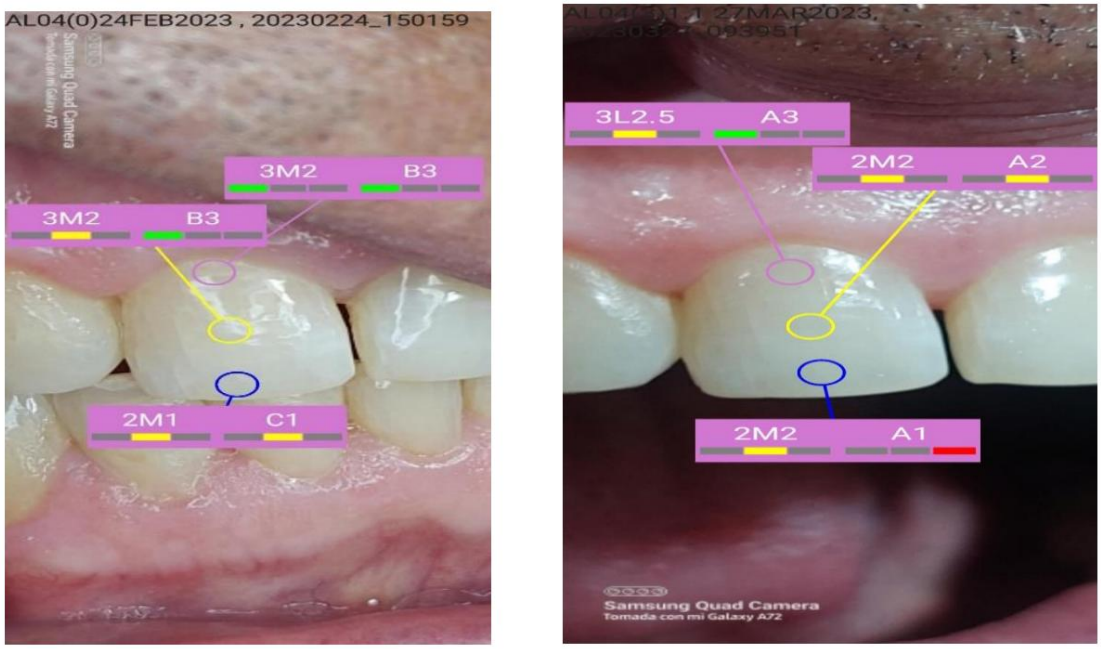


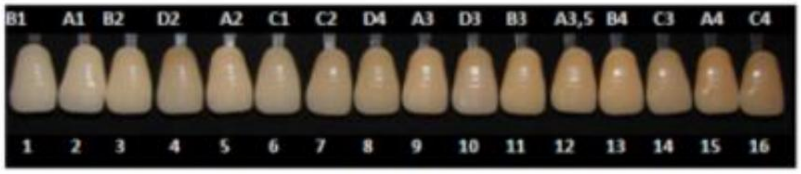
Color improves from D3 (#10) to A2 (#5) in gingival, remains at B2 in the middle third, and improves from C1 (6) to B2 (3) in incisal.



• Figure 3: Numerical conversion of the Classic Vita™ Scale

Shade improves from B3 (#11) to A3 (#9) in gingiva, improves from B3 (#11) to A2 (#5) in the middle third, and improves from C1 (#6) to A1 (#2) in incisal.

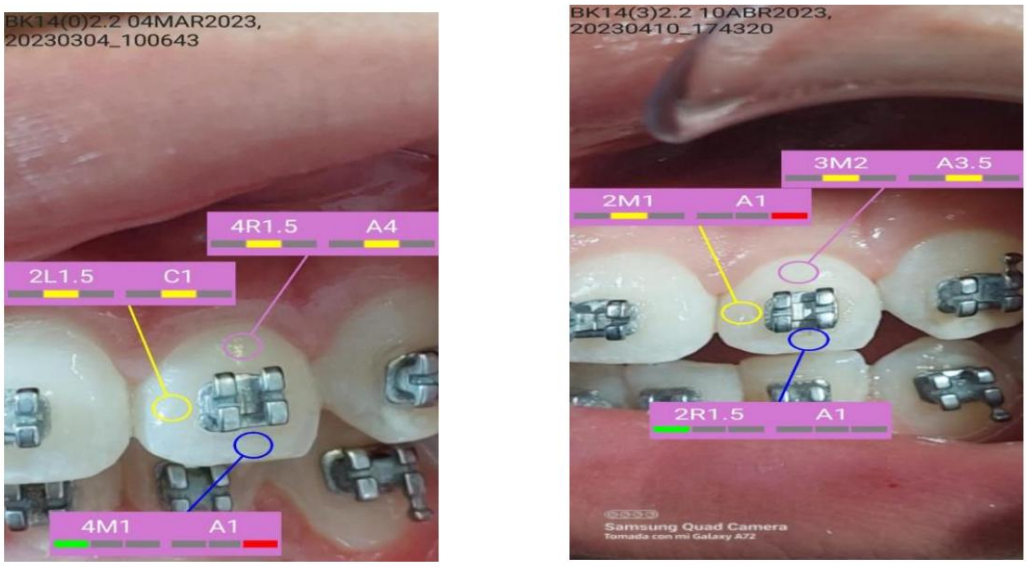




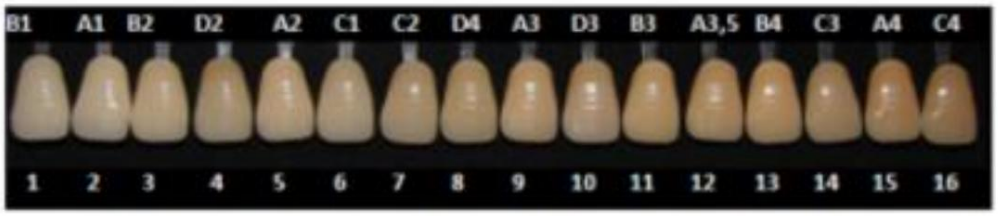
• Figure 3: Numerical conversion of the Classic Vita™ Scale

Shade improves from B3 (#11) to A3 (#9) in gingiva, improves from B3 (#11) to A2 (#5) in the middle third, and improves from C1 (#6) to A1 (#2) in incisal.

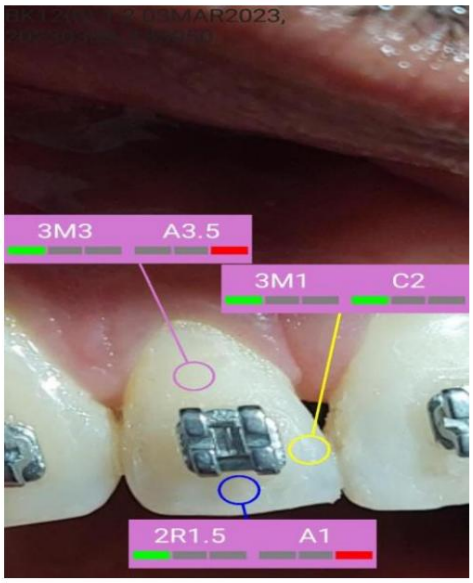
Results obtained with OrthoFoam.

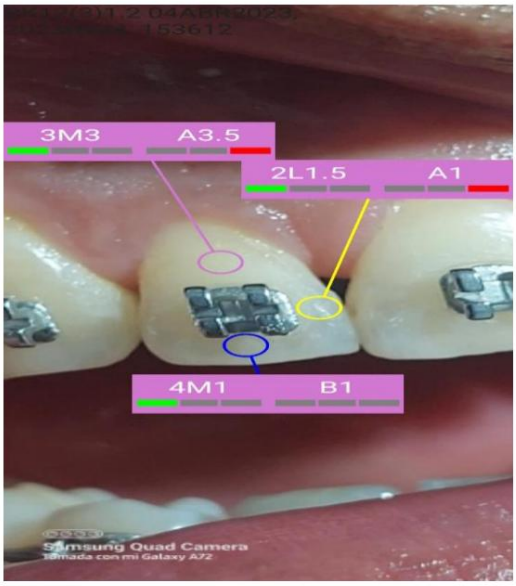


Color improved from A4 (15) to A3.5 (12) in gingival, improved from C1 (6) to A1 (2) in the middle third and remained at A1 in incisal.

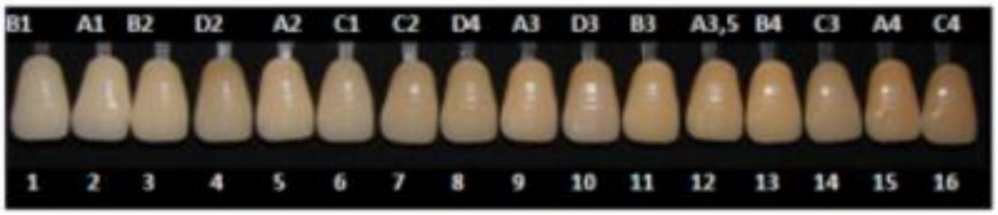


• Figure 3: Numerical conversion of the Classic Vita™ Scale





The color is maintained at A3.5 in the gingiva, improvement from C2 (7) to A1 (2) in the middle third and improvement from A1 (2) to B1 (1)



• Figure 3: Numerical conversion of the Classic Vita™ Scale

15. Conclusions

The treatment groups with AlignerFresh and OrthoFoam were completed with 20 patients in each group, the groups were adequately balanced by gender, weight, height and by BMI, age was evaluated by conforming age groups of 10 years.

Based on the interpretation of the amplitude expressed as a percentage, which corresponds to the number of patients treated in whom positive color changes were evidenced. And the interpretation of the magnitude expressed in number, corresponds to the values of change towards lower tones than those found as basal data.

Amplitude and magnitude analyzes were carried out, identifying 3 variables, gender, age and tooth, and each of them involved detailed analyzes regarding the shade taking area, due to the goodness of the VITA Easyshade V digital spectrophotometer that allowed for each *tooth .* take the color of the gingival area, middle area and the incisal edge.

The measurements made allowed us to identify 3 teeth (incisors, canine and premolar) and additionally in each tooth 3 areas were measured (Gingival, middle area and incisal) for this reason a total of 9 measurements were taken in each patient, we followed up at 10 , 20 and 30 days in each patient where changes in tooth color can be identified.

The main conclusions after using AlignerFresh 3 times a day for 4 weeks are presented below:

- In women and men, clearances were evidenced with an amplitude of 80% and 81% respectively.
- The magnitude of clearance by gender was 3.5 for both.
- The range of clearance by age showed broader results for ages 12-19 years and 20-29 years.
- The amplitude of whitening in the incisal edges was 100% at all ages, the gingival areas had the lowest percentages between 58% and 83% at different ages.
- The magnitude of whitening by age showed an average of 3.7 among all age groups with values between 2.8 (20-29 years) and 5.2 (12-19 years)
- The width of whitening per tooth showed greater whitening in the incisors (91%) and lower in the canines (73%)
- The teeth that showed the greatest amount of whitening were the premolars (4.0) and those that had the lowest response was evidenced by the incisors and canines with 3.0.

The main conclusions after using OrthoFoam 3 times a day for 4 weeks are presented below:

•In women and men, there was evidence of clearance with an amplitude of 75% and 62%respectively.

•The magnitude of clearance by gender was 3.8 and 3.9 for women and men respectively.

•The range of clearance by age showed broader results for ages 20-29 years and 50-59 years

•The amplitude of clearance in the incisal edges was between 66% and 86%, the gingival areas showed 55%and 91% clearance and the middle areas between 58% and 88% at different ages.

•The magnitude of whitening by age showed an average of 3.9 among all age groups with values between 3.8(20-29 years) and 4.1 (50-59 years)

•The width of whitening per tooth showed greater whitening in the incisors (83 %) and minor in the premolars (68%)

•The teeth that showed the greatest magnitude of whitening were the Incisors 3.9 and those that showed the least response were the canines with 3.1

•Patients with braces used for orthodontic treatment accumulate a greater amount of bacterial plaque and developed gingival problems, mainly gingivitis induced by dental biofilm. This pathology decreased in patients who presented this condition and continued treatment with AlignerFresh or OrthoFoam. , the severity of the pathology was not quantified but the conclusion was reached evidencing the photographic evolution obtained to evaluate the color before and after the treatment.

16. Discussion

•The results presented show a satisfactory trend of dental whitening, in accordance with whatresults promised by the evaluation product.

•Obtaining minor changes in whitening in the gingival areas is consistent with the amount of dentin and its natural color, in relation to the amount of enamel available in these areas of the tooth. When finding a smaller amount of enamel, whitening decreases and the dentin is it shines through easier.

• There is evidence of greater whitening in patients who use aligners compared to the use of brackets, this could be explained in the contact time between the product and the dental surface, the aligner allows more contact time of the product than brushing in the tooth. bracket case.

• Teeth with white colors initially show less change than teeth with more pigmentation.

•The whitening results are satisfactory for a product for home use, improving not only the color of the teeth but also the periodontium and gingival health.

• Although it was not the objective of the study, it was an interesting observation, patients who use brackets generally have inflammations since the appliances favor the accumulation of bacterial plaque, during the evaluations it was observed that gingival inflammation decreased with the use of OrthoFoam, this result was also evident in the use of AlignerFresh.

16.1 Limitations

- Treatment was limited for both AlignerFresh and OrthoFoam to 4 weeks, so expecting more extensive color changes was limited.
- The shade was taken independently using a *VITA Easyshade V* digital spectrophotometer . This avoided bias on the part of the researcher
- The shade was taken using the spectrophotometer but the presence of brackets in the third middle of the tooth could limit the color accuracy at this point.

16.2 Financing

Costs of materials, staining equipment, grooming kits, and other costs associated with this study were covered by the sponsor EVERBRANDS INC.

- There was no conflict of interest on the part of the principal investigator (Orthodontist) or the statistical advisor or the research advisor responsible for the study.

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Annex 1 Study protocol.

Annex 2 Informed consent

Appendix 3 Approval Ethics Committee

Annex 1 Study protocol.

Protocolo ALIGNERFRESH-ORIGINAL-001-2022.pdf

Annex 2 Informed consent

Minors Alignerfresh.pdf

CI Parents Alignerfresh.pdf

or CI Elderly age Caregiver Alignerfresh.pdf

Appendix 3 Approval Ethics Committee

Approval Ethics Committee.pdf