Field: MATERIALS ENGINEERING

DOCTORAL THESIS
– SUMMARY –

MATERIALS USED IN DENTAL AESTHETICS. COST AND EFFICIENCY

PhD - Student: **Scientific supervisor:**
Ionela-Amalia Mazilu(married Moldovan)   **Prof. Dr. Eng. Violeta Popescu**

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SUMMARY

The color of the teeth is a combination of the intrinsic colors and the extrinsic stains found on the surface of the enamel. Therefore, teeth whitening products must either remove extrinsic stains or change the intrinsic color. Most commercial bleaching products typically contain hydrogen peroxide or carbamide peroxide.

The originality and novelty of this doctoral thesis consists in the creation of new dental whitening agents in the form of gels, based on natural whitening compounds (organic acids that favor redox reactions, natural extracts enriched in such components, as well as enzymes), which do not require an added amount of peroxide in the formulation. This approach, by virtue of avoiding the 2-30% peroxide concentrations commonly used in commercial whitening gels, ensures healthier and less corrosive enamel whitening with natural color restoration.

The doctoral thesis is structured in two parts: *Current State of Knowledge* and *Personal Contributions*, to which a list of bibliographic references used and a list of publications were added.

In the *Current State of Knowledge*, general aspects regarding the materials used in dental aesthetics, the methods and mechanism of tooth whitening and the factors that influence teeth whitening are presented. One of the chapters from this first part of the literature study includes the study of obtaining gels and their characterization. The literature study part, amongst others, included a marketing study that covers a description of the stomatology services market, an identification of the market and its trends. In the second part of the thesis, the *Personal Contribution* is structured in three personal research studies.

Keywords: biomaterials, whitening gels, dental aesthetics, proteolytic enzymes, natural extracts, micro and nanostructure.

STUDY 1

Realization and characterization of natural gels used in dental aesthetics

Study 1 focuses on the characterization of gels obtained from natural extracts of plants or fruits, which offer, in addition to a tooth whitening effect, protection of the dental hard tissues after the whitening procedure.

Taking into account the literature data and the evolution of materials in the field of dental aesthetics, 4 whitening gels based on carefully selected fruit juices (noted: G19, G28, G29, G30) were developed, within the framework of this study and a further 2 gels based on green tea extracts (noted: G40, G41). The natural juices were extracted from fruits (quince, pineapple, strawberry, sea buckthorn), and the extract from the green tea leaves was obtained after its infusion.

The gels were characterized by HPLC chromatography for the determination of: organic acids, flavonoids and phenolic acids from the content of the gels with fruit extracts and fruit juice. Analyzing the obtained results, we notice that the acidity of the investigated experimental gels depends on the type of natural extract used to obtain them. The content of organic acids varies between 8.7403 mg/g and 328.228 mg/g.

The antibacterial, anti-inflammatory and antioxidant properties of experimental whitening gels with a natural content of fruit juices or medicinal plants are given by flavonoids (catechin, epicatechin, rutin and quercetin) and phenolic acids (vanillic acid, p-coumaric acid, ferulic acid) . The total content of phenolic acids in the studied samples is 193.04 µg/g gel for Gel 29 sample and 25.27 µg/g gel for Gel 41 sample.

The effectiveness of the obtained experimental gels was studied by measuring the color changes of some surfaces after the application. In order to determine the color records, samples of experimental dental composites and samples of dental enamel were investigated and 3 investigation methods were used:

1. Jasco UV-VIS spectrophotometer equipped with an integrating sphere (figure 1).

2. digital, by uploading some images (initial and after bleaching), in an original software called "Dentcolor" - created and optimized at Babeş-Bolyai University, Cluj-Napoca.

3. digital, with the Vita Easy Shade spectrophotometer.

The surfaces subjected to the whitening process using the 6 experimental gels were stored both in artificial saliva and in coffee (in order to be able to better observe the effect of coloring and discoloration) for 10 days and 30 days respectively. All the results were compared to a commercial whitening gel called Opalence 16%. The color difference (ΔE*) was obtained by comparing the initial samples (before the bleaching treatment) to the final ones(after the bleaching process) (figure 1).
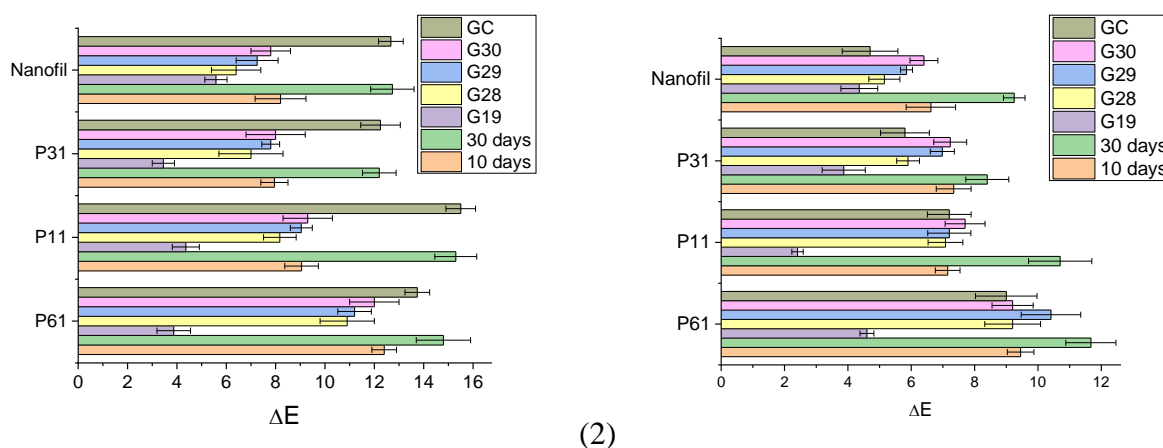

(1)   (2)

Figure 1. ΔEab values measured by UV-Vis spectrophotometry, on dental composites: (1) in coffee; (2) in artificial saliva.

The whitening activity of each experimental gel was tested and measured with the help of the two test methods, on samples of extracted teeth (ΔE values being represented by the average value of the 5 measurements ± standard deviation). The highest value of tooth enamel discoloration was obtained when using the commercial gel, Opalescence 16%, followed by the gels G40 - with green tea and G41 - with green tea and carbamide peroxide in its composition.

The effectiveness of the experimental gels obtained through the *microstructural analysis of enamel surfaces and restorative composites* was analyzed. Therefore both the enamel and the composite surfaces were analyzed in their initial state and after the whitening process by using the following two techniques: scanning electron microscopy (SEM, (figure 2)) and atomic force microscopy (AFM, (figure 3)). For the dental enamel, the SEM images indicate that after the bleaching treatment a remineralization and an uniform whitening of the enamel is observed. The enamel sample treated with G28 (quince and sea buckthorn extract, SiO2, HA-F, HA-Zn) has the most uniform microstructure amongst all of the tested samples. When analysing the G41 Gel, which contains active components of green tea extract and carbamide peroxide, we note the total removal of the mocrostructural clusters and the thin

film of coffee, which indicates a high degree of effectiveness in removing stains, but unfortunately a weak demineralization of the surface layer of the enamel is also observed.
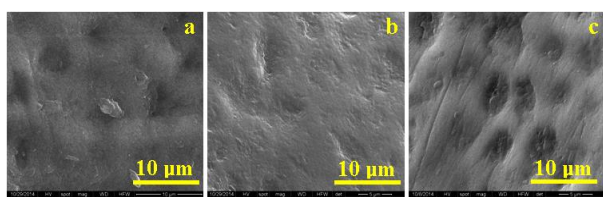

Figure 2. SEM images of enamel: a) control sample stained with coffee, b) bleached with G40 gel and c) bleached with G41 gel
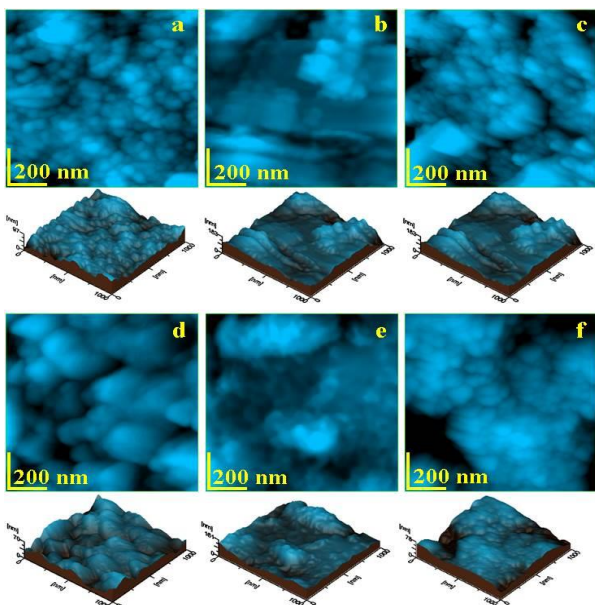

Figure 3. AFM topographic images of the nanostructure of the samples: (a) untreated enamel; (b) enamel stained in coffee; (c) enamel treated with G28 gel; (d) enamel treated with G41 gel; (e) enamel treated with commercial Opalescence gel; (f) enamel treated with G40 gel. The images of the two-dimensional profiles on the horizontal median of the topographic images, which represent the roughness at the nanostructural level (Ra nano)

Cytotoxicity analysis of whitening gels from fruit juices was evaluated as a % percentage of the untreated control, on human dental follicle stem cell strains in comparison with the Opalescence 16% gel. The viability of untreated cultures increased significantly with exposure time, for 24 hours (p = 0.018). The most important effect of decreasing viability was recorded for the commercial gel Opalescence 16% on fibroblasts, and the experimental gel G28 has the lowest cytotoxic effect.

Cytotoxicity was measured by exposing the restorative composite samples to dental follicular stem cell cultures, after treating them with the whitening gel. The least cytotoxic gel is G28, for 24 and 72 hours. All restorative composite samples exposed to cell cultures showed cell viability close to the control sample. The only composite sample (P11), exposed for 24 hours to culture, showed a minor decrease in cell viability, while the P31 composite had a high cell growth effect in the first 24 hours, followed by the commercial Nanofill composite.

The antibacterial effect was studied on 4 different culture environments: Peptostreptococcus anarobius (ATCC 27337), Corynebacterium xerosis (ATCC 373), Streptococcus mutans (ATCC 25175), Candida albicans (ATCC 10231), representative of the oral cavity. The determinations were made by calculating the diameter of the bacterial inhibition zone (mm). The most significant antimicrobial activity was found in the case of Peptostreptococcus anaerobius culture, for the G41 gel. These strains, present in the oral environment, are responsible for 90% of periodontal diseases. Against C, albicans, the experimental gels Gel 40 and Gel 41, based on green tea extract, had no antifungal effects, a fact demonstrated by other studies in the literature which claim that the antifungal effect of green tea is given only at a pH higher greater than 6.

STUDY 2

Realization and characterization of gels based on enzymes in dental aesthetics

In study 2, 4 new whitening products, with enzymatic action, derived from natural products (pineapple and papaya plant or fruit) were developed and characterized.

The most recent studies show that bromelain and papain, two proteolytic enzymes originating from plants (extracted from the fruit/stem), can provide a reduction in tooth sensitivity, in addition to the whitening effect, if incorporated into products used in dental aesthetics. Taking these aspects into consideration, we prepared 4 whitening gels with proteolytic enzymes (papain: Gel 1, Gel 2 and bromelain: Gel 3 and Gel 4) in the form of nanocapsules. These nanocapsules are obtained by using the interfacial deposition method, in which a polymer (polycaprolactone) is used as a precursor. The polymer has the role of a polymeric membrane, surrounding the liquid which is the active substance and allowing a controlled release of the active substance through the diffusion phenomenon.

The four gels obtained were characterized by:

UV-VIS absorption spectrometry (figure 4), which shows that all the gels absorb in the ultraviolet range, with a monotonous and smooth decrease up to 800 nm starting at 350 nm. This decreasing slope can be explained by the presence of PEG 400 polymers. From the presented spectra it can be seen that the bromelain and papain nanocapsules, in the control samples, give a maximum signal at 280 nm, with the observation that the bromelain signal is almost 2 times more intense than the papain signal.
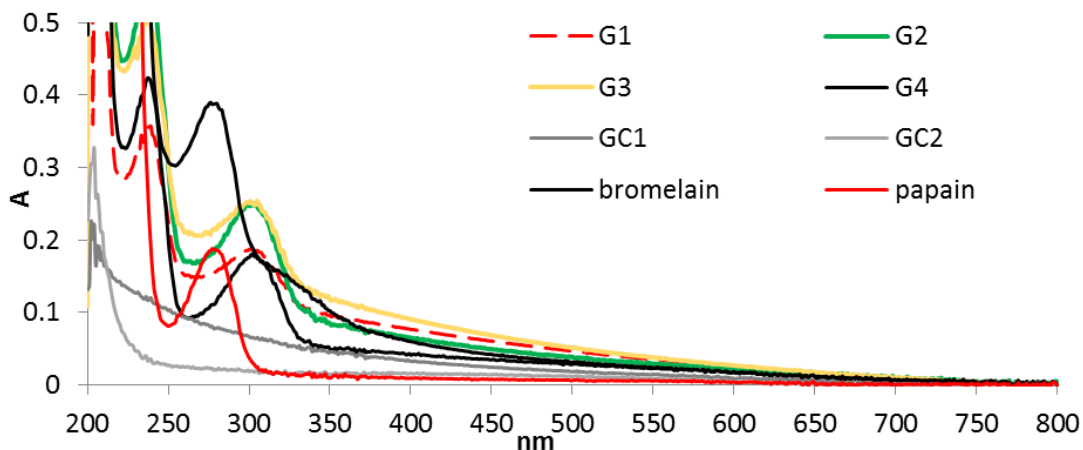

Figure 4. UV-vis spectra of gels and pure protease solutions, which were diluted 1:100 in phosphate buffer pH 7.

In order to examine the stability of the protein components in the gels we analyzed the *electrophoresis in polyacrylamide gel and denaturing conditions* (SDS-PAGE), following an electrophoresis and quantitative analysis protocol previously applied to milk and whey. With the help of the Gelanalyzer program, reference samples containing only the active agent, bromelain or papain, can be detected at the expected molecular masses. In the case of gels Gel1 and Gel2 (with papain), in which the intensity of papain is analyzed, the protein signals are very weak.

The *FT-IR spectra* of the gel samples and the raw materials used (polyethylene glycol, aerosil and carbopol), were analyzed in both wet and dry conditions. The wet ones show a broad absorption band in the range of 3000-3400 cm-1. The wet gels samples based on SiO2

show the maximal absorption rates due to the vibrations of the O-H bonds in the water, with values of around 3386 cm-1 and 1635 cm-1. These maximal absorption rates decrease in intensity following the evaporation of water, allowing an increase in intensity of the maximal absorption bands corresponding to the -CH2 groups from 2928 cm-1.

The broad band that remained after water evaporation at high wavenumbers 3711 and 303 cm-1 (which is not very visible in the spectra) is due to the contributions of several components in the gel, thus they can be attributed to the vibrations of the Si-O bonds in the aerosil, O-H vibrations from polyethylene glycol and amide A vibrations from whey. The maximum of this band underwent a shift to higher wavenumbers following the drying process.

We analyzed the *antibacterial effect* on 5 strains (table2). Depending on the investigated antimicrobial compound, the diameter of the inhibition zone of the experimental gels varied from 9 mm to 13 mm. The greatest antibacterial effect was registered against the bacterial strain Porphyromonas gingivalis, in all 4 of the investigated gels, followed by the Enterococcus faecalis strain. The best inhibition was recorded for the Gel2 gel with papain for all media investigated.

Table 2. Diameters of bacterial inhibition zones (mm)

Bacterial strain /Sample code	Gel 1	Gel 2	Gel 3	Gel 4	GC1 control gel SiO$_2$	GC2 Control gel Lubrizol
Streptococcus mutans	13	13	0	0	0	11
Porphyromonas gingivalis	9	13	9	11	0	10
Enterococcus faecalis	8	13	8	12	0	11
Escherichia coli	0	12	0	9	0	0
Staphylococcus aureus	9	13	7	11	0	10

In the case of these gels, we measured the *efficiency* by determining the color changes using a commercial ESCom 100 photopolymerizable nanocomposite and dental enamel as a test surface. The surfaces were subjected to a coloring process by immersing the samples in 2 storage conditions: Tedi juice and coffee, for 4 h/day, 5 consecutive days. Afterwards they were washed and immersed in artificial saliva at 37°C. After the bleaching process, following the application of the experimental whitening gels, the color difference was analyzed using the Vita Easyshade spectrophotometer (figure 5). The smallest color difference was recorded on composites bleached with Gel1, followed by Gel3, which contain papain and bromelain. For the enamel, the experimental products do not generate constant variations, while the commercial product generates color variation towards lower values of the parameter b* (blue).
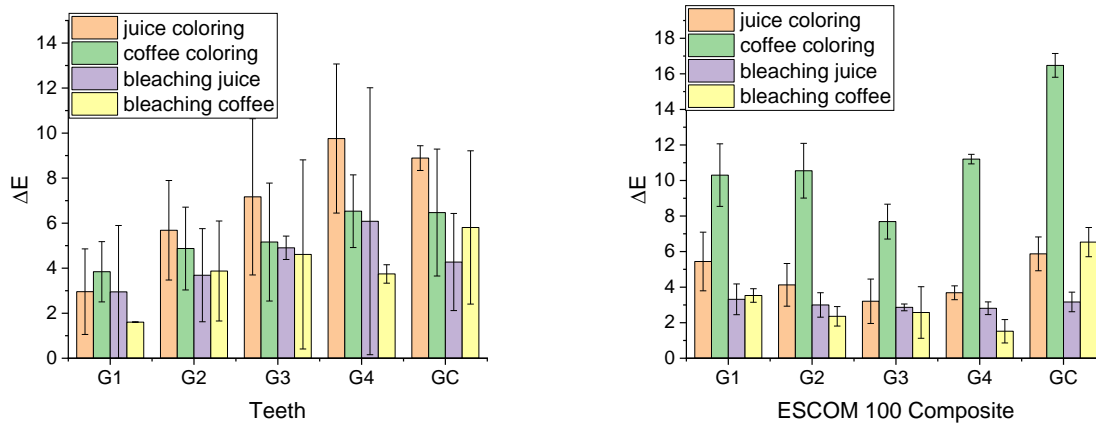
Figure 5. ΔEab values measured with Vita Easy Shade Advance 4.0.

The efficiency of these enzyme-based gels was measured by scanning electron microscopy (SEM) and atomic force microscopy (AFM). The micro and nanostructure of an EsCOM 100 dental composite was analyzed, before and after the coloring-whitening process.

The roughness variation histogram, as compared to the bleaching treatment applied to the samples, indicate the higher values for the coffee colored sample and slightly lower values, following discoloration. Considering the roughness as an indicator of the gels' efficiency in color removal, we can observe the fact that, in the case of the nanohybrid composite, the best effect was obtained by the Gel1, gel containing papain nonocapsules.

The micro-nanostructural analysis of the enamel by SEM (figure 6) and AFM (figure 7), after the application of the Gel1 gel, shows that a great part of the microstructural deposits of fruit pulp that covered the enamel is eliminated. The micro and nanostructured aspects regarding colored enamel with natural juice, from the AFM images, shows that the most beneficial effect was obtained by Gel3 gel due to the synergistic effect between bromelain and SiO_2 nanoparticles, closely followed by the Gel1 gel based on papain and SiO_2 . The Gel4 gel showed a less effective action compared to Gel1 and Gel3 from a micro and nanostructural effect point of view, but a much more delicate action on the enamel was observed, being ideal for sensitive and/or deeply attacked teeth.
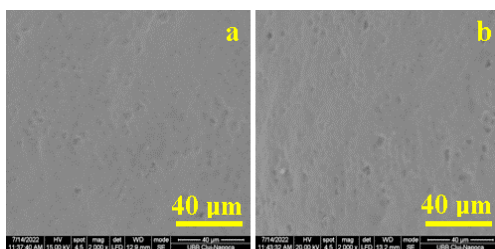

Figure 6. SEM images with the overall microstructural aspect of coffee-stained enamel: a) bleached with G1; b) bleached with G3.
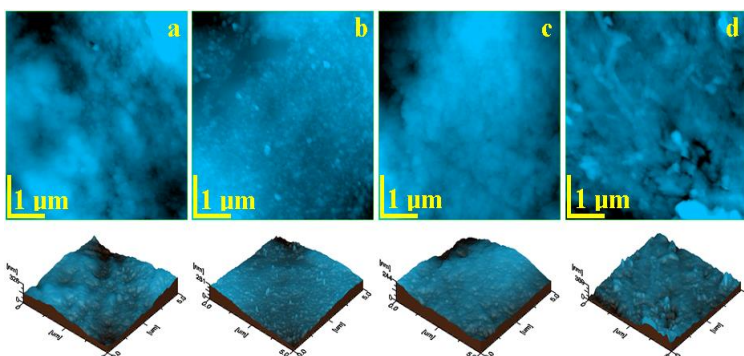

Figure 7. AFM images for the fine microstructure of coffee colored enamel: a) control gel, b) bleached with G1; c) bleached with G3; d) bleached with G4.

Two culture environments were used for the cytotoxicity analysis: mesenchymal stem cells from human dental pulp (dMSC) and human keratinocyte HaCaT cell lines. The results did not reveal cytotoxicity in any of the studied environments and for any of the investigated bleaching gels. According to international standards, if a material has cell viability values below 70%, it is considered cytotoxic. Given that these enzymes are widely used for their anti-inflammatory properties, the experimental whitening gels formulated in this study might be able to reduce tooth sensitivity because the reactive oxygen species are not produced during the tooth whitening process.

STUDY 3

Evaluating aspects of tooth whitening procedures, cost and effectiveness, amongst both dentists and patients

Marketing is the first step in patient education. The emergence of new treatment methods, new minimally invasive and cosmetic dental techniques reflects the fact that dentistry is a dynamic field within oral health care.

To evaluate the cost and efficiency, two questionnaires were created on the Internet, in 3 languages (Romanian, English and French) designed and distributed through Microsoft Forms.

The first questionnaire contains 20 questions and is addressed to dentists, and the second contains 24 questions and is addressed to patients.

For this study, we received approval from the Ethics Committee of the University of Medicine and Pharmacy Cluj-Napoca, with approval number 134/11 May 2021.

1. The group consisted of 120 patients, aged between 18 and 57 years, who received consultation and/or treatment in dental clinics during this study.

2. The group was formed by 127 dentists, endodontists and doctors in general dentistry aged between 25 and 54 years.

In total, the study was based on 247 subjects who fully responded to the questionnaire.

Investigated variables included the following: practitioner age, use and preferences for various specific materials, recommendations in various clinical scenarios, indications, side effects, patient satisfaction, whitening efficacy, cost, and number of sessions required for treatment.

We started from the null hypothesis that there would be no statistical differences between the answers provided by dentists and patients.

When analyzing the parameters related to dentists' choices in selecting the indicated tooth whitening method for their patients, the one-way repeated measures ANOVA test showed a statistically significant difference in both the patient group ($p < 0.0001$) and the group dentists ($p < 0.0001$); consequently, regarding the appropriateness of the therapy and the preferred treatment methods, the results were very varied (figure 8, 9).
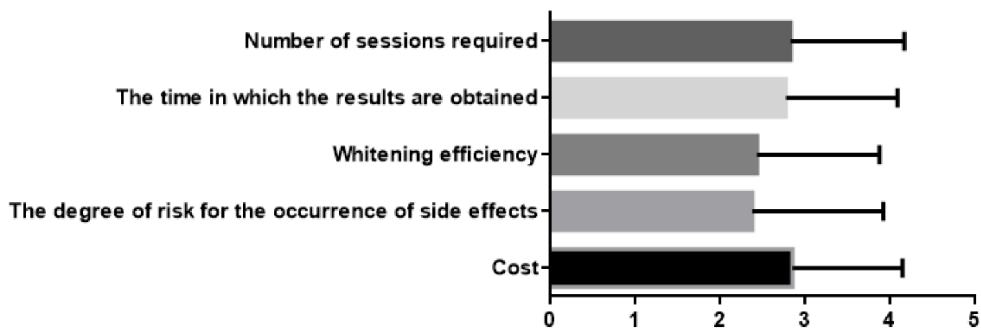
Figure 8. Statistical analysis of the parameters of the in-office whitening method, in relation to the listed criteria (where 1 is the most important and 4 is the least important of the criteria).
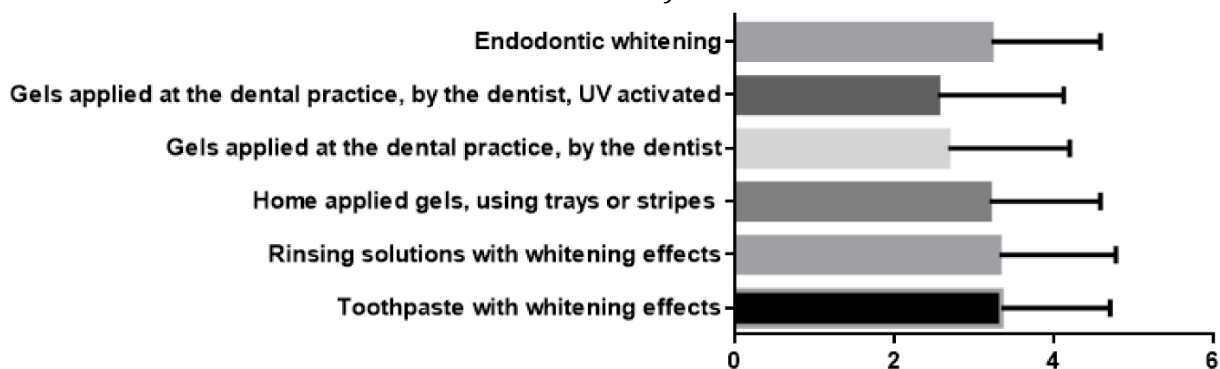

Figura 9. Statistical analysis of the choice by doctors of the teeth whitening procedure (where: 1 – the most frequent; 5 – the least frequent).

The least important criteria according to the dentists was the profit, followed by the cost per patient, the number of necessary sessions, the time in which results were obtained and the whitening efficiency. The most important criteria was the degree of risk associated with side effects.

If we follow the whitening methods recommended by doctors, we notice that toothpastes with whitening effects were the least indicated, followed by mouthwash with whitening effect, internal whitening of endodontically treated teeth, whitening gels applied by the patient at home, the use of individual strips and whitening gels with a high concentration of carbamide peroxide applied by the doctor in the dental office. The most common procedure that doctors chose for teeth whitening was treatment with light-activated whitening gels.

Analysis of the frequency of whitening procedures performed by dentists in dental practices showed that most practitioners performed up to 10 whitening treatments in the last 2 years of practice.

The results are predictable because most patients are not trained to have unbiased opinions about different treatment options unless they are given professional advice. Also, emphasis should be placed on the amount of money patients spend on bleaching procedures.

The most important factor in choosing the most suitable whitening procedure was the cost of the treatment, followed by the number of sessions required, the time in which the results were obtained, the effectiveness of the whitening and the degree of risk associated with side effects.

A strong statistical correlation was observed between patients' age and their satisfaction with the appearance of their teeth after treatment (younger patients were more satisfied). Comparing the whitening procedure with the frequency of use by patients, whitening toothpaste took the first position, followed by whitening rinses; at-home whitening procedures using custom trays and in-cabinet whitening using light-activated or laser-

activated whitening gels. Also, the questionnaires in this study included questions about the participants' knowledge of teeth whitening and their perception of the cost and treatments of teeth whitening.

| Questions Patient/Dentist	1. Endodontic whitening/tooth/ session	2. Treatment at home with individualized mouthguards.	3. Treatament at the office/session	4. Treatament at the office with lamp-activated gels/session
What was the price of the whitening treatment at the dental office?	10-19 EUR 20-29 EUR 30-39 EUR Over 40 EUR	100-124 EUR 125-144 EUR 145-164 EUR 165-199 EUR Over 200 EUR	100-124 EUR 125-144 EUR 145-164 EUR 165-199 EUR Over 200 EUR	165-184 EUR 185-199 EUR 200-224 EUR 225-264 EUR 265-299 EUR Over 300 EUR

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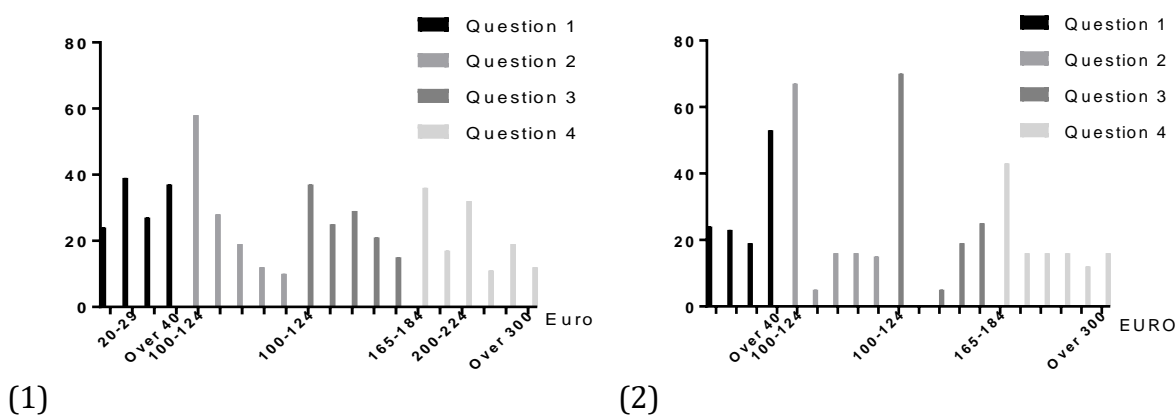
Figure 10. Statistical analysis of cost choice regarding bleaching methods among (1) doctors and (2) patients.

As for the doctors, the variations in the answers regarding the cost of bleaching treatment probably vary according to the general status of their patient and the characteristics of the dyschromia, with the distribution of the cost depending on the treatment being uniform (figure 10). The patients, on the other hand, went for the cheapest option, both for the treatment at home (under 20 Euros) and for the one in the cabinet (100-124 Euros), the most expensive option being the endodontic whitening treatment/tooth/session.

Overlaying the questions related to the cost in relation to the treatments offered and carried out by dentists for teeth whitening, we can see that most patients opt for treatments carried out in the cabinet (60%) or for a treatment carried out at home with customized trays (58%) and then monitored in the cabinet, both procedures at a minimal cost/session. Of these two procedures, doctors firstly recommend the treatment performed at home with personalized mouthguards (45%) followed by the treatments performed in the office (29%), at the same price as the patients (100-124 Euro).

Conclusions: The creation of a new class of materials used in dental aesthetics with remineralization effects and diminished corrosive properties, due to the peroxide-free formulation, are expected to have a synergistic effect on the tooth whitening process.

The degree of complexity in this doctoral thesis consists of several formulas of stable whitening gels, using several natural components as active agents, and the effectiveness of these tooth whitening gels correlates proportionally with the exposure time. The development of these dental biomaterials was based on using original methods of nanofilling synthesis, new natural extracts and original methods of analysis.

Another original aspect focused on obtaining comparative information on the perception of aspects related to tooth whitening procedures, cost and efficiency, amongst both dentists and patients. The study was complex, being addressed to both patients and dentists, but their opinion, resulting from the survey, was the most important aspect of this social research.

The research of this doctoral thesis leads us to the conclusion that alternatives must be found for aesthetic treatments, but without causing unwanted side effects. Also, the appearance of new whitening products is expected, at a lower price point and which will be effective and accessible to all categories of patients.