



**FACULTAD DE CIENCIAS DE LA SALUD
CARRERA PROFESIONAL DE ESTOMATOLOGÍA**

**“COMPARACIÓN DE LA LIBERACIÓN DE FLÚOR DE
BARNICES FLUORADOS EXPUESTOS A BEBIDAS DE
CONSUMO COMÚN: ESTUDIO IN VITRO”**

Tesis para optar el grado académico de:
MAESTRO EN ESTOMATOLOGÍA

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ACTA DE SUSTENTACIÓN DE TESIS

Lima, 07 de junio de 2023.

Los integrantes del Jurado de tesis:

Presidente: Dra. Evelyn Alvarez Vidigal
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Se reúnen para evaluar la tesis titulada:

“COMPARACIÓN DE LA LIBERACIÓN DE FLÚOR DE BARNICES FLUORADOS EXPUESTOS A BEBIDAS DE CONSUMO COMÚN: ESTUDIO IN VITRO”

Presentada por el estudiante/bachiller:

- Sophia Alejandra Casimiro Iriarte

Para optar el Grado Académico de Maestro en Estomatología

Asesorada por: Dra. Lourdes Rosa Chiok Ocaña

Luego de haber evaluado el informe final de tesis y evaluado el desempeño del estudiante de la Carrera de Estomatología en la sustentación, se concluyen de:

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Tesis: Sophia Alejandra Casimiro Iriarte		Nota (en letras): Dieciocho (18)	
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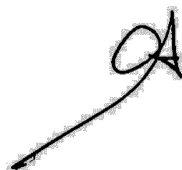
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UNIVERSIDAD CIENTÍFICA DEL SUR
POSGRADO ESTOMATOLOGÍA
MAESTRÍA EN ESTOMATOLOGÍA
DECLARACIÓN DE ORIGINALIDAD DE CONTENIDO DE INFORME FINAL DE TESIS¹³

Lima, 07 de junio del 2023

Señor,
Carlos Zavalaga Reyes

Director General de Investigación, Desarrollo e Innovación
Universidad Científica del Sur

Presente.-

De nuestra consideración,

Yo: Sophia Alejandra Casimiro Iriarte, egresada de la Maestría en Estomatología de la carrera de Estomatología de la Universidad Científica del Sur, en conjunto con la asesora de tesis Rosa Lourdes Chiok Ocaña, declaramos que este informe final de tesis titulado: Comparación de la liberación de flúor de barnices fluorados expuestos a bebidas de consumo común: estudio in vitro, sustentado para obtener el Grado Académico de Maestro en Estomatología es original.

Es decir, no contiene plagio parcial ni total, cuando se utilizó información de fuentes externas se reconoció la autoría mediante la adecuada citación y los resultados obtenidos son producto entero de mi investigación y no han sido falseados ni fabricados. Todo esto en cumplimiento del Código de ética en la investigación, Reglamento del Comité de Integridad Científica, Reglamento de Propiedad Intelectual, Normas y procedimientos de los trabajos de investigación para la obtención de títulos profesionales y grados académicos¹⁴, que afirmamos conocer en su totalidad.

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INFORME DE REVISIÓN DE ORIGINALIDAD

Título del documento evaluado. Comparación de la liberación de flúor de barnices fluorados expuestos a bebidas de consumo común: estudio in vitro

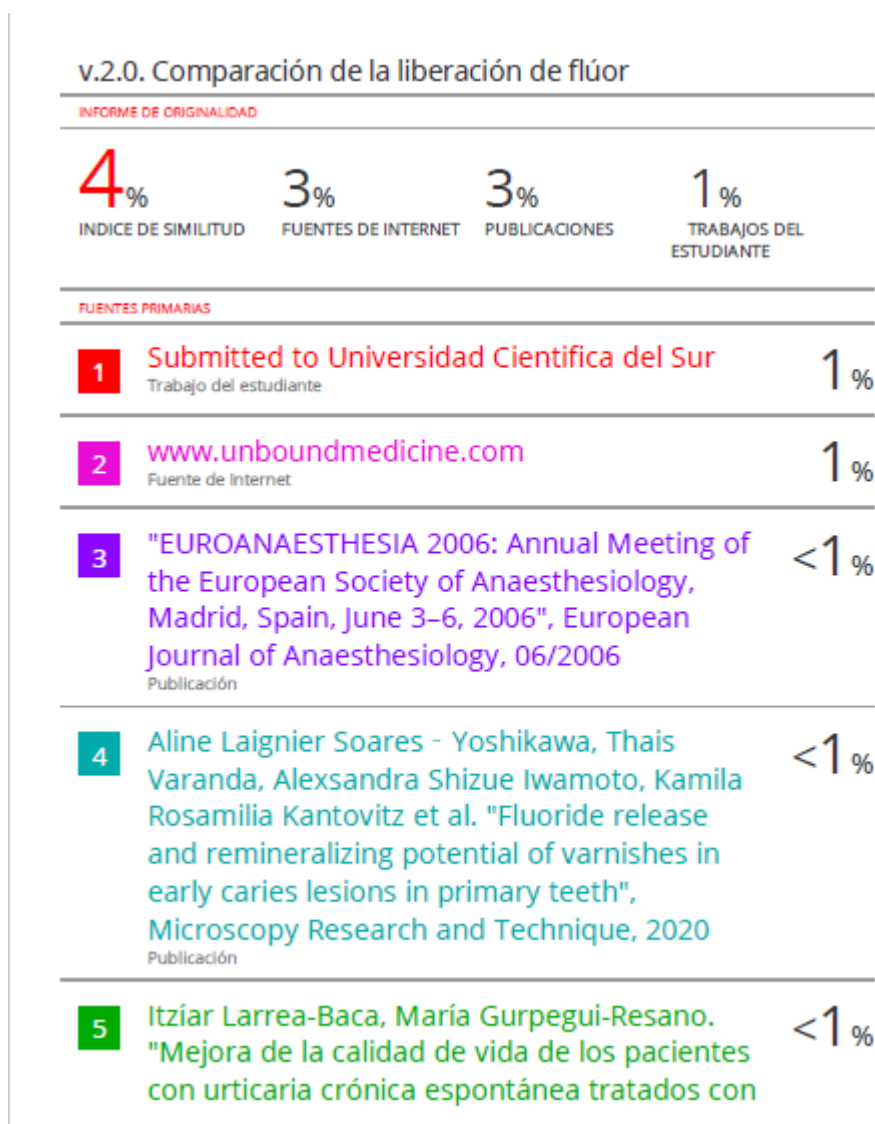
Autores. Sophia Alejandra Casimiro Iriarte.

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Comentarios sobre la revisión. Filtros usados: excluir fuentes de menos de 12 palabras.



Dedicatoria

A mamá, papá y

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por su apoyo incondicional.

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realización de esta investigación.

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realización de esta investigación.

RESUMEN

Objetivo: El objetivo de este estudio fue comparar la liberación de flúor *in vitro* de barnices fluorados expuestos a bebidas de consumo común.

Materiales y métodos: Ciento veinte bloques de acrílico fueron divididos aleatoriamente en 10 grupos de estudio (n=12 por grupo). Para la ejecución, se prepararon 24 bloques de acrílico para cada barniz fluorado (Duraphat®, Duofluorid XII®, Clinpro™, MI Varnish™ y Profluorid Varnish®). Los bloques se colocaron en saliva artificial por 30 minutos y en bebida carbonatada y jugo de fruta hasta las 24 horas. La saliva artificial y las bebidas fueron evaluadas para determinar la liberación de flúor mediante un electrodo ion-selectivo de flúor. Los resultados fueron analizados usando las pruebas de ANOVA F, Friedman y Kruskal Wallis para el análisis bivariado; y ANOVA de tres vías (barniz fluorado, bebidas, tiempo de exposición).

Resultados: Al comparar los barnices fluorados según cada tiempo de exposición, se encontró una diferencia estadísticamente significativa entre todos los barnices fluorados para cada tiempo evaluado en bebida carbonatada y jugo de frutas. MI Varnish™ presentó la mayor liberación de flúor en bebida carbonatada (94.44 ± 5.47 ppm) y en jugo de fruta (126.16 ± 8.89 ppm) a las 8 horas. Duraphat® presentó la menor liberación de flúor en el tiempo basal (0.44 ± 0.08 ppm) para el grupo expuesto a bebida carbonatada. La comparación de tres vías entre liberación de flúor, tiempo de exposición y barniz fluorado fue estadísticamente significativa ($p < 0.001$). Al evaluar el efecto de las tres variables independientes en conjunto sobre la liberación del flúor, se encontró que las variables barniz fluorado ($p < 0.001$) y tiempo de exposición ($p < 0.001$) fueron las que aportaron en la liberación del flúor.

Conclusiones: El tipo de barniz fluorado y el tiempo de exposición posterior a la aplicación contribuyen al modelo de liberación de flúor.

Palabras claves: Fluorides, Topical; Sodium Fluoride; Beverages

ABSTRACT

Background: The aim of this study was to compare the in vitro fluoride release from fluoride varnishes exposed to commonly consumed beverages.

Material and methods: One hundred and twenty acrylic blocks were divided randomly into ten experimental groups (n= 12 per group). For the experiment, 24 blocks were prepared for each fluoride varnish (Duraphat®, Duofluorid XII®, Clinpro™, MI Varnish™ and Profluorid®). The blocks were placed into artificial saliva for 30 minutes and in carbonated beverage or fruit juice for up to 24 hours. Artificial saliva and beverages were evaluated to determine the fluoride release using an ion-selective electrode. Data were analyzed using ANOVA F, Friedman and Kruskal Wallis test for bivariate analysis; and three-way ANOVA (fluoride varnish, beverages, exposure time).

Results: When the fluoride varnishes were compared according to each exposure time, a statistically significant difference was found between all the fluoride varnishes for each evaluation time on carbonated beverage and fruit juice. MI Varnish™ presented the highest fluoride release in carbonated beverage (94.44±5.47ppm) and fruit juice (126.16±8.89ppm) at 8 hours. Duraphat® presented the lowest fluoride release at baseline (0.44±0.08ppm) for carbonated beverage group. A three-way comparison between fluoride release, exposure time and fluoride varnish were statistically significant (p<0.001). When evaluating the effect of the three independent variables together on fluoride release, it was found that the variables fluoride varnish (p<0.001) and exposure time (p<0.001) contributed to the release of fluoride.

Conclusions: The type of fluoride varnish and the time after the application contribute to the fluoride release model.

Keywords: Fluorides, Topical; Sodium Fluoride; Beverages

I. VERSIÓN APROBADA PARA PUBLICAR

1. Introduction

Sodium fluoride is used in dentistry in various fluoride materials as a sensitivity treatment, as well as in dental caries prevention through topical applications, especially in children and teenagers. In 2018, the U.S. Food and Drug Administration (FDA) approved fluoride varnishes (FV) for anticaries treatment (1). Also, the recommendation of FV for individuals at risk of dental caries has been described in clinical practice guidelines and health care programs around the world (2-4). In addition, FV are a modality of topical fluoride, a term that describes delivery systems that provide fluoride to dental surfaces exposed to them, at high concentrations for a local protective effect (5). Since FV have an important role in caries prevention and is commonly used in dental practice, it is important to determine the concentration of fluoride that varnishes released.

On the other hand, it is highly common the massive consumed of beverages and food with acidic pH level from children and teenagers (6). Moreover, manufacturers do not mention the type of diet the patient should consumed after FV application. Manufacturer's instructions suggest time in which food should not be consumed and the recommended hours of a soft diet however, there are no instructions related to the pH of the diet post application (7). Hence, information that describes if an acidic condition could affect fluoride release (FR) it is necessary.

The condition to which FV is exposed could affect their FR. However, while FR has been extensive studied from different varnishes, *in vitro* and *in vivo* (8-14), there is not enough information whereas if the acidic condition modifies the release (7). Moreover, there has not been studies to determine if non-alcohol beverages could affect FR, since these commercial drinks have as part of their composition citric acid, phosphoric and malic acid (15). Thus, the aim of this study was to compare the *in vitro* release of fluoride from FV exposed to commonly consumed beverages.

2. Material and methods

Details about the composition of evaluated FV and their manufacturer's dietary instructions related to beverages are on table 1.

For sample size calculation, the formula of compare K means 1 way-ANOVA for carbonate beverage and fruit juice was used conducted at 5 % significance level and a

95% power, from the pilot study. The pilot study was based on the final sample size of a previous study ($n=3$) (7) obtaining a sample of 1 specimen per group. One hundred and twenty acrylic (methyl methacrylate) blocks (25mmx25mmx9mm) were used as study substrates and divided randomly into ten experimental groups ($n= 12$ per group):

G1: 5% NaF (gold standard: Duraphat® Colgate-Palmolive, Hamburg, Germany) exposed to carbonated beverage

G2: 6% NaF + 6% CaF₂ (Duofluorid XII® FGM, Joinville, Brazil) exposed to carbonated beverage

G3: 5% NaF + TCP (Clinpro™ 3M ESPE™, Saint Paul, USA) exposed to carbonated beverage

G4: 5% NaF + CPP-ACP (MI Varnish™ GC®, Tokyo, Japan) exposed to carbonated beverage

G5: 5% NaF (Profluorid® Voco, Cuxhaven, Germany) exposed to carbonated beverage

G6: 5% NaF (gold standard: Duraphat®) exposed to fruit juice

G7: 6% NaF + 6% CaF₂ (Duofluorid XII®) exposed to fruit juice

G8: 5% NaF + TCP (Clinpro™) exposed to fruit juice

G9: 5% NaF + CPP-ACP (MI Varnish™) exposed to fruit juice

G10: 5% NaF (Profluorid®) exposed to fruit juice

The blocks were cleaned with deionized water and the FV were applied in a smooth surface of each acrylic block. The amount of FV applied in each block was determined after measuring the unit-dose packages using an analytical balance (ED 224S, Sartorius GA, Göttingen, Germany). It was determined to use 40mg in each acrylic block using the manufacture's applicator for Clinpro™ and MI Varnish™, and a similar brush for the other three FV according to the manufacturer's instructions. For the FV in individual unit-dose package, they were thoroughly mixed prior to use with the manufacture's applicator for ten seconds to homogenize the FV since components of sodium fluoride varnishes can separate during storage (7).

For the experiment, 24 blocks were prepared for each FV: half of the acrylic blocks for carbonated beverage (Coca-Cola®) and the other half for fruit juice (Pulp®). To measure

the pH level of both beverages, a pH benchtop meter (inoLab® pH 7310, WTW™ Xylem Analytics, Weilheim, Germany) was used and the measurements were in triplicate immediately after opening at 20°C, the mean of those lectures were recorded as the pH level (15). The main characteristics of the beverages are showed in table 2.

After the FV application, each block was submerged individually in 30ml of artificial saliva in a 100ml plastic beaker (16). The beakers were placed onto an orbital shaker at 100 rpm for 30 minutes. Then, the blocks were removed from the beakers and the artificial saliva was preserved for the measure of fluoride concentration (7).

Immediately after, each block was fully submerged in the beakers with the beverages. Half of the blocks were exposed to 30ml of carbonated beverage (Coca-Cola®; pH 2.57) and the other half were exposed to 30ml of fruit juice (Pulp®; pH 3.61), the beakers were placed onto the orbital shaker at 100 rpm for 30, 420 (7 hours) and 960 minutes (16 hours). The beverages were decanted in a plastic container and renewed at 60 minutes (1 hour) and 480 minutes (8 hours) the FV application (7,13).

2.1 Measure the fluoride release

The FR was determined using an ion-selective electrode (9609 BNWP-Orion Research Inc., Beverly, MA, USA) coupled with a benchtop meter (Orion Star A214, Orion Research Inc.). The calibration of the electrode was performed using a calibration curve with standard solutions of known fluoride concentrations: 0.1, 1, 10, 40 and 100 ppm obtained from a standard solution of 100 ppm F⁻ (Thermo Scientific Orion) (13,17).

The values for the standard curve were obtained from 750 µL of Total Ionic Strength Adjustor Buffer (TISAB II, Thermo Scientific Orion) and 750 µL of the fluoride standard solutions. The fluoride concentration was calculated using a linear regression equation between de electrical conductivity (mV) and the logarithm of the fluoride concentration of the standard solutions (10,17). A new calibration was performed before reading the solutions (mean $r^2=0.9999$) and checked every hour with solutions of know fluoride concentrations: 1, 2 and 10 µg F⁻/mL (Thermo Scientific Orion) with a 2.41% of coefficient of variation between the expected and the calculated concentrations through the whole experiment.

The aliquots of each solution: artificial saliva, carbonated and fruit juice were mixed with TISAB II in a 1:1 proportion and the concentrations were obtained compared to the

standard curve, by transforming the mV to ppm. The samples were read duplicate and the mean of the two readings was recorded as the fluoride concentration (ppm) (7,17).

The statistical analyses were performed using the statistical software Stata 21.0. ANOVA F test for repeated measurements and Bonferroni post-hoc test; and Friedman test and Wilcoxon post-hoc test were used for comparisons between FR and exposure time. Kruskal Wallis and U de Mann Whitney post-hoc test were used for comparisons between FR and FV. An ANOVA three-ways was used for the effect of FV, exposure time and beverages on the FR.

3. Results

For the carbonated beverage, the FR increased from 30 minutes at baseline on artificial saliva to 8 hours for Duraphat®, Duofluorid XII® and MI Varnish™; time after which the FR decreased. On the other hand, the FR increases numerically from 30 minutes on artificial saliva to 24 hours for Clinpro™. Profluorid® also had a different tendency, with a decrease on FR from 30 minutes on artificial saliva to 1 hour, an increase from 1 to 8 hours and a final decrease on FR to 24 hours (Figure 1).

On fruit juice, FR for MI Varnish™ was similar to carbonated beverage. The FV Duraphat®, Clinpro™ and Profluorid® showed a decrease on FR from 30 minutes at baseline on artificial saliva to 1 hour, then an increase was shown from 1 to 24 hours. Duofluorid XII® presented a similar tendency than the one presented by Profluorid® exposed to carbonated beverages (Figure 2).

When the FV were compared according to each time of exposure, a statistically significant difference was found between all the FV for each of the evaluation times on carbonated beverages (Table 3) and fruit juice (Table 4). A three-way comparison between FV, exposure time and commonly consumed beverage FV was statistically significant ($p < 0.001$). When evaluating the effect of the three independent variables (FV, beverages and exposure times) together on the release of fluoride, it was found that the variables FV ($p < 0.001$) and exposure time ($p < 0.001$) were those that contributed to the release of fluoride, whereas the type of beverage was not statically significant ($p = 0.458$).

4. Discussion

The present *in vitro* study shows that each FV vary its FR in artificial saliva and commonly consumed beverages depending on the exposure time. The design of the

experiment was made to mimic the clinical conditions after a FV application. This was also important to define post-application indications and the clinician's decision to use a specific FV according to the FR needed.

The beverages used, with a pH level of 2.57 and 3.61 for Coca-Cola® and Pulp®, respectively, did not influence the FR of the tested FV. On the other hand, both exposure time and type of FV, contributed to the FR model. This is similar to other studies that compared FV under a neutral pH level (8-14,17-20).

Duraphat® and Profluorid® contain 5% NaF without another active ingredient but the FR for them in both beverages was different. Furthermore, the tendency for both FV differs under carbonated beverage but a similar tendency was shown under fruit juice, even though both FV have the same type of carriers. Duraphat® contains 5% NaF with a rosin and ethanol carrier and Profluorid® is also a colophony based FV (21). So, this difference could be associated to the manufacturer's formulation¹⁴ of the FV and to the ingredients of the beverages.

Duraphat® presented its highest FR on carbonated beverage at 8 hours and on fruit juice at 24 hours. Despite this, Duraphat® is the FV that presented less FR overall compared to the other FV which is consistent with other studies (8-13). Duraphat® is considered the "clinical reference" because it is a commonly used FV and it has been tested in similar experiments (8). Additionally, Carvalho et al described that Duraphat® exhibited the greatest preventive effect on demineralization in contrast to its low FR compared to other FV. As a result, they hypothesized that a high FR does not indicate a high cariostatic effect of the FV (13).

MI Varnish™ showed the highest FR under carbonated beverage and fruit juice, which is similar to other studies (8, 20). Dehailan et al found that, for total FR, MI Varnish™ released significantly more fluoride over a 24-hour period than all other FV (20). Also, Cochrane et al described that the FR from MI Varnish™ was significantly higher ($p < 0.05$) at 4, 24, 72 and 168 hours' time points when compared with other FVs tested (8). This FV has as part of its formula casein phosphopeptide – amorphous calcium phosphate (CPP-ACP) that contains protein and phosphate, which are essential for reducing the solubility of calcium fluoride in aqueous solutions (22), such as the ones in this experiment.

Moreover, the highest FR for MI Varnish™ was at 8 hours in both carbonated beverages and fruit juice. Cochrane et al considered this a fast release of fluoride ions. The authors attributed the fast FR to the high water solubility of CPP-ACP complexes (8).

Duofluorid XII® presented the second highest FR in carbonated beverage and fruit juice. This could be associated with its calcium fluoride and sodium fluoride composition. Cochrane et al described that all tested calcium-containing varnishes in their study have a cumulative FR at 24 hours similar or better than Duraphat® (8). Another reason could be the viscosity of the FV. For instance, Carvalho et al found that Duraphat® was more viscous than Duofluorid XII®, so Duofluorid XII® could spread over a larger area on the surface of the acrylic blocks which increased the contact with the solutions to which they were exposed probably leading to a higher FR (13).

The fluoride strength of all the tested varnishes was 22 600 ppm F⁻. All the same, the maximum fluoride release was for MI Varnish™ at 8 hours in carbonated beverage (94.44±5.47 ppm F⁻) and on fruit juice (126.168.89 ppm F⁻) with a statistically significant decrease at 24 hours. On the other hand, Clinpro™, had their peak fluoride release at 24 hours on carbonated beverage (5.12±1.13 ppm F⁻). The same happened on fruit juice to Duraphat® (1.86±0.21 ppm F⁻), Clinpro™ (4.18±1.05 ppm F⁻) and Profluorid® (8.20±0.48 ppm F⁻). In an *in vitro* study by Chhatwani et al, one of the studied varnishes, Fluor Protector S, showed its higher FR at time 1 (after 20 days of thermal cycling). For this reason, Fluor Protector S is considered by the authors to have a high initial level of FR in the oral cavity, but afterward fall to a minimum (23). This is similar to our findings where the FR was statistically different for exposure time according to each varnish.

In spite of the results from FR, it cannot be concluded that this is a measure to determine cariostatic effect of a FV. This because the constant presence at low concentrations of fluoride in the fluid phases of the oral cavity is considered its primary action in caries prevention (13). Furthermore, based on their results, Dehailan et al described that FR cannot be used as a predictive measure for enamel fluoride uptake which is why one should be careful in relating FR to FV efficacy (20). Also, an *in situ* study by Attin et al showed fluoride deposition close to the area where Duraphat® (KOH soluble) was applied, within the first day after the application. This means that the preventive action of the FV is limited to the application area (24).

Lippert concluded in a previous *in vitro* study that the pH level of the solutions in which FV were submerged affects the FR. According to this study, some FV were prone to low

pH fluoride loss compared to FR in artificial saliva (pH level = 7.0) (7). However, in the present study the beverages used, with a pH level of 2.57 and 3.61, did not influenced in the FR of the tested FV. This could be related to the solutions used, Lippert used anhydrous citric acid with pH levels of 2.27 y 3.75 (7), while we used commonly consumed commercial beverages. The ingredients of the beverages, such as phosphoric acid and acidity regulators, could also influenced in this difference as well as the exposure time evaluated.

Clinically, FV post-application instructions advice to not brush the teeth surface for approximately 24 hours, which is the main reason why we decided to evaluate for this long period of time. Additionally, Carvalho et al decided to study FR at 1, 8 and 12 hours, which they considered clinically relevant, because they described that FV only remains in contact with enamel for a few hours. However, they also decided to include other time points to determine if there was FR for longer periods of time (13). In the same way, Dehailan et al carried out an *in vitro* study in 14 FV and determined the FR on artificial saliva for a prolonged period of 24 hours in order to a better simulation of the clinical situation (20).

Bezerra et al evaluated *in vitro* different fluoridated products and observed the higher concentration of fluoride in saliva at the first hour post application. Over time (1 to 48 hours), the concentration of fluoride increased while the FR tax decreased (17). On the other hand, Carvalho et al found the highest FR during the first 8 hours post application. Also, the amount of FR from the tested FV containing CaGP was significantly higher compared to the commercial FV studied. However, after the first 8 hours, the FR of the FV containing CaGP significantly decreased (13). Virupaxi et al, conducted an *in vitro* research for 6 months and found that in the 1st week Clinpro™ XT Varnish released 18.78 ± 7.35 ppm F⁻ which decreased to 9.78 ± 4.11 ppm F⁻ after 6 months. Whilst the FR of this FV decreased, it maintained a constant FR for 6 months in comparison to Fluoritop SR which showed the highest FR at the 1st week, 66.92 ± 16.30 ppm F⁻, but rapidly decreased to 0.61 ± 0.36 ppm F⁻ after 6 months (12).

From those results, it is concluded that the recommended time between fluoride applications will depend on the fluoride varnish. Furthermore, according to our findings, the type of fluoride varnish contributes to the fluoride release model of the fluoride varnishes ($p < 0.001$).

Downey et al conducted an *in vivo* study of FR from varnishes with different compositions (5% NaF, 5% NaF + TCP and 5% NaF + ACP) in unstimulated saliva. The authors attribute the variation of the FR of the varnishes to the differences in formulation (18). Also, Dehailan et al carried out an *in vivo* study to investigate the concentrations of fluoride in biofilm, centrifuged and whole saliva after a single application of three 5% NaF varnishes (19). From the results of these studies, it is concluded that the formulation of other ingredients in FV can affect the concentration of fluoride in saliva which agrees with our findings in artificial saliva.

However, despite our effort to mimic the clinical scenario we cannot over interpret the results; because, as Bezerra et al described, the dynamics of human saliva as well as other physiological conditions, such as swallowing, chewing and body temperature, can interfere with the FR (17). Likewise, Manarelli et al defined the evaluation of the dynamics of human saliva and pH fluctuations in the oral environment as the main limitation of their *in vitro* study, since these can affect the rate of FR (14).

Low pH beverages, such as carbonated beverages and fruit juices, are highly consumed, especially by children and teenagers (15,25,26). That is why it is necessary a better understanding of FR from FV under various acidic conditions. Despite, the lack of statistically difference in this study, more experimentations must be done to determine if other formulas or a bigger difference between the pH level of the beverages might modify the outcomes. Furthermore, fluoride can be toxic only in highly elevated concentrations (5mg/kg) (27) for this reason, FV does not pose a risk of toxicity (7). However, the fluoride present in other products such as toothpaste, juices, cereal, salt and more (28) should also be taken into consideration in the risk of fluoride exposure. Though, it is necessary to determine if beverages different from the ones tested could affect the FR. This is essential, since, undoubtedly, it will be the patient who will ingest these concentrations. In addition, excessive fluoride intake has been linked to detrimental health effects such as the development of dental and skeletal fluorosis, increased bone fractures, and deficits in cognitive development in children (28-30).

5. Conclusions

The present study shows that the *in vitro* FR had a significant difference related to each type of FV and exposure times. Thus, it is concluded that the type of FV and the time after the first FV application contribute to the fluoride release model. Nevertheless, this

study presents limitations due to the fact that it is an *in vitro* experimentation and it only intended to mimic what occurs clinically. For this reason, clinical studies are necessary to confirm the results of this *in vitro* research.

6. Acknowledgments

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Table 1. Dietary directions for the tested FV related to beverages.

FV Commercial Name	Composition	Batch number	After treatment dietary directions
Duraphat® Colgate®	5% sodium fluoride (NaF)	1218-01	Do not eat solid foods for at least 4 hours after the application.
Duofluorid XII® FGM®	6% sodium fluoride (NaF) and 6% calcium fluoride (CaF ₂)	080121	
Clinpro™ White Varnish 3M®	5% sodium fluoride (NaF) + Tri-Calcium Phosphate (TCP)	NC70555	Eat soft foods and do not consume hot drinks or alcohol during the treatment period.
MI Varnish™ GC®	5% sodium fluoride (NaF) + Caesin Phosphopeptide – Amorphous Calcium Phosphate (CPP-ACP)	2003111	Avoid hard, hot or sticky foods and alcohol beverages while the FV is on the teeth.
Profluorid Varnish® Voco®	5% sodium fluoride (NaF)	2002304	Avoid solids and alcohol for 4 hours after applications.

Table 2. Main characteristics of the solutions.

Beverage	Ingredients	pH
Carbonated beverage: Coca-Cola®	Carbonated water, sucrose, caramel color SIN 150d, phosphoric acid SIN 338, natural flavors and caffeine.	2.57
Fruit juice: Pulp®	Treated water, concentrated peach puree, sugar, stabilizers INS 417, INS 440, peach flavor identical to nature, acidity regulator INS 330, vitamin C, sweeteners INS 955, INS 960, vitamin B6 and vitamin B12.	3.61
Artificial saliva	Calcium (0.1169 g of calcium hydroxide/L of deionized water); 0.9 mM of phosphorus and potassium (0.1225 g potassium phosphate monobasic/L of deionized water); 20 mM TRIS buffer (2.4280 g TRIS buffer/L of deionized water).	7.0

Table 3. Mean (DS) of Fluoride Release (ppm F-) of tested fluoride varnishes exposed to artificial saliva at baseline and to carbonated beverage (pH = 2.57) at 1, 8 and 24 hours after the first exposure.

Fluoride Varnish	30 min (Artificial saliva) Mean(SD)	1 hour Mean(SD)	8 hours Mean(SD)	24 hours Mean(SD)	p
Duraphat®	0.44(0.08)a A	0.48(0.12)aA	1.15(0.05)bA	1.01(0.07)cA	p<0.001*
Duofluorid XII®	12.08(2.06)a B	18.79(1.21)b B	49.52(12.56)c B	18.15(1.65)bd B	p<0.001* *
Clinpro™ White Varnish	1.26(0.18)a C	1.48(0.30)bC	3.83(1.30)cC	5.12(1.13)dC	p<0.001* *
MI Varnish™	5.05(0.74)a D	28.17(3.52)b D	94.44(5.47)c D	76.25(8.98)d D	p<0.001* *
Profluorid Varnish®	2.43(0.73)aE	1.08(0.09)bE	3.94(0.29)cC E	3.35(0.22)dE	p<0.001*
p	p<0.001+	p<0.001+	p<0.001+	p<0.001+	

*ANOVA F test for repeated samples, pairwise comparisons with Bonferroni test; **

Friedman test, pairwise comparisons with Wilcoxon test; +Kruskal Wallis test, pairwise comparisons with Mann Whitney U test.

Different lowercase letters indicate statistically significant differences in the comparisons of exposure times according to each varnish (row comparison).

Different capital letters indicate statistically significant differences in the comparisons of the varnishes according to each exposure time (comparison in column).

Table 4. Mean (DS) of Fluoride Release (ppm F-) of tested fluoride varnishes exposed to artificial saliva at baseline and to fruit juice (pH = 3.61) at 1, 8 and 24 hours after the first exposure.

Fluoride Varnish	30 min (Artificial saliva) Mean(SD)	1 hour Mean(SD)	8 hours Mean(SD)	24 hours Mean(SD)	p
Duraphat®	0.58(0.09)a A	0.55(0.10)aA	1.82(0.20)bA	1.86(0.21)bc A	p<0.001*
Duofluorid XII®	11.29(2.57)a B	8.11(2.34)bB	42.78(7.07)cB	14.21(7.86)a B	p<0.001* *
Clinpro™ White Varnish	1.21(0.20)aC	0.45(0.11)bC	1.36(0.53)aC	4.18(1.05)cC	p<0.001*
MI Varnish™	4.59(0.82)a D	20.12(3.98)b D	126.16(8.89)c D	91.92(8.88)d D	p<0.001*
Profluorid Varnish®	2.37(0.71)aE	2.11(0.14)aE	8.15(0.59)bE	8.20(0.48)bc E	p<0.001*
p	p<0.001+	p<0.001+	p<0.001+	p<0.001+	

*ANOVA F test for repeated samples, pairwise comparisons with Bonferroni test; **

Friedman test, pairwise comparisons with Wilcoxon test; +Kruskal Wallis test, pairwise comparisons with Mann Whitney U test.

Different lowercase letters indicate statistically significant differences in the comparisons of exposure times according to each varnish (row comparison).

Different capital letters indicate statistically significant differences in the comparisons of the varnishes according to each exposure time (comparison in column).

Figure 1. Fluoride release (ppm F-) of FV exposed to carbonated beverage.

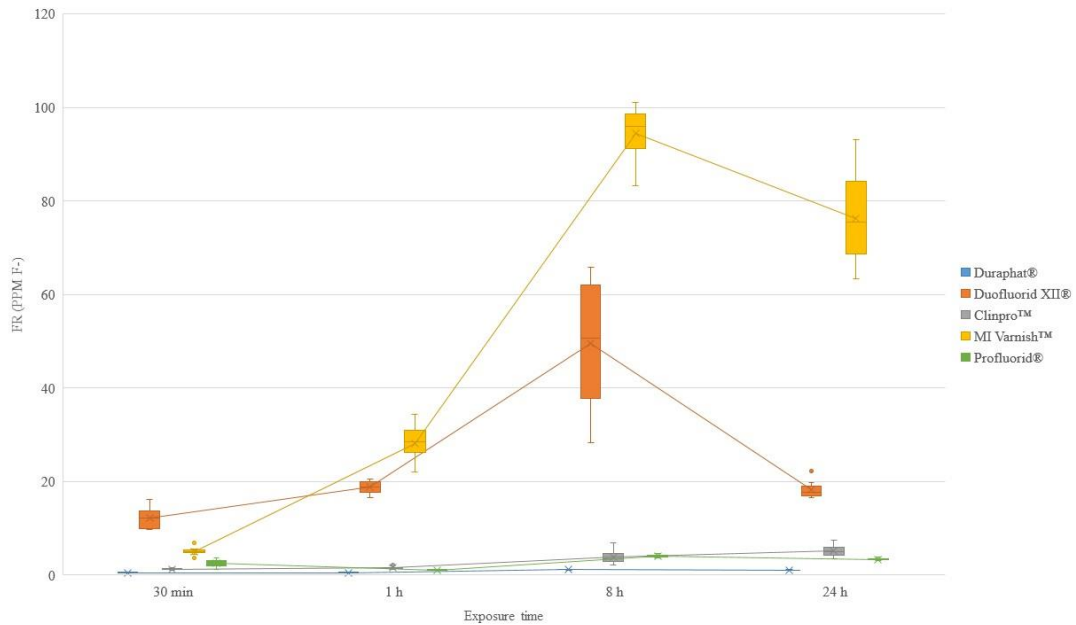
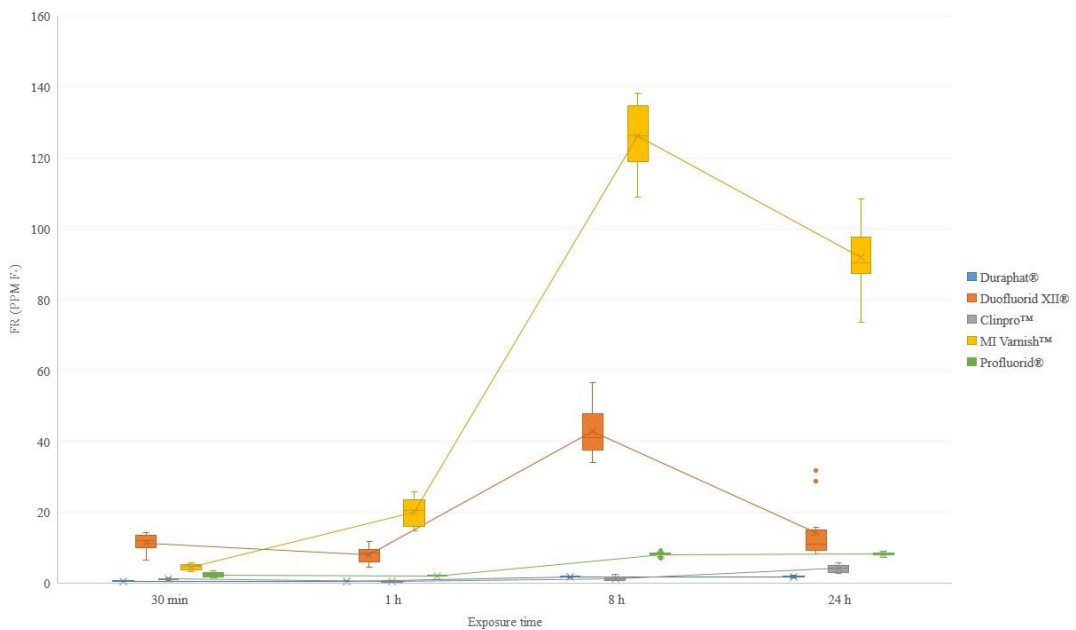


Figure 2. Fluoride release (ppm F-) of FV exposed to fruit juice.



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II. VERSIÓN INICIAL ENVIADA A PUBLICAR

1. Introduction

Sodium fluoride is used in dentistry in various fluoride materials as a sensitivity treatment, as well as in dental caries prevention through topical applications, especially in children and teenagers. In 2018, the U.S. Food and Drug Administration (FDA) approved fluoride varnishes (FV) for anticaries treatment (1). Also, the recommendation of FV for individuals at risk of dental caries has been described in clinical practice guidelines and health care programs around the world (2-4). In addition, FV are a modality of topical fluoride, a term that describes delivery systems that provide fluoride to dental surfaces exposed to them, at high concentrations for a local protective effect (5). Since FV have an important role in caries prevention and is commonly used in dental practice, it is important to determine the concentration of fluoride that varnishes released.

On the other hand, it is highly common the massive consumed of beverages and food with acidic pH level from children and teenagers (6). Moreover, manufacturers do not mention the type of diet the patient should consumed after FV application. Manufacturer's instructions suggest time in which food should not be consumed and the recommended hours of a soft diet however, there are no instructions related to the pH of the diet post application (7). Hence, information that describes if an acidic condition could affect fluoride release (FR) it is necessary.

The condition to which FV is exposed could affect their FR. However, while FR has been extensive studied from different varnishes, *in vitro* and *in vivo* (8-14), there is not enough information whereas if the acidic condition modifies the release (7). Moreover, there has not been studies to determine if non-alcohol beverages could affect FR, since these commercial drinks have as part of their composition citric acid, phosphoric and malic acid (15). Thus, the aim of this study was to compare the *in vitro* release of fluoride from FV exposed to commonly consumed beverages.

2. Material and methods

Details about the composition of evaluated FV and their manufacturer's dietary instructions related to beverages are on table 1.

One hundred and twenty acrylic (methyl methacrylate) blocks (25mmx25mmx9mm) were used as study substrates and divided randomly into ten experimental groups (n= 12 per group):

G1: 5% NaF (gold standard: Duraphat® Colgate-Palmolive, Hamburg, Germany) exposed to carbonated beverage

G2: 6% NaF + 6% CaF₂ (Duofluorid XII® FGM, Joinville, Brazil) exposed to carbonated beverage

G3: 5% NaF + TCP (Clinpro™ 3M ESPE™, Saint Paul, USA) exposed to carbonated beverage

G4: 5% NaF + CPP-ACP (MI Varnish™ GC®, Tokyo, Japan) exposed to carbonated beverage

G5: 5% NaF (Profluorid® Voco, Cuxhaven, Germany) exposed to carbonated beverage

G6: 5% NaF (gold standard: Duraphat®) exposed to fruit juice

G7: 6% NaF + 6% CaF₂ (Duofluorid XII®) exposed to fruit juice

G8: 5% NaF + TCP (Clinpro™) exposed to fruit juice

G9: 5% NaF + CPP-ACP (MI Varnish™) exposed to fruit juice

G10: 5% NaF (Profluorid®) exposed to fruit juice

The blocks were cleaned with deionized water and the FV were applied in a smooth surface of each acrylic block. The amount of FV applied in each block was determined after measuring the unit-dose packages using an analytical balance (ED 224S, Sartorius GA, Göttingen, Germany). It was determined to use 40mg in each acrylic block using the manufacture's applicator for Clinpro™ and MI Varnish™, and a similar brush for the other three FV according to the manufacturer's instructions. For the FV in individual unit-dose package, they were thoroughly mixed prior to use with the manufacture's applicator for ten seconds to homogenize the FV since components of sodium fluoride varnishes can separate during storage (7).

For the experiment, 24 blocks were prepared for each FV: half of the acrylic blocks for carbonated beverage (Coca-Cola®) and the other half for fruit juice (Pulp®). To measure the pH level of both beverages, a pH benchtop meter (moLab® pH 7310, WTW™ Xylem

Analytics, Weilheim, Germany) was used and the measurements were in triplicate immediately after opening at 20°C, the mean of those lectures were recorded as the pH level (15). The main characteristics of the beverages are showed in table 2.

After the FV application, each block was submerged individually in 30ml of artificial saliva in a 100ml plastic beaker (16). The beakers were placed onto an orbital shaker at 100 rpm for 30 minutes. Then, the blocks were removed from the beakers and the artificial saliva was preserved for the measure of fluoride concentration (7).

Immediately after, each block was fully submerged in the beakers with the beverages. Half of the blocks were exposed to 30ml of carbonated beverage (Coca-Cola®; pH 2.57) and the other half were exposed to 30ml of fruit juice (Pulp®; pH 3.61), the beakers were placed onto the orbital shaker at 100 rpm for 30, 420 (7 hours) and 960 minutes (16 hours). The beverages were decanted in a plastic container and renewed at 60 minutes (1 hour) and 480 minutes (8 hours) the FV application (7,13).

2.1 Measure the fluoride release

The FR was determined using an ion-selective electrode (9609 BNWP-Orion Research Inc., Beverly, MA, USA) coupled with a benchtop meter (Orion Star A214, Orion Research Inc.). The calibration of the electrode was performed using a calibration curve with standard solutions of known fluoride concentrations: 0.1, 1,10, 40 and 100 ppm obtained from a standard solution of 100 ppm F⁻ (Thermo Scientific Orion) (13,17).

The values for the standard curve were obtained from 750 µL of Total Ionic Strength Adjustor Buffer (TISAB II, Thermo Scientific Orion) and 750 µL of the fluoride standard solutions. The fluoride concentration was calculated using a linear regression equation between de electrical conductivity (mV) and the logarithm of the fluoride concentration of the standard solutions (10,17). A new calibration was performed before reading the solutions (mean r²=0.9999) and checked every hour with solutions of know fluoride concentrations: 1, 2 and 10 µg F⁻/mL (Thermo Scientific Orion) with a 2.41% coefficient of variation between the expected and the calculated concentrations through the whole experiment.

The aliquots of each solution: artificial saliva, carbonated and fruit juice were mixed with TISAB II in a 1:1 proportion and the concentrations were obtained compared to the

standard curve, by transforming the mV to ppm. The samples were read duplicate and the mean of the two reading was recorded as the fluoride concentration (ppm) (7,17).

The statistical analyses were performed using the statistical software Stata 21.0. ANOVA F test for repeated measurements and Bonferroni post-hoc test; and Friedman test and Wilcoxon post-hoc test were used for comparisons between FR and exposure time. Kruskal Wallis and U de Mann Whitney post-hoc test were used for comparisons between FR and FV. An ANOVA three-ways was used for de effect of FV, exposure time and beverages on the FR.

3. Results

For the carbonated beverage, the FR increased from 30 minutes at baseline on artificial saliva to 8 hours for Duraphat®, Duofluorid XII® and MI Varnish™; time after which the FR decreased. On the other hand, the FR increases numerically from 30 minutes on artificial saliva to 24 hours for Clinpro™. Profluorid® also had a different tendency, with a decrease on FR from 30 minutes on artificial saliva to 1 hour, an increased from 1 to 8 hours and a final decrease on FR to 24 hours (Figure 1).

On fruit juice, FR for MI Varnish™ was similar to carbonated beverage. The FV Duraphat®, Clinpro™ and Profluorid® showed a decreased on FR from 30 minutes at baseline on artificial saliva to 1 hour, then an increased was showed from 1 to 24 hours. Duofluorid XII® presented a similar tendency than the one presented by Profluorid® exposed to carbonated beverages (Figure 2).

When the FV were compared according to each time of exposure, a statistically significant difference was found between all the FV for each of the evaluation times on carbonated beverages (Table 3) and fruit juice (Table 4). A three-way comparison between FV, exposure time and commonly consumed beverage FV was statistically significant ($p < 0.001$). When evaluating the effect of the three independent variables (FV, beverages and exposure times) together on the release of fluoride, it was found that the variables FV ($p < 0.001$) and exposure time ($p < 0.001$) were those that contributed to the release of fluoride, whereas the type of beverage was not statically significant ($p = 0.458$).

4. Discussion

The present *in vitro* study shows that each FV vary its FR in artificial saliva and commonly consumed beverages depending on the exposure time. The design of the

experiment was made to mimic the clinical conditions after a FV application. This was also important to define post-application indications and the clinician's decision to use a specific FV according to the FR needed.

The beverages used, with a pH level of 2.57 and 3.61 for Coca-Cola® and Pulp®, respectively, did not influence the FR of the tested FV. On the other hand, both exposure time and type of FV, contributed to the FR model. This is similar to other studies that compared FV under a neutral pH level (8-14,17-20).

Duraphat® and Profluorid® contain 5% NaF without another active ingredient but the FR for them in both beverages was different. Furthermore, the tendency for both FV differs under carbonated beverage but a similar tendency was shown under fruit juice, even though both FV have the same type of carriers. Duraphat® contains 5% NaF with a rosin and ethanol carrier and Profluorid® is also a colophony based FV (21). So, this difference could be associated to the manufacturer's formulation¹⁴ of the FV and to the ingredients of the beverages.

Duraphat® presented its highest FR on carbonated beverage at 8 hours and on fruit juice at 24 hours. Despite this, Duraphat® is the FV that presented less FR overall compared to the other FV which is consistent with other studies (8-13). Duraphat® is considered the "clinical reference" because it is a commonly used FV and it has been tested in similar experiments (8). Additionally, Carvalho et al described that Duraphat® exhibited the greatest preventive effect on demineralization in contrast to its low FR compared to other FV. As a result, they hypothesized that a high FR does not indicate a high cariostatic effect of the FV (13).

MI Varnish™ showed the highest FR under carbonated beverage and fruit juice, which is similar to other studies (8,20). Dehailan et al found that, for total FR, MI Varnish™ released significantly more fluoride over a 24-hour period than all other FV (20). Also, Cochrane et al described that the FR from MI Varnish™ was significantly higher ($p < 0.05$) at 4, 24, 72 and 168 hours' time points when compared with other FVs tested (8). This FV has as part of its formula casein phosphopeptide – amorphous calcium phosphate (CPP-ACP) that contains protein and phosphate, which are essential for reducing the solubility of calcium fluoride in aqueous solutions (22), such as the ones in this experiment.

Moreover, the highest FR for MI Varnish™ was at 8 hours in both carbonated beverages and fruit juice. Cochrane et al considered this a fast release of fluoride ions. The authors attributed the fast FR to the high water solubility of CPP-ACP complexes (8).

Duofluorid XII® presented the second highest FR in carbonated beverage and fruit juice. This could be associated with its calcium fluoride and sodium fluoride composition. Cochrane et al described that all tested calcium-containing varnishes in their study have a cumulative FR at 24 hours similar or better than Duraphat® (8). Another reason could be the viscosity of the FV. For instance, Carvalho et al found that Duraphat® was more viscous than Duofluorid XII®, so Duofluorid XII® could spread over a larger area on the surface of the acrylic blocks which increased the contact with the solutions to which they were exposed probably leading to a higher FR (13).

The fluoride strength of all the tested varnishes was 22 600 ppm F⁻. All the same, the maximum fluoride release was for MI Varnish™ at 8 hours in carbonated beverage (94.44±5.47 ppm F⁻) and on fruit juice (126.168.89 ppm F⁻) with a statistically significant decrease at 24 hours. On the other hand, Clinpro™, had their peak fluoride release at 24 hours on carbonated beverage (5.12±1.13 ppm F⁻). The same happened on fruit juice to Duraphat® (1.86±0.21 ppm F⁻), Clinpro™ (4.18±1.05 ppm F⁻) and Profluorid® (8.20±0.48 ppm F⁻). In an *in vitro* study by Chhatwani et al, one of the studied varnishes, Fluor Protector S, showed its higher FR at time 1 (after 20 days of thermal cycling). For this reason, Fluor Protector S is considered by the authors to have a high initial level of FR in the oral cavity, but afterward fall to a minimum (23). This is similar to our findings where the FR was statistically different for exposure time according to each varnish.

In spite of the results from FR, it cannot be concluded that this is a measure to determine cariostatic effect of a FV. This because the constant presence at low concentrations of fluoride in the fluid phases of the oral cavity is considered its primary action in caries prevention (13). Furthermore, based on their results, Dehailan et al described that FR cannot be used as a predictive measure for enamel fluoride uptake which is why one should be careful in relating FR to FV efficacy (20). Also, an *in situ* study by Attin et al showed fluoride deposition close to the area where Duraphat® (KOH soluble) was applied, within the first day after the application. This means that the preventive action of the FV is limited to the application area (24).

Lippert concluded in a previous *in vitro* study that the pH level of the solutions in which FV were submerged affects the FR. According to this study, some FV were prone to low

pH fluoride loss compared to FR in artificial saliva (pH level = 7.0) (7). However, in the present study the beverages used, with a pH level of 2.57 and 3.61, did not influenced in the FR of the tested FV. This could be related to the solutions used, Lippert used anhydrous citric acid with pH levels of 2.27 y 3.75 (7), while we used commonly consumed commercial beverages. The ingredients of the beverages, such as phosphoric acid and acidity regulators, could also influenced in this difference as well as the exposure time evaluated.

Clinically, FV post-application instructions advice to not brush the teeth surface for approximately 24 hours, which is the main reason why we decided to evaluate for this long period of time. Additionally, Carvalho et al decided to study FR at 1, 8 and 12 hours, which they considered clinically relevant, because they described that FV only remains in contact with enamel for a few hours. However, they also decided to include other time points to determine if there was FR for longer periods of time (13). In the same way, Dehailan et al carried out an *in vitro* study in 14 FV and determined the FR on artificial saliva for a prolonged period of 24 hours in order to a better simulation of the clinical situation (20).

Bezerra et al evaluated *in vitro* different fluoridated products and observed the higher concentration of fluoride in saliva at the first hour post application. Over time (1 to 48 hours), the concentration of fluoride increased while the FR tax decreased (17). On the other hand, Carvalho et al found the highest FR during the first 8 hours post application. Also, the amount of FR from the tested FV containing CaGP was significantly higher compared to the commercial FV studied. However, after the first 8 hours, the FR of the FV containing CaGP significantly decreased (13). Virupaxi et al, conducted an *in vitro* research for 6 months and found that in the 1st week Clinpro™ XT Varnish released 18.78 ± 7.35 ppm F⁻ which decreased to 9.78 ± 4.11 ppm F⁻ after 6 months. Whilst the FR of this FV decreased, it maintained a constant FR for 6 months in comparison to Fluoritop SR which showed the highest FR at the 1st week, 66.92 ± 16.30 ppm F⁻, but rapidly decreased to 0.61 ± 0.36 ppm F⁻ after 6 months (12).

From those results, it is concluded that the recommended time between fluoride applications will depend on the fluoride varnish. Furthermore, according to our findings, the type of fluoride varnish contributes to the fluoride release model of the fluoride varnishes ($p < 0.001$).

Downey et al conducted an *in vivo* study of FR from varnishes with different compositions (5% NaF, 5% NaF + TCP and 5% NaF + ACP) in unstimulated saliva. The authors attribute the variation of the FR of the varnishes to the differences in formulation (18). Also, Dehailan et al carried out an *in vivo* study to investigate the concentrations of fluoride in biofilm, centrifuged and whole saliva after a single application of three 5% NaF varnishes (19). From the results of these studies, it is concluded that the formulation of other ingredients in FV can affect the concentration of fluoride in saliva which agrees with our findings in artificial saliva.

However, despite our effort to mimic the clinical scenario we cannot over interpret the results; because, as Bezerra et al described, the dynamics of human saliva as well as other physiological conditions, such as swallowing, chewing and body temperature, can interfere with the FR (17). Likewise, Manarelli et al defined the evaluation of the dynamics of human saliva and pH fluctuations in the oral environment as the main limitation of their *in vitro* study, since these can affect the rate of FR (14).

Low pH beverages, such as carbonated beverages and fruit juices, are highly consumed, especially by children and teenagers (15, 25, 26). That is why it is necessary a better understanding of FR from FV under various acidic conditions. Despite, the lack of statistically difference in this study, more experimentations must be done to determine if other formulas or a bigger difference between the pH level of the beverages might modify the outcomes. Furthermore, fluoride can be toxic only in highly elevated concentrations (5mg/kg) (27) for this reason, FV does not pose a risk of toxicity (7). However, the fluoride present in other products such as toothpaste, juices, cereal, salt and more (28) should also be taken into consideration in the risk of fluoride exposure. Though, it is necessary to determine if beverages different from the ones tested could affect the FR. This is essential, since, undoubtedly, it will be the patient who will ingest these concentrations. In addition, excessive fluoride intake has been linked to detrimental health effects such as the development of dental and skeletal fluorosis, increased bone fractures, and deficits in cognitive development in children (28-30).

5. Conclusions

The present study shows that the *in vitro* FR had a significant difference related to each type of FV and exposure times. Thus, it is concluded that the type of FV and the time after the first FV application contribute to the fluoride release model. Nevertheless, this

study presents limitations due to the fact that it is an *in vitro* experimentation and it only intended to mimic what occurs clinically. For this reason, clinical studies are necessary to confirm the results of this *in vitro* research.

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Table 1. Dietary directions for the tested FV related to beverages.

FV Commercial Name	Composition	Batch number	After treatment dietary directions
Duraphat® Colgate®	5% sodium fluoride (NaF)	1218-01	Do not eat solid foods for at least 4 hours after the application.
Duofluorid XII® FGM®	6% sodium fluoride (NaF) and 6% calcium fluoride (CaF ₂)	080121	
Clinpro™ White Varnish 3M®	5% sodium fluoride (NaF) + Tri-Calcium Phosphate (TCP)	NC70555	Eat soft foods and do not consume hot drinks or alcohol during the treatment period.
MI Varnish™ GC®	5% sodium fluoride (NaF) + Caesin Phosphopeptide – Amorphous Calcium Phosphate (CPP-ACP)	2003111	Avoid hard, hot or sticky foods and alcohol beverages while the FV is on the teeth.
Profluorid Varnish® Voco®	5% sodium fluoride (NaF)	2002304	Avoid solids and alcohol for 4 hours after applications.

Table 2. Main characteristics of the solutions.

Beverage	Ingredients	pH
Carbonated beverage: Coca-Cola®	Carbonated water, sucrose, caramel color SIN 150d, phosphoric acid SIN 338, natural flavors and caffeine.	2.57
Fruit juice: Pulp®	Treated water, concentrated peach puree, sugar, stabilizers INS 417, INS 440, peach flavor identical to nature, acidity regulator INS 330, vitamin C, sweeteners INS 955, INS 960, vitamin B6 and vitamin B12.	3.61
Artificial saliva	Calcium (0.1169 g of calcium hydroxide/L of deionized water); 0.9 mM of phosphorus and potassium (0.1225 g potassium phosphate monobasic/L of deionized water); 20 mM TRIS buffer (2.4280 g TRIS buffer/L of deionized water).	7.0

Table 3. Mean (DS) of Fluoride Release (ppm F-) of tested fluoride varnishes exposed to artificial saliva at baseline and to carbonated beverage (pH = 2.57) at 1, 8 and 24 hours after the first exposure.

Fluoride Varnish	30 min (Artificial saliva) Mean(SD)	1 hour Mean(SD)	8 hours Mean(SD)	24 hours Mean(SD)	p
Duraphat®	0.44(0.08)a A	0.48(0.12)aA	1.15(0.05)bA	1.01(0.07)cA	p<0.001*
Duofluorid XII®	12.08(2.06)a B	18.79(1.21)b B	49.52(12.56)c B	18.15(1.65)bd B	p<0.001* *
Clinpro™ White Varnish	1.26(0.18)a C	1.48(0.30)bC	3.83(1.30)cC	5.12(1.13)dC	p<0.001* *
MI Varnish™	5.05(0.74)a D	28.17(3.52)b D	94.44(5.47)c D	76.25(8.98)d D	p<0.001* *
Profluorid Varnish®	2.43(0.73)aE	1.08(0.09)bE	3.94(0.29)cC E	3.35(0.22)dE	p<0.001*
p	p<0.001+	p<0.001+	p<0.001+	p<0.001+	

*ANOVA F test for repeated samples, pairwise comparisons with Bonferroni test; **

Friedman test, pairwise comparisons with Wilcoxon test; +Kruskal Wallis test, pairwise comparisons with Mann Whitney U test.

Different lowercase letters indicate statistically significant differences in the comparisons of exposure times according to each varnish (row comparison).

Different capital letters indicate statistically significant differences in the comparisons of the varnishes according to each exposure time (comparison in column).

Table 4. Mean (DS) of Fluoride Release (ppm F-) of tested fluoride varnishes exposed to artificial saliva at baseline and to fruit juice (pH = 3.61) at 1, 8 and 24 hours after the first exposure.

Fluoride Varnish	30 min (Artificial saliva) Mean(SD)	1 hour Mean(SD)	8 hours Mean(SD)	24 hours Mean(SD)	p
Duraphat®	0.58(0.09)a A	0.55(0.10)aA	1.82(0.20)bA	1.86(0.21)bc A	p<0.001*
Duofluorid XII®	11.29(2.57)a B	8.11(2.34)bB	42.78(7.07)cB	14.21(7.86)a B	p<0.001* *
Clinpro™ White Varnish	1.21(0.20)aC	0.45(0.11)bC	1.36(0.53)aC	4.18(1.05)cC	p<0.001*
MI Varnish ™	4.59(0.82)a D	20.12(3.98)b D	126.16(8.89)c D	91.92(8.88)d D	p<0.001*
Profluorid Varnish®	2.37(0.71)aE	2.11(0.14)aE	8.15(0.59)bE	8.20(0.48)bc E	p<0.001*
p	p<0.001+	p<0.001+	p<0.001+	p<0.001+	

*ANOVA F test for repeated samples, pairwise comparisons with Bonferroni test; **

Friedman test, pairwise comparisons with Wilcoxon test; +Kruskal Wallis test, pairwise comparisons with Mann Whitney U test.

Different lowercase letters indicate statistically significant differences in the comparisons of exposure times according to each varnish (row comparison).

Different capital letters indicate statistically significant differences in the comparisons of the varnishes according to each exposure time (comparison in column).

Figure 1. Fluoride release (ppm F-) of FV exposed to carbonated beverage.

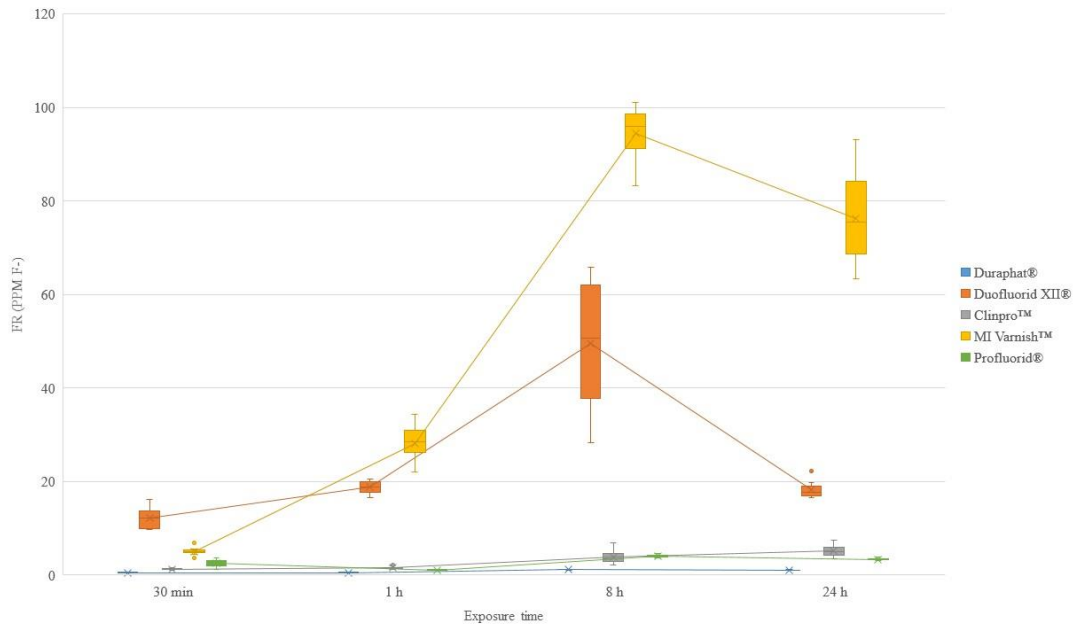
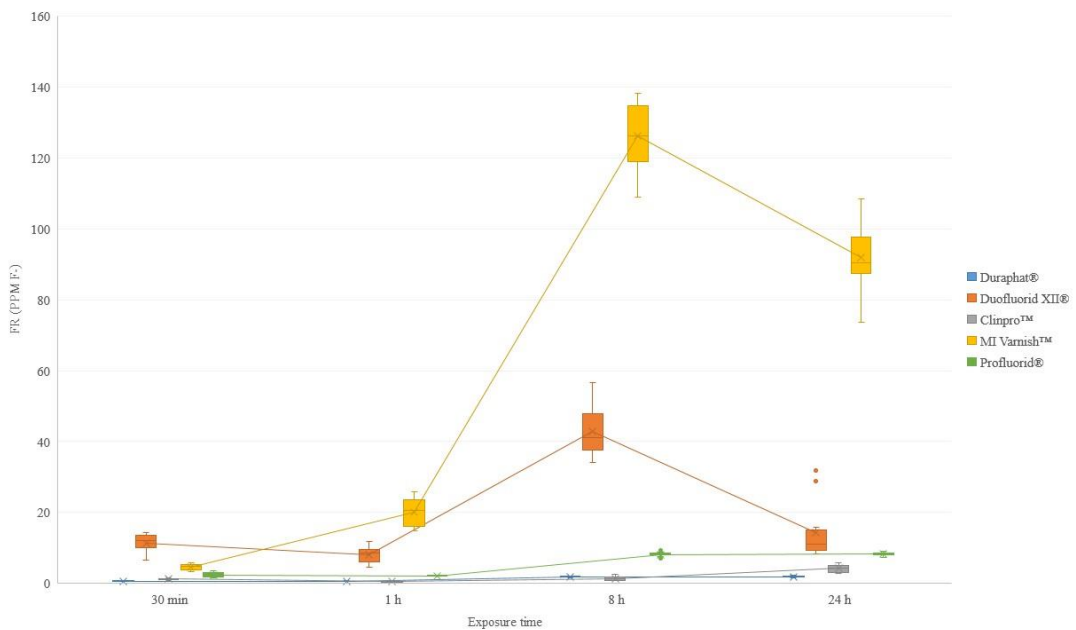


Figure 2. Fluoride release (ppm F-) of FV exposed to fruit juice.



Conflict of interest: The authors declare no conflicts of interest.

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Authors contribution: Sophia A. Casimiro-Iriarte: Conceptualization, Data curation, Formal analysis, Supervision, Writing – original draft, Writing – review & editing.
Lourdes R. Chiok-Ocaña: Conceptualization, Formal analysis, Supervision, Writing – review & editing.

III. RESPUESTA A OBSERVACIONES DE LOS REVISORES

De: jced.es <jced@jced.es>

Date: mié, 2 de nov de 2022, 5:00 a. m.

Subject: JCED_Ref.60022_Date.2022-11-01

To: <sophia.casimiroiriarte@gmail.com>

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Title: Fluoride release from fluoride varnishes exposed to commonly consumed beverages: an in vitro study

Dr. Sophia Casimiro-Iriarte

Dear Dr.

We inform you that your article has been evaluated by the reviewers; however, a series of changes are required before publication is possible (see the report below).

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Inform of the reviewers

MANUSCRIPT: "Fluoride release from fluoride varnishes exposed to commonly consumed beverages: an in vitro study".

REVIEWER'S REPORT (Final checklist):

Does the article comply with the requirements of the journal-instructions for authors?

Yes

Does the paper satisfy the aims and scope of the journal?

Yes

Is the manuscript of the required length?

Yes

Is the title adequate?

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Does the summary comply with the requirements of the journal (150-300 words, structure)?

Yes

Are the key words and running title adequate?

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Introduction: Are the references sufficient and updated?

Yes

Introduction: Are the study hypothesis and objectives clearly stated?

Yes

Material and Methods: Inclusion/exclusion criteria?

Yes

Material and Methods: Are sample selection, number of cases, and controls adequate?

Yes

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Yes

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Material and Methods: Is Ethics adequate?

Yes

Results: Are the results well described

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Results: Are statistical tests and significance stated?

Yes

Results: Are the tables and figures cited in the text?

Yes

Discussion: Is sufficient contrast made with other studies?

Yes

Discussion: Are the interpretations of the results well founded?

Yes

Tables and figures: Do they comply with the requirements of the journal?

Yes

Tables and figures: Are there too many?

No

Tables and figures: Are the abbreviations clearly defined?

Yes

Tables and figures: Are they referenced in the results section?

Yes

References: Do they comply with the requirements of the journal?

Yes

Originality of the contribution?

Yes

Are there problems of grammar?

No

Comments to the authors:

The manuscript entitled "Fluoride release from fluoride varnishes exposed to commonly consumed beverages: an in vitro study" is a very well conducted experimental study,

with good bias control and an appropriate methodology. It is an interesting article that could be published for JCED and only one point should be improved or clarified:

-Add a sample size calculation.

“For sample size calculation, the formula of compare K means 1 way-ANOVA for carbonate beverage and fruit juice was used conducted at 5 % significance level and a 95% power, from the pilot study. The pilot study was based on the final sample size of a previous study (n=3) (7) obtaining a sample of 1 specimen per group.”

IV. VERSIÓN EN ESPAÑOL

1. Introducción

El fluoruro de sodio es utilizado en odontología en distintos productos fluorados para el tratamiento de la sensibilidad dental, como también, en la prevención de caries dental mediante aplicaciones tópicas, especialmente en niños y adolescentes. En 2018, la Food and Drug Administration (FDA) de Estados Unidos aprobó el uso de los barnices fluorados (FV) en el tratamiento anticaries.¹ Además, la recomendación de utilizar FV en pacientes con riesgo de caries dental aparece a nivel mundial en distintas guías de prácticas clínicas y programas de salud oral.²⁻⁴ Asimismo, los FV son una modalidad de fluoruro tópico, un término que describe a los sistemas que proveen de flúor a las superficies dentarias expuestas a ellos, a altas concentraciones para un efecto local de protección.⁵ Por ello, ya que el FV tiene un importante rol en la prevención de caries dental y es ampliamente utilizado por los clínicos, es importante definir el aporte de flúor que proveen estos barnices.

Por otro lado, es habitual el consumo masivo de bebidas y alimentos con un pH ácido por parte de niños y adolescentes.⁶ En este sentido, las marcas comerciales no hacen mención del tipo de dieta que el paciente deberá consumir post aplicación del FV. Si bien las indicaciones del fabricante hacen referencia al tiempo en el que no se ingerirá alimentos y la cantidad de horas en las cuales se deberá tener una dieta blanda, no se tienen indicaciones claras con respecto al pH de la dieta post tratamiento.⁷ Es así que se requiere información con respecto a si el medio ácido post tratamiento podría afectar la liberación de flúor (FR).

El medio al cual está expuesto el FV podría afectar su FR. No obstante, mientras que la FR ha sido ampliamente estudiada en diversas marcas comerciales, tanto *in vitro* como *in*

vivo,⁸⁻¹⁴ existe poca información con respecto a si el medio ácido modifica esta liberación.⁷ Asimismo, no se han realizado investigaciones para determinar si las bebidas no alcohólicas afectan la FR, ya que estas bebidas comerciales tienen como aditivos el ácido fosfórico, ácido cítrico y ácido málico.¹⁵ Por ello, el objetivo de esta investigación fue comparar *in vitro* la FR de FV expuestos a bebidas de consumo común.

2. Materiales y métodos

Los detalles sobre los FV evaluados y sus respectivas instrucciones dietéticas relacionadas con las bebidas se encuentran en la tabla 1.

Para la determinación del tamaño muestral, se utilizó la fórmula de comparación de k medias one-way ANOVA para bebidas carbonatadas y jugo de frutas con un nivel de significancia de 5% y poder de 95%, en base a la prueba piloto. La prueba piloto se basó en el tamaño muestral final de un estudio previo ($n=3$)⁷ obteniéndose como tamaño muestral final un espécimen por grupo. Se utilizaron 120 bloques de acrílico (metacrilato de metilo 25mm×25mm×9 mm) como los sustratos de estudio los cuales fueron divididos aleatoriamente en 10 grupos de estudio ($n=12$ por grupo):

Grupo 1: 5% NaF (gold estándar: Duraphat® Colgate-Palmolive, Hamburgo, Alemania) expuesto a bebida carbonatada

Grupo 2: 6% NaF + 6% CaF₂ (Duofluorid XII® FGM, Joinville, Brasil) expuesto a bebida carbonatada

Grupo 3: 5% NaF + TCP (Clinpro™ 3M ESPE™, Saint Paul, EEUU) expuesto a bebida carbonatada

Grupo 4: 5% NaF + CPP-ACP (MI Varnish™ GC®, Tokio, Japón) expuesto a bebida carbonatada

Grupo 5: 5% NaF (Profluorid® Voco, Cuxhaven, Alemania) expuesto a bebida carbonatada

Grupo 6: 5% NaF (gold estándar: Duraphat® Colgate-Palmolive, Hamburgo, Alemania) expuesto a jugo de fruta

Grupo 7: 6% NaF + 6% CaF₂ (Duofluorid XII® FGM, Joinville, Brasil) expuesto a jugo de fruta

Grupo 8: 5% NaF + TCP (Clinpro™ 3M ESPE™, Saint Paul, EEUU) expuesto a jugo de fruta

Grupo 9: 5% NaF + CPP-ACP (MI Varnish™ GC®, Tokio, Japón) expuesto a jugo de fruta

Grupo 10: 5% NaF (Profluorid® Voco, Cuxhaven, Alemania) expuesto a jugo de fruta

Los bloques se limpiaron con agua desionizada y los FV fueron aplicados sobre una de las superficies lisas de cada bloque. La cantidad de FV aplicado en cada bloque fue determinado luego de pesar las dosis individuales mediante una balanza analítica (ED 224S, Sartorius GA, Alemania). Se determinó utilizar 40mg en cada bloque usando el aplicador del fabricante para el caso de Clinpro™ y MI Varnish™, y un pincel similar para los otros tres barnices según las indicaciones del fabricante. Para los FV en dosis individual, se mezcló el barniz utilizando el aplicador del fabricante durante al menos 10 segundos para homogeneizar el FV, ya que durante el almacenamiento puede haber ocurrido la sedimentación del fluoruro de sodio y la separación de fases.⁷

Para la ejecución, se prepararon 24 bloques de acrílico para cada barniz fluorado: la mitad del número de bloques para la bebida carbonatada (Coca-Cola®) y la otra mitad para el jugo de fruta (Pulp®). Para medir el pH de cada bebida se utilizó un potenciómetro digital

(inoLab® pH 7310, WTW™ Xylem Analytics, Weilheim, Alemania) en triplicado inmediatamente después de abierta a 20°C, y la media de las tres lecturas se registró como el nivel de pH.¹⁵ Las principales características de las bebidas se encuentran en la tabla 2.

Después de la aplicación de los FV, cada bloque fue sumergido individualmente en 30 ml de saliva artificial en un beaker de plástico de 100 ml.¹⁶ Los beakers fueron llevados a un agitador orbital a 100 rpm por 30 minutos. Luego, los bloques fueron retirados de los recipientes y la saliva artificial de cada beaker se preservó para la medición de la concentración de flúor.⁷

Inmediatamente después, cada bloque se sumergió por completo en los beakers con las bebidas. La mitad de los bloques fueron expuestos a 30ml de bebida carbonatada Coca-Cola®; pH 2.57) y la otra mitad fue expuesta a 30ml de jugo de fruta (Pulp®; pH 3.61), los beakers fueron llevados al agitador orbital a 100 rpm por 30, 420 (7 horas) y 960 minutos (16 horas). Las bebidas se decantaron recipientes de plástico y fueron renovadas a los 60 minutos (1 hora) y 480 minutos (8 horas) después de la aplicación de FV.^{7,13}

2.1. Medición de la liberación de flúor

La FR de las soluciones se determinó usando un electrodo ion selectivo de flúor (9609 BNWP-Orion Research Inc., Beverly, MA, EEUU), y un analizador de iones y lector de milivoltaje (Orion Star A214, Orion Research Inc.). La calibración del equipo se realizó con una curva de calibración con con soluciones estándar de concentraciones conocidas de flúor: 0.1, 1,10, 40 and 100 ppm obtenidas de una solución estándar de 100ppm F⁻ (Thermo Scientific Orion).^{13,17}

Los valores de la curva estándar se obtuvieron a partir de 750 µl de solución tampón de ajustador de fuerza iónica total (TISAB II, Thermo Scientific Orion) y 750 µl de las soluciones estándar de flúor. La concentración de fluoruro se calculó mediante una

ecuación de regresión lineal entre la conductividad eléctrica (mV) y el logaritmo de la concentración de flúor de las soluciones estándar.^{10,17} Se realizó una nueva calibración antes de la lectura de las soluciones (media $r^2=0.9999$) y se verificó cada hora con soluciones de flúor de concentraciones conocidas: 1, 2 y 10 ppm F- (Thermo Scientific Orion) con un coeficiente de variación entre 2.41% las concentraciones esperadas y las calculadas durante todo el experimento.

Las alícuotas de cada solución: saliva artificial, bebida carbonatada y jugo de fruta fueron mezcladas en proporción 1:1 con solución TISAB II y analizadas sobre su contenido de flúor en comparación con la curva estándar preparada, al transformar de mV a ppm. Las muestras fueron leídas en duplicado y la media de las dos lecturas se registró como la concentración de flúor (ppm).^{7,17}

El análisis estadístico se realizó mediante el programa estadístico Stata® versión 21.0. Se utilizó las pruebas F de ANOVA para muestras repetidas con comparaciones por pares con prueba de Bonferroni; y prueba de Friedman con comparaciones por pares con prueba de Wilcoxon para comparaciones entre FR y tiempo de exposición. La prueba de Kruskal Wallis con comparaciones por pares con U de Mann Whitney se utilizó para comparaciones entre FR y FV. La prueba F de ANOVA de tres vías fue utilizado para el efecto de los FV, tiempo de exposición y bebidas en la FR.

3. Resultados

En la bebida carbonatada, la FR aumentó numéricamente desde los 30 minutos del tiempo basal en saliva artificial hasta las 8 horas para los barnices Duraphat®, Duofluorid XII® y MI Varnish™; tiempo después del cual la liberación del flúor disminuyó. Por otro lado, la FR aumentó numéricamente desde los 30 minutos en saliva artificial hasta las 24 horas Clinpro™. Profluorid® también presentó una tendencia diferente, con una disminución

en la FR desde los 30 minutos en saliva artificial hasta 1 hora, un aumento de 1 a 8 horas y una disminución final a las 24 horas (Gráfico 1).

En el jugo de fruta, la FR de MI Varnish™ fue similar a la presentada en la bebida carbonatada. Los FV Duraphat®, Clinpro™ y Profluorid® presentaron una disminución de la FR desde los 30 minutos del tiempo basal en saliva artificial hasta 1 hora, luego hubo un aumento desde 1 hora hasta las 24 horas. Duofluorid XII® presentó una tendencia similar a la presentada por Profluorid® expuesto a bebida carbonatada (Gráfico 2).

Cuando se compararon los FV según cada tiempo de exposición, se encontró una diferencia estadísticamente significativa entre todos los FV para cada uno de los tiempos de evaluación en bebida carbonatada (Tabla 3) y en jugo de fruta (Tabla 4). La comparación de tres vías entre FV, tiempo de exposición y bebidas de consumo común fue estadísticamente significativa ($p < 0.001$). Al evaluar el efecto de las tres variables independientes (barniz, bebida y tiempos de exposición) en conjunto sobre la liberación del flúor, se encontró que las variables barniz ($p < 0.001$) y tiempo de exposición ($p < 0.001$) fueron aquellas que aportaron en la liberación del flúor, mientras que el tipo de bebida no fue estadísticamente significativo ($p = 0.458$).

4. Discusión

La presente investigación *in vitro* muestra que cada FV varía su FR en saliva artificial y en bebidas de consumo común dependiendo del tiempo de exposición. El diseño de estudio se realizó para imitar las condiciones clínicas después de la aplicación de FV. Esto es importante para definir las indicaciones post-aplicación y la decisión clínica de utilizar un FV específico según la necesidad de FR.

Las bebidas utilizadas, con un nivel de pH de 2,57 y 3,61 para Coca-Cola® y Pulp®, respectivamente, no influyeron en la FR de los FV evaluados. Por otro lado, el tiempo de exposición y el tipo de FV contribuyeron al modelo de FR. Esto es similar a otros estudios que compararon FV expuestos a un nivel de pH neutro.^{8-14,17-20}

Duraphat® y Profluorid® contienen un 5% de NaF sin ningún otro ingrediente activo; sin embargo, la FR de ambos FV fue diferente en las bebidas. Además, la tendencia de ambos FV difiere al ser expuestos a bebida carbonatada, pero se mostró una tendencia similar al ser expuestos a jugo de fruta, a pesar de que ambos FV tienen el mismo tipo de vehículo. Duraphat® contiene 5% de NaF con un soporte de colofonia y etanol y Profluorid® también es un FV a base de colofonia.²¹ Por lo que esta diferencia podría estar asociada a la formulación del fabricante del FV¹⁴ y a los ingredientes de las bebidas.

Duraphat® presentó su FR más alta en bebidas carbonatadas a las 8 horas y en jugo de frutas a las 24 horas. A pesar de esto, Duraphat® es el FV que presentó menos FR en general en comparación con los otros FV, lo que es consistente con otros estudios.^{8,13}

Duraphat® se considera la "referencia clínica" porque es un FV de uso común y ha sido estudiado en experimentos similares.⁸ Además, Carvalho et al describieron que Duraphat® exhibió el mayor efecto preventivo sobre la desmineralización en contraste con su bajo FR en comparación con otros FV. Como resultado, plantearon la hipótesis de que una alta FR no indica un alto efecto cariostático.¹³

MI Varnish™ mostró la FR más alta en bebida carbonatada y jugo de fruta, lo cual es similar a otros estudios.^{8,20} Dehailan et al encontraron que, para la FR total, MI Varnish™ liberó significativamente más fluoruro que todos los demás FV evaluados durante un período de 24 horas.²⁰ Además, Cochrane et al describieron que la FR de MI Varnish™ fue significativamente mayor ($p < 0,05$) a las 4, 24, 72 y 168 horas en comparación con

otros FV evaluados.⁸ Este FV tiene como parte de su fórmula fosfopéptido de caseína-fosfato de calcio amorfo (CPP-ACP) que contiene proteína y fosfato, los cuales son esenciales para reducir la solubilidad del fluoruro de calcio en soluciones acuosas,²² como las de este experimento.

Además, la FR más alta para MI Varnish™ fue a las 8 horas tanto en bebida carbonatada como en jugo de fruta. Cochrane et al consideraron esto como una liberación rápida de iones de fluoruro. Los autores atribuyeron la FR rápida a la alta solubilidad en agua de los complejos CPP-ACP.⁸

Duofluorid XII® presentó la segunda FR más alta en bebida carbonatada y jugo de fruta. Esto podría estar asociado a su composición de fluoruro de calcio y fluoruro de sodio. Cochrane et al describieron que todos los barnices evaluados en su estudio que contienen calcio tienen una liberación acumulada de iones de fluoruro a las 24 horas similar o mejor que Duraphat®.⁸ Otra posible razón podría ser la viscosidad del FV. Por ejemplo, Carvalho et al descubrieron que Duraphat® era más viscoso que Duofluorid XII®, por lo que Duofluorid XII® se podía esparcir sobre un área más grande en los bloques de acrílico. Esto aumentó su área de superficie de contacto con el agua circundante y posiblemente condujo a una mayor FR.¹³

La concentración de fluoruro de todos los barnices probados fue de 22 600 ppm F-. Asimismo, la liberación máxima de fluoruro fue para MI Varnish™ a las 8 horas en bebidas carbonatadas (94.44±5.47 ppm F-) y en jugo de frutas (126.168.89 ppm F-) con una disminución estadísticamente significativa a las 24 horas. Por otro lado, Clinpro™, tuvo su pico de liberación de fluoruro a las 24 horas en bebidas carbonatadas (5.12±1.13 ppm F-). Lo mismo sucedió en el jugo de frutas para Duraphat® (1.86±0.21 ppm F-), Clinpro™ (4.18±1.05 ppm F-) y Profluorid® (8.20±0.48 ppm F-). En un estudio *in vitro*

realizado por Chhatwani et al, uno de los barnices estudiados, Fluor Protector S, mostró una liberación máxima de fluoruro en el tiempo 1 (después de 20 días de ciclos térmicos). Se puede suponer que Fluor Protector puede entregar un alto nivel inicial de liberación de fluoruro al entorno de la cavidad oral circundante, pero luego los niveles de fluoruro caen al mínimo.²³ Esto es similar a nuestros hallazgos donde la FR fue estadísticamente diferente para el tiempo de exposición según cada barniz.

A pesar de los resultados de FR, no se puede concluir que esta sea una medida para determinar el efecto cariostático de un FV. Esto debido a que la acción principal del flúor en la prevención de caries está más relacionada con su presencia en las fases líquidas de la cavidad oral, donde el flúor debe estar constantemente presente en bajas concentraciones.¹³ Además, con base en sus resultados, Dehailan et al describieron que FR no se puede utilizar como una medida predictiva de la captación de fluoruro en el esmalte, por lo que se debe tener cuidado al relacionar la eficacia de FR con la de FV.²⁰ Además, el estudio *in situ* de Attin et al dejó claro que dentro del primer día después de la aplicación de un altamente concentrado FV (Duraphat® - KOH soluble) el fluoruro se deposita en proximidad al sitio de fluorización. Esto significa que la acción preventiva de los FV es limitada al área sobre el cual ha sido aplicado.²⁴

Lippert F concluyó en su estudio *in vitro* que el nivel de pH de las soluciones en las que se sumergieron los FV afectó la FR. De acuerdo con este estudio, algunos FV fueron propensos a la pérdida de flúor al ser expuestos a un pH bajo en comparación con la FR en la saliva artificial (nivel de pH = 7,0).⁷ Sin embargo, en el presente estudio las bebidas utilizadas, con un nivel de pH de 2,57 y 3,61, no influyeron en la FR de los FV evaluados. Esto podría estar relacionado con las soluciones utilizadas, Lippert F utilizó ácido cítrico anhidro con niveles de pH de 2.27 y 3.75,⁷ mientras que en el presente estudio se utilizaron bebidas comerciales de consumo común. Los ingredientes de las bebidas, como

el ácido fosfórico y los reguladores de la acidez, también pudieron influir en esta diferencia, así como el tiempo de exposición evaluado.

Clínicamente, las instrucciones posteriores a la aplicación de FV indican no cepillar la superficie de los dientes durante aproximadamente 24 horas. Esta es la razón principal por la que decidimos evaluar por este prolongado período de tiempo. Asimismo, Carvalho et al decidieron estudiar periodos de tiempo clínicamente relevantes, con intervalos de 1, 8 y 12 horas ya que describieron que los FV solo permanecen en contacto con el esmalte por unas pocas horas. Sin embargo, también decidieron incluir otros puntos de tiempo para determinar si los FV podían liberar flúor por períodos más prolongados.¹³ De la misma manera, Dehailan et al realizaron un estudio *in vitro* en 14 FV y determinaron la FR en saliva artificial durante un período prolongado de 24 horas para una mejor simulación de la situación clínica.²⁰

Bezerra et al encontraron en su estudio *in vitro* de diferentes productos fluorados que se observó una mayor concentración de fluoruro en la saliva una hora después de la aplicación. La concentración de fluoruro aumentó con el tiempo (1-48 horas), mientras que la tasa de liberación de fluoruro disminuyó.¹⁷ Por otro lado, Carvalho et al observaron que la mayor FR se produjo durante las primeras 8 horas después de la aplicación, y la cantidad de FR de los barnices probados que contenían CaGP fue significativamente mayor que la de los barnices de flúor comerciales estudiados. Sin embargo, encontraron que después del primer período de 8 horas, la cantidad de FR de estos barnices disminuyó sustancialmente.¹³ Virupaxi et al, realizaron una investigación *in vitro* durante 6 meses y descubrieron que en la primera semana Clinpro™ XT Varnish liberó $18,78 \pm 7,35$ ppm F- que disminuyó gradualmente a $9,78 \pm 4,11$ ppm F- después de 6 meses. El FR de este barniz se redujo pero aún así mantuvo una liberación constante de fluoruro hasta los 6 meses en contraste con Fluoritop SR que mostró la mayor liberación de fluoruro para la

primera semana 66.92 ± 16.30 ppm F⁻ que disminuyó rápidamente a 0.61 ± 0.36 ppm F⁻ al final de los 6 meses.¹²

De esos resultados se concluye que el tiempo recomendado entre aplicaciones de flúor dependerá del barniz de flúor. Además, según nuestros hallazgos, el tipo de FV contribuye al modelo de FR de los FV ($p < 0,001$).

Downey et al realizaron un estudio *in vivo* de FR de FV con diferentes composiciones (5% NaF, 5% NaF + TCP y 5% NaF + ACP) en saliva no estimulada. Los autores atribuyen la variación de la FR de los FV a las diferencias en la formulación.¹⁸ Además, Dehailan et al llevaron a cabo un estudio *in vivo* para investigar las concentraciones de flúor en biopelícula, saliva total y centrifugada después de una sola aplicación de tres barnices de 5% NaF.¹⁹ A partir de los resultados de estos estudios, se concluye que la formulación de otros ingredientes en los FV puede afectar la concentración de flúor en saliva, lo que concuerda con nuestros hallazgos en saliva artificial.

Sin embargo, a pesar de nuestro esfuerzo por imitar el escenario clínico, no podemos sobre interpretar los resultados; ya que, como describen Bezerra et al, la dinámica de la saliva humana puede interferir en la FR, así como otras condiciones fisiológicas propias del ser humano, como la deglución, la masticación y la temperatura corporal.¹⁷ Asimismo, Manarelli et al definieron como principal limitación de su estudio *in vitro* la evaluación de la dinámica de la saliva humana o de las fluctuaciones de pH en el ambiente oral afectando la tasa de liberación.¹⁴

Las bebidas con un pH bajo, como las bebidas carbonatadas y los jugos de frutas, son muy consumidas, especialmente por niños y adolescentes.^{15,25,26} Por eso, es necesario comprender mejor la FR de los FV en diversas condiciones ácidas. A pesar de la falta de diferencia estadística en este estudio, se deben realizar más experimentos para determinar

si otras fórmulas o una mayor diferencia entre el nivel de pH de las bebidas podrían modificar los resultados. Además, el flúor puede ser tóxico solo en concentraciones muy elevadas (5 mg/kg),²⁷ por lo que el FV no presenta riesgo de toxicidad.⁷ Sin embargo, el flúor presente en otros productos como pasta de dientes, jugos, cereales, sal y más²⁸ también debe tenerse en cuenta en el riesgo de exposición a fluoruros. Por ello, es necesario determinar si bebidas diferentes a las evaluadas podrían afectar la FR. Esto es fundamental, ya que, sin duda, será el paciente quien ingerirá estas concentraciones. Asimismo, la ingesta excesiva de flúor se ha relacionado con efectos nocivos para la salud, como el desarrollo de fluorosis dental y esquelética, aumento de las fracturas óseas y deficiencias en el desarrollo cognitivo de los niños.²⁸⁻³⁰

5. Conclusiones

El presente estudio muestra que la FR *in vitro* tiene una diferencia significativa en relación a cada tipo de FV y tiempo de exposición. Por ello, se concluye que el tipo de FV y el tiempo después de la aplicación de FV contribuyen al modelo de FR. Sin embargo, este estudio presenta limitaciones debido a que se desarrolla *in vitro* y que solo intenta imitar lo que ocurre clínicamente. Por este motivo, serán necesarios estudios clínicos que confirmen los resultados de esta investigación *in vitro*.

6. Reconocimientos

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Tabla 1. Instrucciones dietéticas de los FV evaluados en relación a bebidas.

Nombre comercial	Composición	Número de lote	Indicaciones relacionadas a la dieta
Duraphat® Colgate®	5% fluoruro de sodio (NaF)	1218-01	No consumir alimentos duros por lo menos 4 horas después de la aplicación.
Duofluorid XII® FGM®	6% fluoruro de sodio (NaF) y 6% fluoruro de calcio (CaF ₂)	080121	
Clinpro™ White Varnish 3M®	5% fluoruro de sodio (NaF) y fosfato tricálcico (TCP)	NC70555	Comer alimentos suaves y no consumir bebidas calientes ni alcohólicas durante el periodo de tratamiento.
MI Varnish™ GC®	5% fluoruro de sodio (NaC) y fosfopéptido de caseína-fosfato de calcio amorfo (CPP-ACP)	2003111	Evitar alimentos duros, calientes o pegajosos y bebidas alcohólicas mientras el FV está sobre los dientes.
Profluorid Varnish® Voco®	5% fluoruro de sodio (NaF)	2002304	Evitar sólidos y alcohol por 4 horas posteriores a la aplicación.

Tabla 2. Principales características de las soluciones.

Solución	Ingredientes	pH
Bebida carbonatada: Coca-Cola®	Agua carbonatada, azúcar, colorante color caramelo SIN 150d, acidulante ácido fosfórico SIN 338, saborizantes naturales y cafeína.	2.57
Jugo de fruta: Pulp®	Agua tratada, puré concentrado de durazno, azúcar, estabilizantes SIN 417, SIN 440, sabor durazno idéntico al natural, regulador de la acidez SIN 330, vitamina C, edulcorantes SIN 955, SIN 960, vitamina B6 y vitamina B12	3.61
Saliva artificial	Calcio (0.1169 g de hidróxido de calcio/L de agua desionizada); 0.9 mM de fósforo y potasio (0.1225 g fosfato de potasio monobásico/L de agua desionizada); 20 mM TRIS buffer (2.4280 g TRIS buffer/L de agua desionizada).	7.0

Tabla 3. Media (DS) de la liberación de flúor (ppm F-) de los barnices evaluados expuestos a saliva artificial en un tiempo basal y a una bebida carbonatada (pH = 2.57) a 1, 8 y 24 horas después de la primera aplicación.

Barniz fluorado	30 min (Saliva artificial) Media(DS)	1 hora Media(DS)	8 horas Media(DS)	24 horas Media(DS)	p
Duraphat®	0.44(0.08)a A	0.48(0.12)aA	1.15(0.05)bA	1.01(0.07)cA	p<0.001*
Duofluorid XII®	12.08(2.06)a B	18.79(1.21)b B	49.52(12.56)c B	18.15(1.65)bd B	p<0.001* *
Clinpro™ White Varnish	1.26(0.18)a C	1.48(0.30)bC	3.83(1.30)cC	5.12(1.13)dC	p<0.001* *
MI Varnish™	5.05(0.74)a D	28.17(3.52)b D	94.44(5.47)c D	76.25(8.98)d D	p<0.001* *
Profluorid Varnish®	2.43(0.73)aE	1.08(0.09)bE	3.94(0.29)cC E	3.35(0.22)dE	p<0.001*
p	p<0.001+	p<0.001+	p<0.001+	p<0.001+	

*Prueba F de ANOVA para muestras repetidas, comparaciones por pares con Prueba de Bonferroni; ** Prueba de Friedman, comparaciones por pares con Prueba de Wilcoxon; +Prueba de Kruskal Wallis, comparaciones por pares con Prueba U de Mann Whitney.

Letras minúsculas diferentes indican diferencias estadísticamente significativas en las comparaciones de los tiempos de exposición según cada barniz (comparación en fila).

Letras mayúsculas diferentes indican diferencias estadísticamente significativas en las comparaciones de los barnices según cada tiempo de exposición (comparación en columna).

Tabla 4. Media (DS) de la liberación de flúor (ppm F-) de los barnices evaluados expuestos a saliva artificial en un tiempo basal y a un jugo de fruta (pH = 3.61) a 1, 8 y 24 horas después de la primera aplicación.

Barniz fluorado	30 min (Saliva artificial) Media(DS)	1 hora Media(DS)	8 horas Media(DS)	24 horas Media(DS)	p
Duraphat®	0.58(0.09)a A	0.55(0.10)aA	1.82(0.20)bA	1.86(0.21)bc A	p<0.001*
Duofluorid XII®	11.29(2.57)a B	8.11(2.34)bB	42.78(7.07)cB	14.21(7.86)a B	p<0.001* *
Clinpro™ White Varnish	1.21(0.20)aC	0.45(0.11)bC	1.36(0.53)aC	4.18(1.05)cC	p<0.001*
MI Varnish™	4.59(0.82)a D	20.12(3.98)b D	126.16(8.89)c D	91.92(8.88)d D	p<0.001*
Profluorid Varnish®	2.37(0.71)aE	2.11(0.14)aE	8.15(0.59)bE	8.20(0.48)bc E	p<0.001*
p	p<0.001+	p<0.001+	p<0.001+	p<0.001+	

*Prueba F de Anova para muestras repetidas, comparaciones por pares con Prueba de Bonferroni; ** Prueba de Friedman, comparaciones por pares con Prueba de Wilcoxon; +Prueba de Kruskal Wallis, comparaciones por pares con Prueba U de Mann Whitney.

Letras minúsculas diferentes indican diferencias estadísticamente significativas en las comparaciones de los tiempos de exposición según cada barniz (comparación en fila).

Letras mayúsculas diferentes indican diferencias estadísticamente significativas en las comparaciones de los barnices según cada tiempo de exposición (comparación en columna).

Gráfico 1. Liberación de flúor (ppm F-) de FV expuestos a bebida carbonatada.

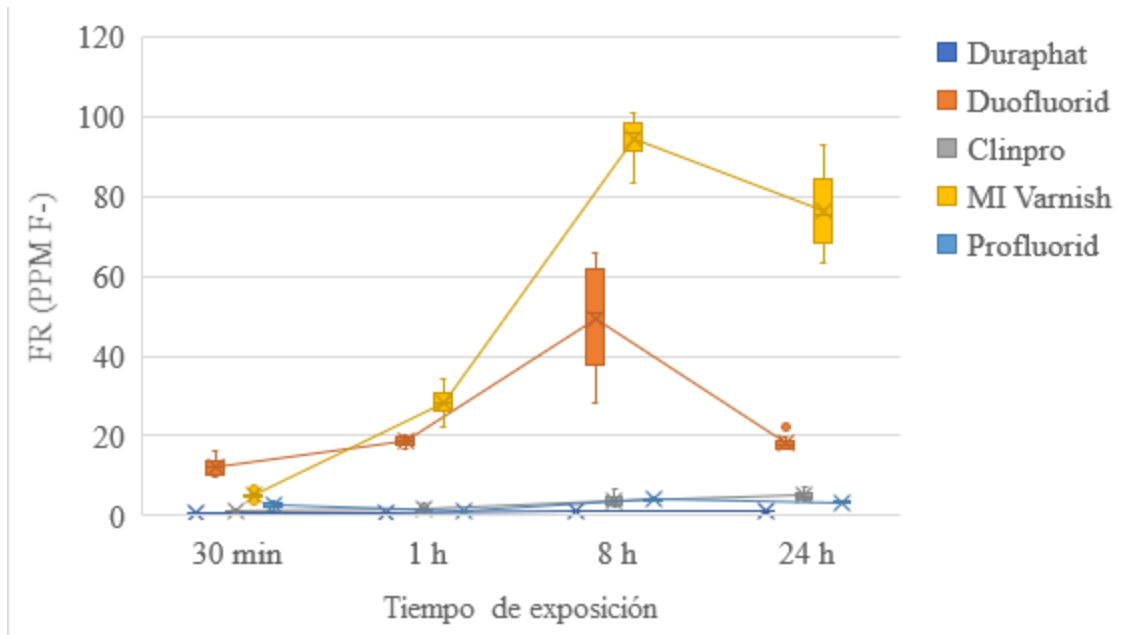
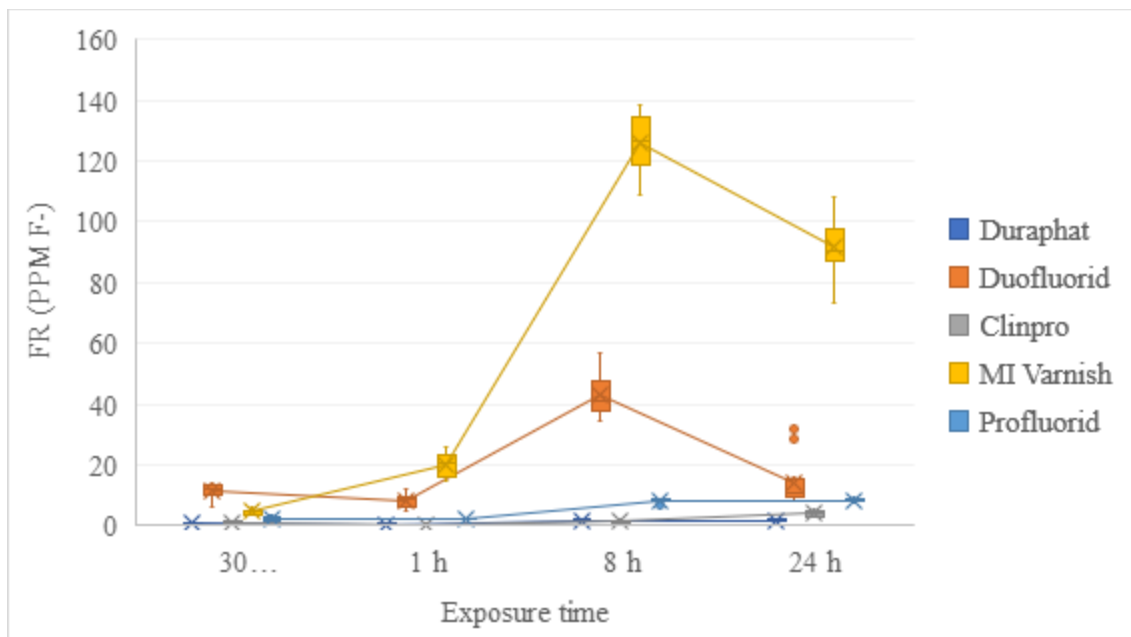


Gráfico 2. Liberación de flúor (ppm F-) de FV expuestos a juego de fruta.



Conflicto de intereses: Los autores declaran no tener conflictos de intereses.

Ética: El proyecto de investigación fue aceptado por el comité de ética de la Universidad Científica del Sur para su ejecución con el número 148-CIEI-CIENTÍFICA-2020.

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Contribución de los autores: Sophia A. Casimiro-Iriarte: Conceptualización, Curación de datos, Análisis formal, Supervisión, Redacción – borrador original, Redacción – revisión y edición. Lourdes R. Chiok-Ocaña: Conceptualización, Análisis formal, Supervisión, Redacción – revisión y edición.

VI. ABREVIATURAS

F.D.A.: Food and Drug Administration

FV: Fluoride varnishes, barnices fluorados

FR: Fluoride release, liberación de flúor

NaF: Fluoruro de sodio

CaF₂: Fluoruro de calcio

TCP: Fosfato tricálcico

CPP: Fosfopéptido de caseína

ACP: Fosfato de calcio amorfo

VII. ANEXOS

1. Prueba de publicación del artículo

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Fluoride release from fluoride varnishes exposed to beverages

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Fluoride release from fluoride varnishes exposed to commonly consumed beverages: An *in vitro* study

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Abstract

Background: The aim of this study was to compare the *in vitro* fluoride release from fluoride varnishes exposed to commonly consumed beverages.

Material and Methods: One hundred and twenty acrylic blocks were divided randomly into ten experimental groups (n= 12 per group). For the experiment, 24 blocks were prepared for each fluoride varnish (Duraphat®, Duofluorid XII®, Clinpro™, MI Varnish™ and Profluorid®). The blocks were placed into artificial saliva for 30 minutes and in carbonated beverage or fruit juice for up to 24 hours. Artificial saliva and beverages were evaluated to determine the fluoride release using an ion-selective electrode. Data were analyzed using ANOVA F, Friedman and Kruskal Wallis test for bivariate analysis; and three-way ANOVA (fluoride varnish, beverages, exposure time).

Results: When the fluoride varnishes were compared according to each exposure time, a statistically significant difference was found between all the fluoride varnishes for each evaluation time on carbonated beverage and fruit juice. MI Varnish™ presented the highest fluoride release in carbonated beverage (94.44±5.47ppm) and fruit juice (126.16±8.89ppm) at 8 hours. Duraphat® presented the lowest fluoride release at baseline (0.44±0.08ppm) for carbonated beverage group. A three-way comparison between fluoride release, exposure time and fluoride varnish were statistically significant (p<0.001). When evaluating the effect of the three independent variables together on fluoride release, it was found that the variables fluoride varnish (p<0.001) and exposure time (p<0.001) contributed to the release of fluoride.

Conclusions: The type of fluoride varnish and the time after the application contribute to the fluoride release model.

Key words: Fluorides, topical, sodium fluoride, beverages.

Introduction

Sodium fluoride is used in dentistry in various fluoride materials as a sensitivity treatment, as well as in dental caries prevention through topical applications, especially

in children and teenagers. In 2018, the U.S. Food and Drug Administration (FDA) approved fluoride varnishes (FV) for anticaries treatment (1). Also, the recommendation of FV for individuals at risk of dental caries has

e187

2. Copia de documento de aprobación de CIEI

COMITÉ INSTITUCIONAL DE ÉTICA EN INVESTIGACIÓN



CONSTANCIA N° 148-CIEI-CIENTÍFICA-2020

El presidente del Comité Institucional de Ética en Investigación de la Universidad Científica del Sur (CIEI-CIENTÍFICA) da constancia que el proyecto de investigación, cuyos datos se registran a continuación, ha sido **aprobado** por el CIEI-CIENTÍFICA.



Código de registro: **634-2019-POS8**
 Título: **Comparación de la liberación de flúor de barnices fluorados expuestos a bebidas de consumo común: estudios in vitro**
 Investigador(a): **Sophia Alejandra Casimiro Iriarte**

La aprobación del proyecto de investigación implica que el documento presentado a evaluación cumple con los lineamientos de Universidad en materia de investigación y ética, los cuales se sustentan en la normativa internacional vigente. Adicionalmente, en el contexto de la pandemia de enfermedad por COVID-19, le recomendamos cumplir con las normas publicadas por el estado peruano.

En tal sentido, esta aprobación carecerá de valor si es que el proyecto de investigación arriba indicado es modificado de cualquier forma. Toda enmienda, añadido, eliminación o eventualidad (eventos adversos, etc.) posterior a la fecha de aprobación debe ser reportado al CIEI-CIENTÍFICA.

La vigencia de la aprobación de este documento es de dieciocho (18) meses (**hasta el 07 de diciembre del 2021**), periodo en el que puede desarrollarse el proyecto. Cualquier trámite para su renovación deberá ser enviado antes del término de la vigencia de este documento, debiendo suspenderse todo desarrollo hasta que la renovación sea aprobada.

Villa El Salvador, 08 de junio de 2020



 Méd. Fernando M. Runzer Colmenares
 Presidente
 Comité Institucional de Ética en Investigación

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 informes@cientifica.edu.pe | • Calle Cantuarias 398, Miraflores

COMITÉ INSTITUCIONAL DE ÉTICA EN
INVESTIGACIÓN



Constancia

Luego de la revisión de la solicitud, el presidente del Comité Institucional de Ética en Investigación de la Universidad Científica del Sur (CIEI-CIENTÍFICA) hace constar que el CIE-CIENTÍFICA ha aprobado de forma expedita la **enmienda/modificación** del proyecto de investigación que se detalla continuación:

Código de registro: **634-2019-POS8**

Título: **Comparación de la liberación de flúor de barnices fluorados expuestos a bebidas de consumo común: estudios in vitro**

Investigador(es): **Sophia Alejandra Casimiro Iriarte**

Constancia de aprobación: **148-CIEI-CIENTÍFICA-2020**

Periodo de aprobación: **8/06/2020 al 07/12/2021**

La **enmienda/modificación** corresponde a los siguientes documentos:

1. **Ampliación de vigencia del periodo de aprobación ética.** Vista la solicitud y considerarse que está justificada, se amplía el periodo de vigencia de seis (6) meses más. Así, la vigencia se extiende hasta el 08 de junio del 2022. Si desea solicitar una extensión adicional, deberá realizar el pedido antes de esta fecha.

Lima, 12 de febrero del 2022.




Mg. Fulton Paul Rivera Albinagorta
Presidente
Comité Institucional de Ética en Investigación

**COMITÉ INSTITUCIONAL DE ÉTICA EN
INVESTIGACIÓN**



Constancia

Luego de la revisión de la solicitud, el presidente del Comité Institucional de Ética en Investigación de la Universidad Científica del Sur (CIEI-CIENTÍFICA) hace constar que el CIE-CIENTÍFICA ha aprobado de forma expedita la **enmienda/modificación** del proyecto de investigación que se detalla continuación:

Código de registro: **634-2019-POS8**

Título: **Comparación de la liberación de flúor de barnices fluorados expuestos a bebidas de consumo común: estudios in vitro**

Investigador(es): **Sophia Alejandra Casimiro Iriarte**

Constancia de aprobación: **Constancia Ref. 148-CIEI-CIENTÍFICA-2020 (12/02/22)**

La **enmienda/modificación** corresponde a los siguiente:

1. **Proyecto de investigación**, solicitud recibida el 06 de junio de 2022. Se detalla los siguientes cambios:
 - a. **Ampliación de vigencia del periodo de aprobación ética.** Vista la solicitud, se da la ampliación de la vigencia de la aprobación ética por un periodo de seis meses; es decir, hasta el 08 de diciembre del 2022. El proyecto solo podrá ejecutarse entre los periodos detallados tanto en la constancia ref. 148-CIEI-CIENTÍFICA-2020 como en esta enmienda.

Todas las demás características de la investigación descritas en el proyecto original permanecen; por lo que cualquier otra modificación deberá informarse a este Comité, para su evaluación.

Lima, 25 de agosto de 2022.



 Mg. Eulter Paul Rivera Albin
 Presidente
 Comité Institucional de Ética en Investigación

3. Copia de resolución de aprobación del proyecto de tesis



RESOLUCIÓN DECANAL DE ACEPTACIÓN E INSCRIPCIÓN DEL PROYECTO DE TESIS Y NOMBRAMIENTO DEL ASESOR

RESOLUCIÓN DECANAL ACADÉMICA DE CARRERA N° 213-DACE-DAFCS-U. CIENTIFICA-2020

Lima 02 de Octubre de 2020

VISTO:

El informe técnico del Comité Institucional de Ética en Investigación de la Universidad Científica con código N° 634-2019-POSS, se aprueba el proyecto de Tesis Titulado: “COMPARACIÓN DE LA LIBERACIÓN DE FLÚOR DE BARNICES FLUORADOS EXPUESTOS A BEBIDAS DE CONSUMO COMÚN: ESTUDIO IN VITRO” presentado por la BACH. SOPHIA ALEJANDRA CASIMIRO IRIARTE.

CONSIDERANDO:

Que de acuerdo al Reglamento para la presentación del proyecto de tesis, para obtener el Grado Académico de MAESTRO EN ESTOMATOLOGÍA en la Carrera de Estomatología de la Universidad Científica, la Bachiller, debe desarrollar la Tesis Titulada: “COMPARACIÓN DE LA LIBERACIÓN DE FLÚOR DE BARNICES FLUORADOS EXPUESTOS A BEBIDAS DE CONSUMO COMÚN: ESTUDIO IN VITRO”

La Bachiller, ha presentado los expedientes correspondientes y efectuado los pagos respectivos.

Estando a lo dispuesto por el Reglamento de Estudios de Posgrado, las disposiciones vigentes y en uso de las atribuciones conferidas al Director de la Carrera

SE RESUELVE:

ART. 1º: APROBAR e inscribir el Proyecto de Tesis titulada: “COMPARACIÓN DE LA LIBERACIÓN DE FLÚOR DE BARNICES FLUORADOS EXPUESTOS A BEBIDAS DE CONSUMO COMÚN: ESTUDIO IN VITRO” de la BACH. SOPHIA ALEJANDRA CASIMIRO IRIARTE; y establecer el inicio de periodo de ejecución del mencionado proyecto.

ART. 2º: NOMBRAR como Asesor del Trabajo de Tesis señalado, a la DRA. LOURDES ROSA CHIOK OCAÑA.

Regístrese, comuníquese y archívese.



Mg. Claudio Peña Soto
Decano de la Carrera de Estomatología

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4. Instrumentos usados en la investigación

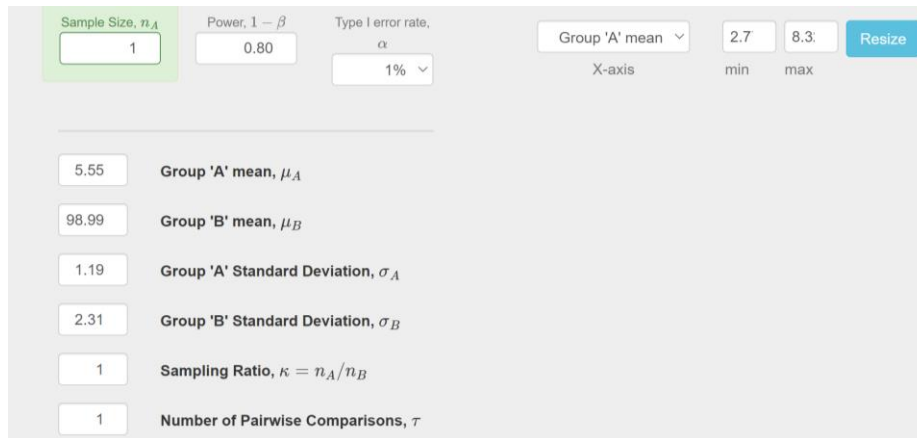
4.1. Modelo de la base de datos

Muestra	Barniz fluorado	Bebida	Tiempo de exposición	mV 1	mV 2	Promedio mV	ppm
n							
n							
n							

4.2. Fórmula de tamaño muestral a partir de la prueba piloto

Grupo A: MI Varnish™ GC® en bebida carbonatada en tiempo basal

Grupo B: MI Varnish™ en bebida carbonatada a las 8 horas



5. Fotografías

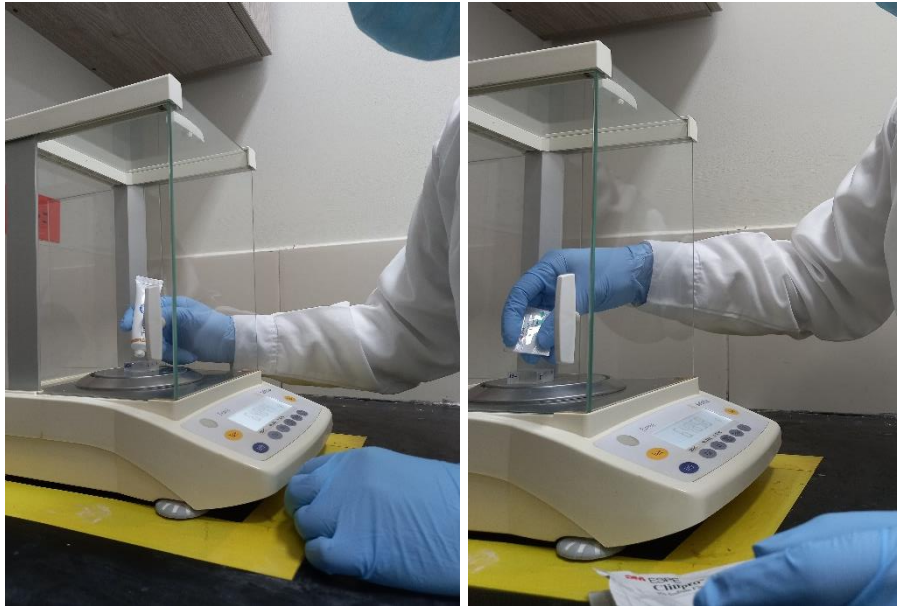
5.1. *Medición del pH de las soluciones*



5.2. *Medición de los 30ml de cada solución para cada matraz de plástico*



5.3. Medición del peso aplicado sobre cada bloque de acrílico



5.4. Aplicación de los FV sobre los bloques de acrílico



5.5. Bloques sumergidos en saliva artificial llevados al agitador orbital



5.6. Cambio de solución de los bloques: De saliva artificial a bebidas de consumo común



5.7. Calibración del electrodo ion selectivo de flúor



5.8. Medición de la concentración de flúor

