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The Abrasive and Remineralising Efficacy of Coturnix Eggshell

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ABSTRACT

Aim: The objective of this research was to determine the abrasive and remineralising efficacy of quail eggshell (Coturnix coturnix) incorporated into a new experimental toothpaste.

Methods: An experimental study was designed to compare the surface microhardness of the dental enamel of bovine lateral incisors exposed to different toothpastes. In relation to microroughness (abrasive efficiency), the unit of analysis was each specimen with 16 polymethylmethacrylate (PMMA) blocks. For the remineralisation variable, the unit of analysis was each bovine lateral incisor tooth. A sample size of 16 was determined for each group.

Results: The unpolished PMMA specimens presented the highest mean surface microroughness with a mean of 4.60 $\pm$ 1.47 $\mu$m, whilst the quail paste, universal polishing paste, control paste, and Diamond Excel Paste showed a mean of 1.16 $\pm$ 0.40 $\mu$m, 0.63 $\pm$ 0.024 $\mu$m, 0.45 $\pm$ 0.22 $\mu$m, and 0.43 $\pm$ 0.17 $\mu$m, respectively, presenting significant differences ($P < .001$).

When comparing the surface microhardness of bovine lateral incisor dental enamel exposed to different toothpastes, the universal polishing paste presented the lowest microhardness, with 192.43 $\pm$ 56.21 kg/mm\textsuperscript{2}, whilst the quail paste presented the greatest remineralising effect, with the highest average of 272 $\pm$ 21.18 kg/mm\textsuperscript{2}, followed by the control paste with 244 $\pm$ 41.43 kg/mm\textsuperscript{2} and the Diamond Excel Paste with 228.72 $\pm$ 43.72 kg/mm\textsuperscript{2}. These differences were statistically significant ($P < .001$).

Conclusions: Significant differences were found in the surface abrasive efficiency (microroughness) of PMMA subjected to different toothpastes. The quail toothpaste presented statistically significant results compared to the control pastes, the Diamond Excel Paste, and the universal polishing paste.

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Introduction

In recent years, there has been a growing interest in finding new sources of calcium carbonate (CaCO\textsubscript{3}). Early studies on the composition of eggshell showed that it is made up by approximately 97% of CaCO\textsubscript{3}. Moreover, eggshell can also provide a great protective barrier against the penetration of microorganisms, and at the same time it is constituted by numerous porous layers permeable to water and gases, which allow the embryo to breathe. In addition, eggshell is composed of a bioceramic composite material to guarantee the calcium necessary for the skeletal formation of the chick.\textsuperscript{1,2}

Fluoride has an effective remineralising effect on caries prevention. However, chronic low-level fluoride exposure can generate urogenital, respiratory, and gastrointestinal conditions. In addition, the application of fluoride ions alone cannot

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completely remineralise carious lesions. Therefore, it is necessary to find an efficient alternative to fluoride for caries prevention and incipient enamel lesion remineralisation.

The loss of minerals due to enamel demineralisation, caries, and erosion of the enamel can lead to multiple health problems. The carious process is a dynamic de- and remineralising process resulting from microbial metabolism, which eventually leads to cavitation. In contrast, the term dental erosion is used when exposure to acid, such as hydrochloric acid from chronic acid reflux, or extrinsic acids, such as fruit juice, affect the enamel of the tooth.5 Tooth surface roughness is important to prevent bacterial plaque retention, staining, and subsequent adverse impacts on oral health. Smooth and well-polished denture surfaces are more prone to intraoral success.6

Thus, the aim of this study was to determine the abrasive and remineralising efficacy of quail eggshell (Coturnix coturnix) incorporated into a new experimental toothpaste.

Materials and methods

Sample size and study design

An in vitro, longitudinal, comparative, experimental design was developed for this study. The unit of analysis was each surface of the polymethylmethacrylate (PMMA) block. Finally, for the remineralization variable, the unit of analysis was each enamel of the bovine lateral incisor tooth. In relation to the variable microroughness, the unit was PMMA blocks. For the remineralization variable, the unit was formed by the bovine lateral incisors for each group. The sample was calculated in relation to the means and standard deviations previously obtained in a pilot test using the formula for comparison of means with the statistical software Stata®15, determining an n=16 for each group evaluated.

Group A: Dental bovine enamel exposed to the experimental toothpaste including CaCO₃ from Coturnix eggshell vs commercial pastes (universal polishing paste, Diamond Excel Paste, and control paste without quail eggshell).

Group B: Polymethyl-methacrylate blocks exposed to the experimental paste based on CaCO₃ from Coturnix eggshell vs commercial pastes (universal polishing paste, Diamond Excel Paste, and control paste without quail eggshell).

Preparation of the toothpaste

Fresh quail eggs were selected and crushed. The broken shells were emptied, and 125 g of eggshell were collected. After selecting a mortar, the other components were added and weighed based on the proportions established in the formulation (tetrasodium, aerosil, pyrophosphate, menthol nipagin, titanium dioxide, and saccharin). First, all the components collected in the mortar were mixed until a homogeneous compound was achieved to combine gum, sodium lauryl ether sulfate, and fluoride solution. When the final compound obtained good viscosity, glycerin was added followed by distilled water. Finally, when the paste obtained good viscosity, the procedure was completed by labeling the product in a container (Table 1).

Sample preparation and demineralisation

Bovine incisors were collected for this study. The enamel blocks were 4 mm long, 4 mm wide, and 2 mm thick and were embedded in acrylic blocks. Moreover, the samples were immersed in a solution of potassium dihydrogen phosphate (KH₂PO₄), calcium chloride dihydrate (CaCl₂ 2H₂O), methylhydroxydiphosphonate, and acetic acid (CH₃COOH) (Merck) at pH 4.95 and placed in an incubator (37.8 °C; BR 6000; Heraeus Kulzer) for 14 days. During the demineralisation period, the solutions were not shaken or changed. The pH values of the demineralising solutions were monitored daily (pHelectrode GE 100 BNC by a pH meter GMH 3510; Greisinger). In addition, small amounts of hydrochloric acid were added to maintain a constant pH value of between 4.94 and 4.96 for the enamel during the demineralisation period.

Remineralisation process

The samples were then stored separately in an eggshell-based experimental paste for 2 and 5 weeks (37 °C). The toothpastes were diluted in a remineralising solution based on the EN ISO 11609 (European standards for preparing artificial saliva/toothpastes) to obtain a homogeneous suspension. These pastes were commercially available (all without fluorides) as active ingredients. After preparation, the suspensions pH values were measured directly. Subsequently, the samples were brushed manually with minimal pressure using a soft toothbrush. Brushing procedures were performed on each subgroup twice a day for 5 seconds each (an additional contact time of 115 seconds with the suspension resulted in 120 seconds of total contact time). After each brushing procedure, the samples were cleaned with deionised water (10 seconds). The remineralising solutions were replenished every 2 days (250 mL each time per group), and the pH values were verified. Three weeks later, half of the

Table 1 – Characteristics of the toothpaste studied.

| Company                        | Formulation                                                                 | Country     |
|--------------------------------|-----------------------------------------------------------------------------|-------------|
| Quail paste                     | Powdered quail eggshell 25.0 g; tetrasodium pyrophosphate 3.75 g; aerosil   | Peru        |
|                                | (hydrophilic fumed silica) 0.20 g; nipagin (sodium methylparaben) 0.15 g;  |             |
|                                | crystallised sodium saccharin 0.20 g; menthol crystals 0.80 g; titanium     |             |
|                                | dioxide (dye) 0.53 g; xanthan gum 1.05 g; sodium lauryl ether sulfate 28%  |             |
|                                | 2.00 g; sodium fluoride (fluoride) 0.32 g; liquid glycerine 24.00 mL;       |             |
|                                | distilled water 42.00 mL.                                                   |             |
| Diamond Excel Paste            | Micronised diamond-based polishing paste with extra-fine grit (2 to 4        | Brazil      |
| Universal Polishing Paste      | microns)                                                                    | Germany     |
|                                | Mixed of the substances listed below with nonhazardous additions.           |             |
|                                | Naphtha (petroleum), hydrotreated heavy (Nota P, <0.1% benzene),            |             |
|                                | Ammoniumoleate                                                             |             |
|                                |                                                                            |             |

1. The abrasives and remineralising efficacy of quail eggshell (Coturnix coturnix) incorporated into a new experimental toothpaste.
Exposed surfaces were varnished with enamel to determine the effect after 2 weeks (intermediate effect).

**Microhardness testing**

The surface microhardness of all the specimens was analysed using Vickers microhardness testing equipment. A load of 25 g was applied for 5 seconds and 5 notches were applied to each specimen with a spacing of 100 microns.

**Preparation of acrylic PMMA specimens**

The acrylic blocks (1 x 1 x 1 cm) used to elaborate the PMMA specimens were prepared. According to the manufacturer’s recommendations, all specimens were polymerised (Vitacryl). Using a milling cutter (tungsten carbide) at 18,000 rpm, the samples were trimmed after the polymerisation procedure. Before achieving the final brilliance, the samples were polished with abrasive paper (silicon carbide CC763) and were finally polished with each of the evaluated pastes.

**Surface roughness analysis**

Using a roughness tester (Mitutoyo), the microroughness surfaces of the acrylic specimens were analysed. The measuring instrument was calibrated with a 0.8-mm cutoff filter, evaluating a length of 4.00 mm with a range of 5.1 mm. Four surface roughness measurements were performed in each specimen, and the mean Ra values were used for evaluation.

**Ethics approval**

This study was reviewed and approved by the Institutional Ethics Committee of Universidad Cientifica del Sur with code (N° 174-CIEI-AB-CIENTÍFICA-2020).

**Statistical analyses**

Descriptive analysis was performed for the numeric variables (antimicrobial, remineralising, and abrasive activity). Normality was determined using the Shapiro–Wilk test. The analysis of variance and Kruskal–Wallis tests were used for bivariate analysis. Finally, the Bonferroni test was selected for post hoc analysis. All statistical analyses were performed with the Stata 15 programme, establishing a significance level of P < .05.

**Results**

It was observed that the unpolished PMMA specimens had the highest mean surface microroughness, with a mean of 4.60 ± 1.47 μm, whilst the quail paste, universal polishing paste, control paste, and Diamond Excel Paste presented means of 1.16 ± 0.40 μm, 0.63 ± 0.024 μm, 0.45 ± 0.22 μm, and 0.43 ± 0.17 μm, respectively. On the other hand, significant differences were observed between the groups when comparing abrasion (surface microroughness; P < .001; Table 2).

| Group                        | Mean  | SD   | Min  | Max  | P     | P     |
|------------------------------|-------|------|------|------|-------|-------|
| Polymethylmethacrylicate, unpolished | 4.60  | 1.47 | 2.74 | 7.65 | .050  | <.001 |
| Quail paste                  | 1.16  | 0.40 | 0.66 | 1.88 | .080  |       |
| Control paste                | 0.45  | 0.22 | 0.16 | .82  | .166  |       |
| Diamond Excel Paste          | 0.43  | 0.17 | 0.16 | .78  | .850  |       |
| Universal polishing paste    | 0.63  | 0.24 | 0.28 | 1.25 | .286  |       |

* Shapiro–Wilk test.  
† Analysis of variance.

All values were expressed in μm and met the assumption of homogeneity of variances by Bartlett test with P > .05 in all the groups evaluated.

The post hoc analysis showed that there were only significant differences amongst the microroughness produced by the quail paste, control paste, and Diamond Excel Paste vs unpolished PMMA (P < .001; Table 3). Comparison of the surface microhardness of the dental enamel of the bovine incisors exposed to the different toothpastes showed that the universal polishing paste presented the lowest microhardness, with 192.43 ± 56.21 kg/mm², whilst the quail paste had the highest remineralising effect with the highest surface microhardness at 272 ± 21.18 kg/mm², followed by the control paste with 244 ± 41.43 kg/mm² and the Diamond Excel Paste at 228.72 ± 43.72 kg/mm² (Table 4).

**Discussion**

By the end of 2025, it is projected that the global market for CaCO₃ will be in the billions. CaCO₃ from eggshells can be used as a substitute for food additives or supplements, for soil conditioning, or as a material for making pharmaceuticals, biomaterials, etc.⁸⁹ Despite the efficacy of different toothpaste formulations with several antibacterial properties,¹⁰¹¹ there has been an increase in the consumption of oral care products containing natural agents.¹² For instance, some natural herbal toothpastes are composed of sodium bicarbonate and other components with medicinal properties such as chamomilla extract with anti-inflammatory properties, echinacea extract which stimulates immune response, sage extract which reduces bleeding, myrrh extract with antiseptic properties, and Mentha piperita extract which has antiseptic and antimicrobial properties.¹³¹⁴ Therefore, there is a promising demand for the use of eggshell in stomatology and medical applications.

Although there seem to be different applications for the use of eggshells, there is limited evidence regarding the abrasive potential of the eggshell powder related to the smoothness of dental prostheses.¹⁵¹⁶ Moreover, CaCO₃ can be used in solid dosage forms as a diluent and is also used for dental and medicinal preparations, dissolutions, and buffering aids in the form of dispensable tablets. Likewise, it can be used in food additives and for calcium supplementation.¹⁷¹⁹

This study showed that the use of eggshell in toothpaste maintained a surface microroughness of 1.16 μm, compared...
Table 3 – Post hoc analysis of the abrasive efficiency (surface microroughness) (µm) of acrylic subjected to different toothpastes during polishing.

| Paste Type                  | Polymethylmethacrylate unpolished | Quail paste | Control paste | Diamond Excel Paste |
|----------------------------|-----------------------------------|-------------|---------------|---------------------|
| Quail paste                | 0.001                             | ---         | 0.056         | ---                 |
| Control paste              | 0.001                             | 0.045       | 1.000         | 1.000               |
| Diamond Excel Paste        | 0.001                             | 0.379       | 1.000         | 1.000               |
| Universal polishing paste  | 0.001                             | 0.379       | 1.000         | 1.000               |

All post hoc analyses were performed using the Bonferroni test.

Table 4 – Comparison of remineralising efficacy (surface microhardness) of bovine tooth enamel subjected to different toothpastes.

| Group                    | Mean  | SD   | Min  | Max  | P*  | P†  |
|--------------------------|-------|------|------|------|-----|-----|
| Untreated tooth          | 290.02| 11.01| 259.10| 302.66| <.001|     |
| Quail paste              | 272.32| 21.18| 206.00| 296.64| .001|     |
| Control paste            | 244.89| 41.43| 133.62| 285.12| .001|     |
| Diamond Excel Paste      | 228.72| 43.72| 165.20| 296.64| .001|     |
| Universal polishing paste| 192.43| 56.21| 1.51  | 249.58| .001|     |

* Shapiro–Wilk test. † Kruskal–Wallis test.

All specimens were expressed in Vickers (kg/mm²).

to other pastes indicated for polishing dental materials such as Diamond Excel Paste and universal polishing paste, with 0.43 and 0.63 µm, respectively. This is congruent with the study by Onwubi et al that showed that surface roughness is an important factor affecting biofilm retention and staining. Therefore, it is essential to note that good polishing of PMMA denture surfaces ensures successful clinical performance. This is supported by several studies that report an increase in microbial adhesion on rough surfaces.6,16,20

According to Onwubi et al,16 microroughness is generally evaluated in Ra (µm) values, and the values reported in their study were under the threshold value of 0.2 µm. Therefore, the eggshell powder was found to be effective in decreasing the microroughness of the polymethylmethacrylate surface. These differences are attributed to the diverse particle sizes included in the experimental paste and can be explained by the suggestion that abrasive materials of smaller particle size quickly wear away surface roughness and thereby have better clinical performance.21

When comparing our results with other abrasive materials (Colgate Great Regular Flavor; Colgate Palmolive Ltd.), it was found that wear occurred after toothbrushing. However, the values were low. On the other hand, the data of another study suggest that abrasion of tooth enamel caused by the diamond particles in the DD2 diamond dentifrice was directly associated with the diamond particle load.22,23

In relation to microhardness, some in vitro studies have mainly shown that different commercial toothpastes have remineralisation capacity on enamel and dentin lesions. In addition, toothpaste with fluoride aggregates showed the least hard tissue remineralisation as well as an increment in lesion depth. Similar to the results of the present study, the literature justifies the use of bovine enamel and dentin samples, as this source represents an accepted surrogate of human enamel. Remineralisation is a complex process involving different ions such as calcium and phosphate, which are returned to the internal structure of the tooth to ensure a strong tooth structure. However, when these minerals are lost the pores are larger, favouring demineralisation of the enamel.24,25

Generally, after the application of remineralising agents on caries lesions, it seems that mineral ions are diffused into the surface layer and thereby clog the surface porosities. The present study showed a significant difference in the remineralisation of the superficial enamel generated by the quail toothpaste (mean of 272.32 kg/mm²), similar to the results of Roza et al,3 who described that the microhardness significantly decreased in the samples after demineralisation and rose significantly after exposure to therapeutic solutions. Microhardness did not significantly differ between groups after pH immersions. Based on our results, the eggshell paste is effective as a remineralising agent in enamel caries-type lesions. Likewise, Kattimani et al demonstrated eggshell biocompatibility, good in vitro material properties, and successful use of eggshell as graft material for bone defect grafting.

It is important to take into account that toothpastes based on pure CaCO₃ from eggshells are a novel and organic alternative. However, more studies are needed to establish the factors associated with antimicrobial efficacy of this product compared to commercial toothpastes using CaCO₃ of synthetic origin. The main limitation of our study was the limited availability of quail eggshells. Nonetheless, the reproduction of microbiological assays using CaCO₃ from eggshells is feasible. Finally, another limitation was the lack of specialised culture media to cultivate some facultative anaerobic bacteria. Nonetheless, the reproduction of microbiological assays was feasible. Finally, another limitation was the large quantity of quail eggshell required vs the small amount available of this natural resource.

Conclusions

Significant differences were found in the surface abrasive efficiency (microroughness) of polymethylmethacrylate subjected to the quail, control, Diamond Excel, and universal polishing pastes during polishing. The post hoc test showed that there were only significant differences in surface microroughness between unpolished polymethylmethacrylate and the other pastes. Finally, comparison of the remineralising efficacy (surface microhardness) of bovine dental enamel subjected to different toothpastes as remineralising agents showed significant differences between the quail vs the control, Diamond Excel, and universal polishing toothpastes.
Conflict of interest

None disclosed.

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REFERENCES

1. Awogbemi O, Inambao F, Onuh EI. Modification and characterization of chicken eggshell for possible catalytic applications. Heliyon 2020;6(10):e05283.

2. Boron L. Citrato de cálcio da casca do ovo: biodisponibilidade e uso como suplemento alimentar. Florianópolis: UFSC; 2004.

3. Roza H, Majid M, Motahare A, Mohammad A. Remineralization effect of eggshell versus nano-hydroxyapatite on caries-like lesions in permanent teeth (in vitro). J Int Oral Health 2016;8(4):435–9.

4. Selwitz RH, Ismail AI, Pitts NB. Dental caries. Lancet 2007;369(9555):51–9.

5. Hornby K, Ricketts SR, Philpotts CJ, Joiner A, Schemehorn B, Willson R. Enhanced enamel benefits from a novel toothpaste and dual phase gel containing calcium silicate and sodium phosphate salts. J Dent 2014;42(Suppl 1):S39–45.

6. Gungor H, Gundogdu M, Yesil Duymus Z. Investigation of the effect of different polishing techniques on the surface roughness of denture base and repair materials. J Prostheth Dent 2014;112(5):1271–7.

7. ReportLinker. Calcium carbonate market research report by type, by industry - global forecast to 2025 - cumulative impact of COVID-19. ReportLinker; 2020. Available from: https://www.reportlinker.com/p05913702/Calcium-Carbonate-Market-Research-Report-by-Type-by-Industry-Global-Forecast-to-Cumulative-Impact-of-COVID-19.html?utm_source=GNW. Accessed October 27, 2020.

8. Yoo S, Hsieh JS, Zou P, Kokoszka J. Utilization of calcium carbonate particles from eggshell waste as coating pigments for ink-jet printing paper. Bioresour Technol 2005;90(24):6416–21.

9. Qasimi MI, Mohibbi H, Nagaoka K, Watanabe G. Accumulation of steroid hormones in the eggshells of Japanese quail (Coturnix japonica). Gen Comp Endocrinol 2018;259:161–4.

10. Bratthall D, Hansel-Pettersson G, Sundberg H. Reasons for the caries decline: what do the experts believe? Eur J Oral Health 2014;6(3):15–9.

11. Gunsolley JC. A meta-analysis of six-month studies of antiplaque and antigingivitis agents. J Am Dent Assoc 2006;137(12):1649–57.

12. Lee SS, Zhang W, Li Y. The antimicrobial potential of 14 natural herbal dentifrices: results of an in vitro diffusion method study. J Am Dent Assoc 2004;135(8):1133–41.

13. Pistorius A, Willershhausen B, Steinmeier EM, Kreisler M. Efficacy of subgingival irrigation using herbal extracts on gingival inflammation. J Periodontal 2003;74(5):616–22.

14. Pannuti CM, Mattos JP, Ranoya PN, Jesus AM, Lotufo RF, Romito GA. Clinical effect of a herbal dentifrice on the control of plaque and gingivitis: a double-blind study. Pesqui Odontol Bras 2003;17(4):314–8.

15. Berger JC, Driscoll CF, Romberg E, Luo Q, Thompson G. Surface roughness of denture base acrylic resins after processing and after polishing. J Prosthodont 2006;15(3):180–6.

16. Onwubu SC, Vahed E, Singh S, Kanny KM. Reducing the surface roughness of dental acrylic resins by using an eggshell abrasive material. J Prostheth Dent 2017;117(2):310–4.

17. Daengprok W, Garnjanagoonchorn W, Mine Y. Fermented pork sausage fortified with commercial or hen eggshell calcium lactate. Meat Sci 2002;62(2):199–204.

18. Schaafsma A, Pakan I, Hofstede GJ, Muskiet FA, Van Der Veer E, De Vries PJ. Mineral, amino acid, and hormonal composition of chicken eggshell powder and the evaluation of its use in human nutrition. Poult Sci 2000;79(12):1833–8.

19. Mony B, Ebenezar AV, Ghani MF, Narayanan A, Anand S, Mohan AG. Effect of chicken egg shell powder solution on early enamel carious lesions: an in vitro preliminary study. J Clin Diagn Res 2015;9(3):ZC30–2.

20. Jefferies SR. The art and science of abrasive finishing and polishing in restorative dentistry. Dent Clin North Am 1998;42(4):613–27.

21. Neshandar Asli H, Rahimabadi S, Babae Hemmati Y, Falahchah M. Effect of different surface treatments on surface roughness and flexural strength of repaired 3D-printed denture base: an in vitro study. J Prostheth Dent 2021;126(4):595.e1–595.e8.

22. Sangpanya A, Fuangtharnthip P, Nimmanon V, Pachimsawat P. Toothbrush-dentifrice abrasion of dental sealants: an in vitro study. Eur J Dent 2021. Epub ahead of print. doi: 10.1055/s-0041-1735798.

23. Tawakoli PN, Becker K, Attin T. Abrasive effects of diamond dentifrices on dentine and enamel. Swiss Dent J 2018;128(1):14–9.

24. Kielbassa AM, Hellwig E, Meyer-Lueckel H. Effects of irradiation on in situ remineralization of human and bovine enamel demineralized in vitro. Caries Res 2006;40(2):130–5.

25. Tschoppe P, Kielbassa AM, Meyer-Lueckel H. Evaluation of the remineralizing capacities of modified saliva substitutes in vitro. Arch Oral Biol 2009;54(9):810–6.

26. Kattimani VS, Chakravarthi PS, Kanumuru NR, et al. Eggshell derived hydroxyapatite as bone graft substitute in the healing of maxillary cystic bone defects: a preliminary report. J Int Oral Health 2014;6(3):15–9.

27. Poma-Castillo L, Espinoza-Poma M, Pinzo L, Funes-Moreno F, Montes AC, Pacheco-Molina MP, Fuentes-Arévalo H, de la Concha-Valdiviezo D, Mayta-Tovalino F. Antifungal activity of Ethanol-extracted Bixa orellana (L) (Achiote) on Candida albicans, at six different concentrations. J Contemp Dent Pract 2019;20(10):1159–63.

28. Mayta-Tovalino F, Sandoval-Baibin G, Romero-Tapia P, et al. Development of new experimental dentifrice of Peruvian Solanum tuberosum (Tocosh) fermented by water stress: antibacterial and cytotoxic activity. J Contemp Dent Pract 2019;20(10):1206–11.

29. Mayta-Tovalino F, Fernández-Giusti A, Del Pino J, et al. Formulation and development of an experimental polishing paste with antimicrobial activity based on Coturnix (Codorniz) eggshell. Int J Dent 2021;2021:9998989.