Enhanced Fluoride Bioavailability with Incorporation of Arginine in Child Dentifrices

Mohammed Nadeem Bijle*/ James Tsoi **/ Manikandan Ekambaram***/ Edward C M Lo****/Clifton M Carey*****/ Cynthia Kar Yung Yiu ******

Objective(s): *To: 1) examine the fluoride concentrations in commercial child formula dentifrices (CFD)s; and 2) investigate the effect of arginine incorporation in CFDs on fluoride bioavailability.*

Study Design: *Five commercial CFDs were examined for fluoride concentrations. Total, total soluble, and insoluble fluorides in CFDs were determined by the modified Taves acid-diffusion method (TAD). Ionic F and MFP were estimated by modified direct method with standard addition technique. L-arginine (L-Arg)/L-arginine monohydrochloride (L-Arg.HCl) were incorporated at 2% w/w in the commercial CFDs. The pH of the toothpaste slurries, buffer capacity of the added Arg, potentially available fluorides (PAF) and 1-min PAF by TAD were determined.* ***Results:*** *The CFDs had 4 to 32% of insoluble fluorides. Addition of L-Arg/L-Arg.HCl significantly improved the fluoride bioavailability in CFDs ($p<0.05$). Incorporation of L-Arg significantly increased the pH of toothpaste slurries ($p<0.05$); while L-Arg.HCl decreased the pH. Principal component analysis showed that L-Arg.HCl decreased the pH of toothpaste slurries due to the presence of Cl in the form of HCl; whereas the inherent elements/molecules (Na/P/Pi/F) remain distinct with unidentified influence on the variables.* ***Conclusion(s):*** *The CFDs containing NaF only have higher concentrations of bioavailable fluoride. Incorporating arginine (L-arginine or L-arginine monohydrochloride) at 2% w/w improves fluoride bioavailability of the child formula dentifrices.*

Keywords: *Arginine, bioavailability, child, dentifrices, fluorides.*

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INTRODUCTION

The Global Burden of Disease Study 2016 estimated that 486 million children worldwide suffer from dental caries.[1] Untreated caries with progressive destruction of teeth have negative impacts on children's quality of life.[2–4] Regular fluoride dentifrice use has considerably reduced the caries incidence globally.[5] Fluoride enhances remineralization and inhibits demineralization due to the formation of fluorapatite crystals. However, there are concerns on the risk of fluorosis with high fluoride dentifrices use (> 1000 ppm F) in very young children (< 6 years).[6,7] Therefore, low fluoride toothpastes (< 1000 ppm F) or child formula dentifrice (CFD)s for very young children are available in the market. The reduced fluoride concentration in the CFDs negatively influences the anti-caries effect of fluoridated dentifrices.[8] Thus, strategies that augment the caries-preventive potential of CFDs are highly desirable.

Caries is a biofilm-mediated disease and novel strategies directed to control oral biofilms are inevitable due to the little sustained effect of fluorides on cariogenic biofilms.[9] Arginine is a naturally occurring prebiotic for commensal alkalogenic bacteria (*Streptococcus sanguinis* and *Streptococcus gordonii*) with discernible effects on oral biofilms. It is metabolized to ammonia via the arginine deiminase (ADS) pathway, which raises the biofilm pH,

rendering the environment less conducive for the growth of cariogenic bacteria, especially *Streptococcus mutans*.[10–13] Recently, a positive correlation has been shown between caries activity and low arginolytic capacity in supra-gingival oral biofilms of children over time.[14]

Arginine is available in commercial toothpastes with high fluoride concentrations (1450 ppm F), demonstrating a superior anti-caries effect compared to fluoride (only)-dentifrices.[15,16] Furthermore, the combination of arginine and fluoride demonstrate potential synergistic effect in maintaining a healthy microbial equilibrium.[17] However, to date there has been no report on the incorporation of arginine in low fluoride CFDs on enhancing its caries-preventive potential.

The commonly used fluorides in children's dentifrices include sodium fluoride (NaF), sodium monofluorophosphate (NaMFP) and AmF (amine fluoride). To ensure bioavailability in the oral cavity for effective caries control, fluorides must be chemically free (soluble) in the dentifrice formulation.[18] However, there have been concerns that the concentration and bioavailability of fluorides in some CFDs are lower than labelled, affecting the anti-caries potential.[19] Hence, when incorporating arginine in CFDs, there is a need to assess its effect on fluoride bioavailability, which ultimately affects the clinical efficacy of CFDs. Thus, the objectives of the present study were two-fold: 1) to examine the bioavailability of fluorides in CFDs; and 2) to investigate the effect of incorporating arginine in CFDs on fluoride bioavailability. The null hypothesis tested in the present study was that the incorporation of arginine in CFDs has no influence on fluoride bioavailability.

MATERIALS AND METHOD

The study included 5 different commercial CFDs as presented in Table 1. The labelled F content of the five studied CFDs were – (i) NaF600 and (ii) MFP600, both were 600 ppm F CFDs and (iii) AmF500, (v) NaF+Xyl500, and (v) NaF500, all were 500 ppm F CFDs. Two arginine variants – L-arginine (L-Arg) and L-arginine monohydrochloride (L-Arg.HCl) at 2% w/w of dentifrices were incorporated in the commercial CFDs based on the results of our previous studies.[20,21] The unmodified CFDs were used as controls. Thus, each dentifrice has three subsequent sub-groups namely; control, incorporated with 2% L-Arg or 2% L-Arg.HCl. Eventually, there were 15 groups tested for toothpaste slurry pH; total (TF), total soluble fluoride or potentially available fluorides (TSF/PAF) and 1-min PAF by acid-diffusion methods; selective molecular and elemental analysis that might impact the anti-caries effect of the formulations.

All toothpaste slurries for the experiments were prepared with deionized water (DIW).[20] All fluoride detection experiments were performed using Fluoride-Ion Selective Electrode (F-ISE) (Orion 9609BNWP, Thermo Scientific, Newport, UK) attached to an ion benchtop meter (Orion 2700, Oakton Eutech Instruments, Singapore) which was calibrated to fresh standards of 0.1, 1, 10, 100, and 1000 µg/g F (Orion, Thermo Scientific, Newport, UK) per experiment per analysis (R^2=0.999). Calibration stability was monitored by checking standards before, during and after the sample measurements.

Total, total soluble, ionic, MFP, and insoluble fluoride in CFDs

In the present study, the terms total fluorides (TF), total soluble or potentially available fluorides (TSF/PAF), ionic fluorides, insoluble fluorides and 1-min PAF were defined as per a previous workshop paper.[22]

Total fluorides (TF) is the total amount of fluorides present in the dentifrices.

Total soluble or potentially available fluorides (TSF/PAF) in toothpastes is the fraction of total fluorides in the dentifrices that is soluble in a solvent accounting to the solubilized fluorides while brushing.

1-minute potentially available fluorides (1-min PAF) amounts for the potentially available fluorides but restricts the extraction

Table 1: Child formula dentifrices used in the study

Experimental Groups	Product	Claimed Active Ingredients	Claimed Inactive Ingredients
NaF600	Colgate kids anticavity toothpaste, Minions	Sodium Fluoride (0.132% w/v.) 600 ppm F	Sorbitol, water, hydrated silica, PEG-12, flavor, cellulose gum, tetrasodium pyrophosphate, sodium lauryl sulfate, sodium saccharin, CI 77019, CI 77891, CI 42090
MFP600	Darlie Jolly Junior	Sodium Monofluorophosphate (0.456%) 600 ppm F	Sorbitol, hydrated silica, flavor, PEG-12, sodium lauryl sulfate, tetrasodium pyrophosphate, carrageenan, benzyl alcohol, xylitol, sodium saccharin, dicalcium phosphate, titanium dioxide (CI 77891), CI 47005
AmF500	Elmex Kinder Zahnpasta	Amine Fluoride 500 ppm F	Aqua, sorbitol, hydrated silica, hydroxyethylcullose, CI 77891, cocamidopropyl betaine, olaflur, aroma, saccharin, limonene
NaF+Xyl500	Lion Kodomo	5% Xylitol and Sodium Fluoride 500 ppm F	-No mention-
NaF500	Oral-B kids toothpaste	Sodium Fluoride 500 ppm F	Sorbitol, aqua, hydrated silica, sodium lauryl sulfate, trisodium phosphate, aroma, cellulose gum, sodium phosphate, sodium saccharin, carbomer, limonene, polysorbate 80, sodium hydroxide, CI 42090

or availability of the fluorides to 1 to 3 dilution. The 1-min PAF simulates the availability of fluorides in the oral cavity after regular tooth brushing.

Ionic fluorides (ionic F) is the readily bioavailable ionic form of fluorides upon dissolution in an aqueous solvent.

Insoluble fluorides (insoluble F) is the chemical fraction of the total available fluorides that is not soluble due to the formulation constraints and thus, is not available for its potential benefits.

The TF, TSF, and insoluble F in the CFDs were determined by a modified Taves[23] diffusion analysis method as described below. Insoluble F was estimated by subtracting the determined TSF from TF. Further, % Insoluble F was computed using the following formula –

$$\% \text{ Insoluble F} = 100 \times [\text{Insoluble F/TF}]$$

To determine ionic F and MFP in the CFDs, a method was adopted as described in a previous study[24] which was modified to estimate F concentrations by standard addition method (ISO 19448). The toothpaste slurries were prepared in 1:100 dilution in DIW and the suspension was subjected to centrifugation at 5000g for 10 min. After centrifugation, the supernatant was acid hydrolyzed with 2 mol/L HCl (0.25 ml) for 1h at 45°C, neutralized with 1 mol/L NaOH (0.5 ml). To determine ionic F and MFP in the CFDs, a method was adopted as described in a previous study[24] which was modified to estimate F concentrations by standard addition method (ISO 19448). The toothpaste slurries were prepared in 1:100 dilution in DIW and the suspension was subjected to centrifugation at 5000g for 10 min. After centrifugation, the supernatant was acid hydrolyzed with 2 mol/L HCl (0.25 ml) for 1h at 45oC, neutralized with 1 mol/L NaOH (0.5 ml) and supplemented with total ionic strength adjustment buffer II (TISAB II) to determine TSF in the toothpaste. The supernatant was serially supplemented with 1 mol/L NaOH, 2 mol/L HCl, and TISAB II to measure ionic F. MFP was calculated by subtracting the ionic F from TSF. Primarily, internal standards were prepared following the same steps as the samples. For the standard addition method, internal standards were prepared to estimate the calibration curve for calculating the measured F concentrations. A series of test solutions with DIW and standards in 4:1 ratio were prepared before supplementing the solutions with TISAB II. A scatter plot of added F for each test solutions to measured F concentration was constructed (R2>0.99) to determine the regression equation whereby the absolute value of x-intercept was the F concentration in the sample.

Toothpaste slurry preparation

The toothpaste slurries were prepared in 1:3 ratio with DIW. For slurry preparation, 10 g of toothpaste was supplemented with/without 2% L-Arg/L-Arg.HCl w/w (as per the experimental group) followed by suspending the combination in 30 mL DIW. The suspension was vortexed for 60 s prior to further analysis. Caution was taken to weigh the slurries on a scale to avoid pipetting errors due to foamy consistency of certain dentifrice slurries. All measurements were made on a single balanced scale to avoid inter-equipment errors.

pH of toothpaste slurry

The pH of toothpaste slurries prepared in 1:3 ratio with DIW was estimated using pH electrode (E-201-C, e-Gizmo Mechatronix, Manila, Philippines) calibrated to 4.01, 7.00, and 10.01 external pH standards through an ion benchtop meter (Orion 2700, Oakton Eutech Instruments, Singapore). The electrode was mounted on a stand with test tube holder and stationed in the center of the tube with micro-stir magnetic bars on a digital magnetic stirrer (Wise-Stir, Daihan Scientific, Seoul, South Korea) to provide continuous stirring during measurements. The potentiometer was set on an auto-read mode and measurements were made in triplicate for each sample.

The buffer capacity of the added arginine (in equiv./L) was calculated using the following formula as per a previous study[25]

$$B = 2.303 \left[\frac{Ka\,[buff][H]}{(Ka + [H])^2} + [H] + \frac{Kw}{[H]}\right]$$

Whereby;

B = buffer capacity of the added arginine

Ka = dissociation constant for the added arginine

[*buff*] = concentration of the added arginine

Kw = dissociation constant for water

[H] = hydrogen ion concentration of slurries with added arginine

Total, potentially and 1-min potentially available fluoride by Taves Diffusion Analysis

The TF, PAF, and 1-min PAF were determined by modified Taves[23] diffusion analysis. The sample preparation method to determine TF was similar to that of toothpaste slurry preparation; whereas the slurries were centrifuged at 5000*xg* for 2 min to obtain the supernatant which was utilized to determine PAF. For 1-min PAF, the slurries were diluted 10-folds and centrifuged for 2 min at 5000*xg* to obtain supernatant which was further diluted 10-folds in DIW to derive the sample for analysis.

The modified Taves diffusion analysis was performed on Conway Diffusion Dishes (Bel-Art, Thermo Fisher Scientific, Stockholm, Sweden) lubricated with petroleum jelly (Vaseline, Unilever, London, UK) that held 1 mol/L $HClO_4$ saturated with 2.5% hexamethyldisiloxane (HMDS) and 1 mol/L KOH at different compartments within the dishes. The samples were added to the acid containing compartments and care was taken to immediately seal the dishes to avoid any leakage of HMDS-F complex. The dishes were subjected to tilt-mix by placing on an orbital shaker (Labnet, Woodbridge, USA) at 60 rpm at room temperature. The dishes were left overnight on the shaker to permit adequate acid diffusion of the samples. Then, the fluoride trapped KOH was neutralized with 1 mol/L HCl and TISAB II (1:2) thoroughly homogenized to estimate fluoride through the calibrated F-ISE. Because the diffusion of F from the sample to the KOH is not 100 % efficient, F standards were prepared and subjected to the same diffusion steps as the samples. These internal standards were used to calculate the sample F concentrations.

The % change in F bioavailability with L-Arg.HCl/L-Arg at PAF and 1-min PAF was calculated using the following formula:

$$\% \text{ increase in F} = [(\text{experimental group F} - \text{control group F})/\text{control group F}] \times 100$$

Molecular (arginine and Pi) and elemental (Ca, P, Na, Cl) analysis

The molecules arginine (o-phthaldialdehyde derivatization method) and inorganic phosphate (Pi) (malachite green phosphate detection kit) were detected using a microplate spectrophotometer. The elemental analysis for Ca, P, Na and Cl was done using ICP-OES (Spectro Arcos, Ametek, Germany). The data was obtained to further evaluate the interaction of the examined variables in the study.

For arginine detection, o-phthaldialdehyde was dissolved in absolute ethanol, β-mercaptoethanol and 50 mmol/L of Na_2CO_3 were added to the dissolved o-phthaldialdehyde which was further diluted with DIW. The working solution was then added to the sample (1:10) in a 96-well microplate along with 8-times serially 2-folds diluted 10 µg/g arginine standards (R^2>0.99). The plate was read using end-point fluorescence with excitation at 340 nm and emission at 455 nm.

The Pi was detected using a malachite green phosphate detection kit (R & D systems, Minneapolis, USA) with high sensitivity microplate assay protocol. A 6-point standard curve was derived using 0.1 mol/L standard phosphate with the reagent kits. The reagents were thoroughly mixed with the samples in a 96-well microplate which was read for end-point absorbance at 620 nm. The Pi content for the samples was computed in µg/g.

The elemental analysis for Ca, P, Na and Cl was done using ICP-OES (Spectro Arcos, Ametek, Kleve, Germany) with 6-point standard curve for each element in 2% v/v HNO_3 as a medium. The spectrum was first calibrated and the study solutions were drawn in triplicate to measure the concentration in µg/g using Smart Analyser Vision, 2014 v.6.01.0943 (Spectro Analytical Instruments, GmBH, Kleve, Germany). The final determined concentrations were presented as an average in µg/g for individual element per sample.

Statistical analysis

All experiments were done in triplicate and *a priori* ensured for reproducibility with at least 3 individually prepared samples (per experiment) from different toothpaste tubes within the group. The data were analyzed using SPSS version 24 (IBM SPSS® Statistics Inc, New York, USA).

Independent t-test was used to analyze differences between the TF and TSF in each group.

Similarly, independent t-test was used to evaluate the differences in the ionic F content between NaF600 and MFP600 – 600 ppm F dentifrices.

While, 1-way ANOVA with Tukey's HSD test was performed to evaluate the differences in ionic F (500 ppm F dentifrices), % insoluble F in CFDs, pH of toothpaste slurries, PAF and 1-min PAF.

Two-way ANOVA with Bonferroni's post-hoc test was performed to evaluate the effect of "CFDs" and "arginine variants" on buffer capacity of the added arginine.

The data at all levels for individual experimental and control groups were arranged to extract variables by dimension reduction based on correlation matrix. Principal component factor analysis (PCA) was done using varimax rotation method to identify the model explaining the variations in the study following incorporation of arginine variants on the elements, pH, detected molecules and its effect on fluoride bioavailability.

RESULTS

TF, TSF, ionic F, insoluble F, MFP and % insoluble F in CFDs

The results for TF, TSF, and insoluble F in CFDs are shown in Figure 1a. The estimated TF for all tested CFDs except NaF500 was less than the labelled F. The estimated TF for all the groups was significantly higher than the TSF ($p<0.05$).

The ionic F and MFP content in CFDs are presented in Figure 1b. Amongst the 600 ppm F dentifrices, the ionic F content for NaF600 was higher than MFP600 ($p<0.05$). Though the ionic F for MFP600 was the least, it had the highest amount of estimated MFP ($p<0.05$). While the ionic F content for NaF500 > NaF+Xyl500 > AmF500 ($p<0.05$), within the 500 ppm F dentifrices.

The % insoluble F in the CFDs is shown in Figure 1c. The % insoluble F for all CFDs ranged from 4 – 32% and was the highest for AmF500, followed by MFP600 > NaF+Xyl500 > NaF600 > NaF500 ($p<0.05$).

Thus, it can be concluded that all CFDs tested in the present study demonstrated insoluble F content. The NaF groups (600 ppm and 500 ppm F) have higher total soluble F, higher ionic F and lower % insoluble F. In contrast, AmF500 has the lowest soluble F, lower ionic F and higher % insoluble F.

pH of toothpaste slurries

Figure 2a shows the pH of toothpaste slurries. Except for AmF500, the incorporation of L-Arg.HCl into toothpaste slurries significantly decreased the pH of the toothpaste slurries ($p<0.05$) (Figure 2a). By contrast, the incorporation of L-Arg in all CFDs significantly increased the pH of the toothpaste slurries as compared to their control counterparts ($p<0.05$) (Figure 2a).

Figure 2b shows the buffer capacity of the added arginine that indicates the resistance to pH change. Two-way ANOVA testing revealed that "arginine variants" ($p<0.001$) and "CFDs" ($p<0.001$) had a significant effect on the buffer capacity of the added arginine. Interaction of the two factors was also significant ($p<0.001$). In general, the buffer capacity of the added L-Arg was significantly higher than the added L-Arg.HCl ($p<0.05$) (Figure 2b). The buffer capacity of the added L-Arg was the highest in NaF600, followed by MFP600 = NaF+Xyl500 > NaF500 = AmF500 ($p<0.$**05) (Figure 2b).** Conversely, the buffer capacity of the added L-Arg.HCl for AmF500 was significantly higher than the rest of the CFDs ($p<0.05$ (**Figure 2b**).

Overall, the incorporation of L-Arg significantly increases the pH of the toothpaste slurries with a higher buffer capacity of added L-Arg; while L-Arg.HCl decreases the slurry pH in CFDs.

PAF and 1-min PAF in control and experimental toothpaste slurries

Table 2 shows the results for PAF determined in CFDs and their experimental counterparts after incorporation of L-Arg and L-Arg. HCl by acid-diffusion method; whereas Table 3 shows the results for 1-min PAF determined using acid-diffusion method in experimental and control CFDs.

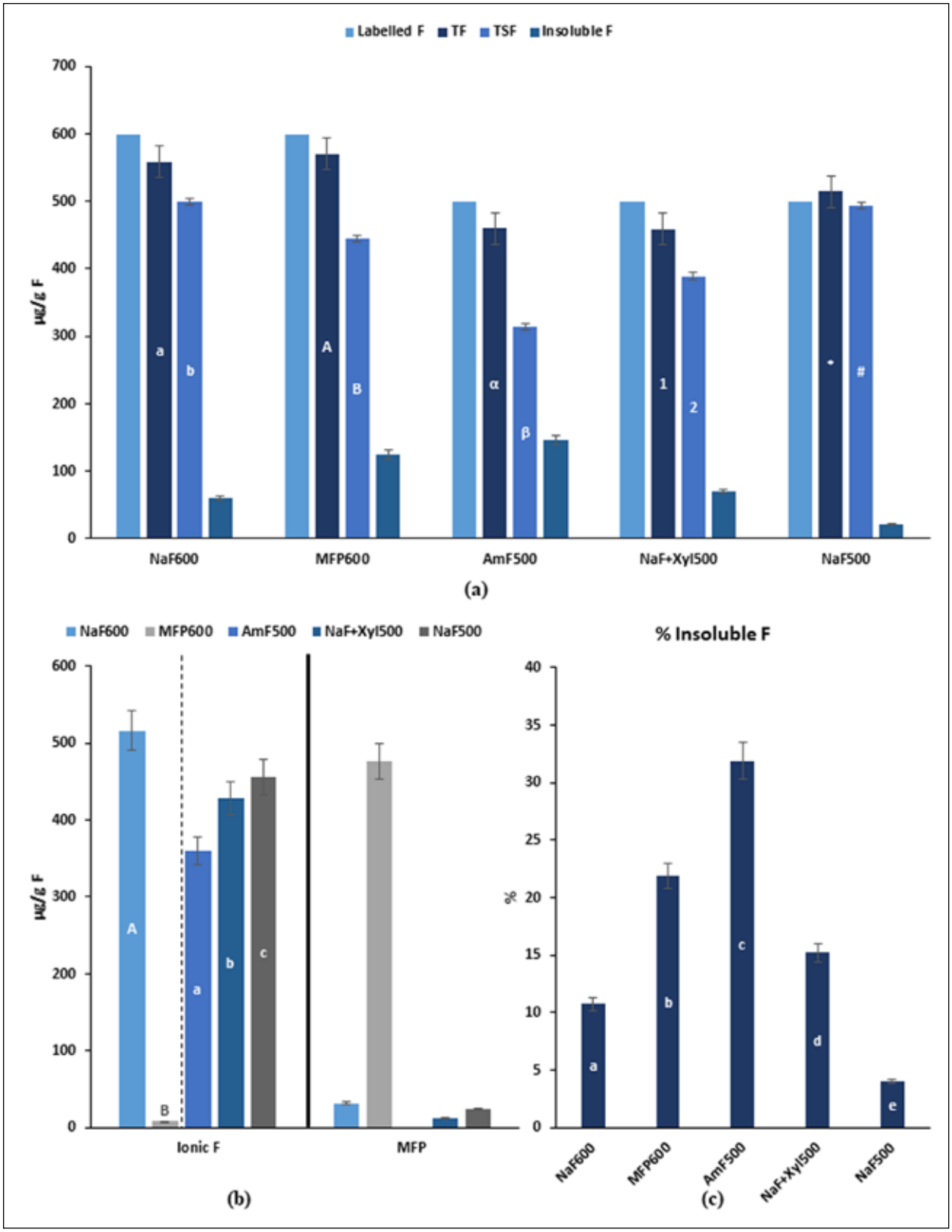


Figure 1: (a) Labelled, total, total soluble, and insoluble fluoride estimated by Taves Diffusion Analysis (n=3) (Differences between total and total soluble fluorides in each groups are shown by either small alphabets (a,b), capital alphabets (A,B), Greek alphabets (α,β), numbers (1,2), and signs (*,#); (b) ionic fluoride and MFP estimated by modified direct method with standard addition technique (n=3) (Capital alphabets – A & B indicate differences analyzed using independent t-test; small alphabets – a, b, c indicate differences analyzed using 1-way ANOVA with Tukey's HSD post-hoc test) ; and (c) % insoluble fluoride in child formula dentifrices (n=3) (small alphabets – a, b, c, d, e indicate differences analyzed using 1-way ANOVA with Tukey's HSD post-hoc test).

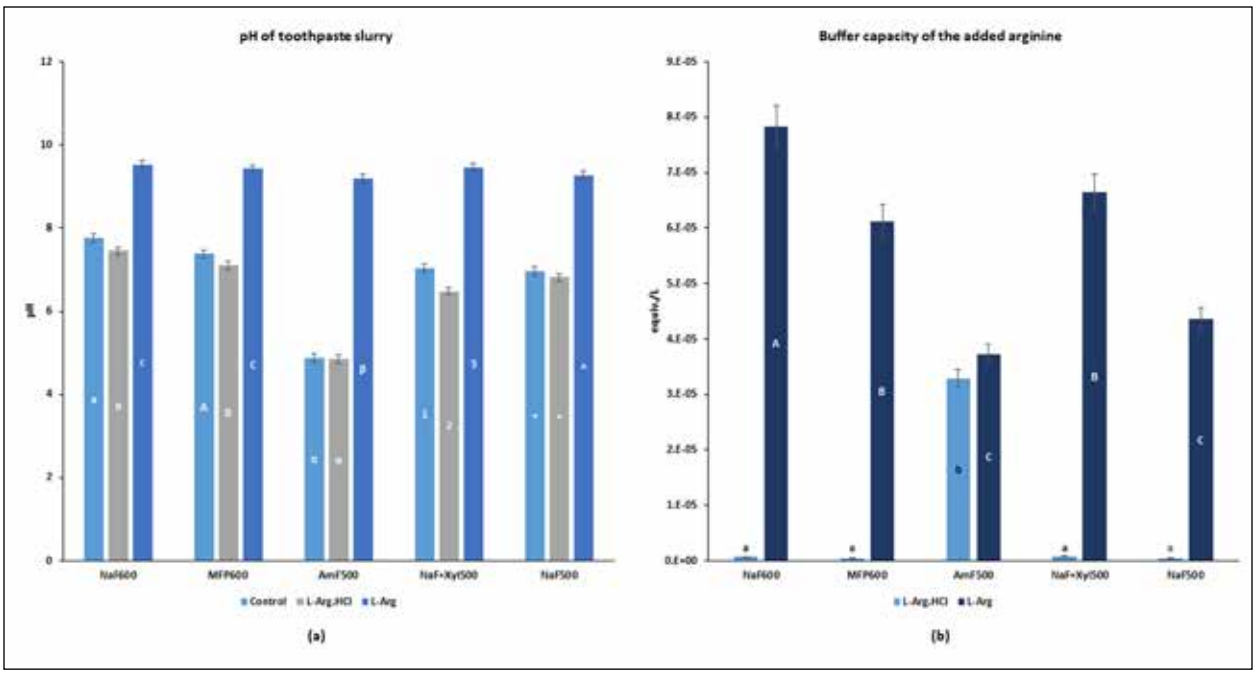


Figure 2: (a) pH of control and experimental toothpaste slurries (n=3) (small alphabets, capital alphabets, Greek alphabets, numbers, and signs indicate differences between the experimental and control pH slurries in each group. The differences were discerned using 1-way ANOVA with Tukey's post-hoc test); and (b) Buffer capacity of the added arginine (n=3) (Small alphabets indicate differences between groups after incorporating L-Arg.HCl; while capital alphabets indicate differences between groups after incorporating L-Arg in the dentifrices. The differences were discerned using 2-way ANOVA with Bonferroni's post-hoc test).

Table 2: Potentially available fluorides (n=3) in the experimental and control groups (small alphabets indicate differences between the experimental and control groups identified by 1-way ANOVA with Tukey's HSD test in each row).

PAF in µg/g F (Mean ± SD)			
Groups	**Control**	**+ L-Arg. HCl**	**+ L-Arg**
NaF600	499.20 ± 1.77^{a}	516.19 ± 2.57^{b}	513.98 ± 0.73^{c}
MFP600	445.66 ± 1.37^{a}	453.28 ± 2.37^{b}	449.87 ± 0.95^{c}
AmF500	313.38 ± 1.15^{a}	324.19 ± 0.97^{b}	329.28 ± 3.43^{c}
NaF+Xyl500	389.17 ± 3.80^{a}	401.25 ± 1.70^{b}	405.81 ± 2.43^{c}
NaF500	493.59 ± 2.79^{a}	506.54 ± 2.40^{b}	501.55 ± 1.06^{c}

The incorporation of L-Arg and L-Arg.HCl in CFDs significantly increased PAF and 1-min PAF as compared to their controls ($p<0.05$) (Table 2 and 3). No significant difference in 1-min PAF was observed between the L-Arg and L-Arg.HCl incorporated NaF600 and NaF+Xyl500 CFDs ($p>0.05$) (Table 3).

Figure 3a shows the % increase in PAF and 1-min PAF with incorporation of L-Arg.HCl. The % increase in PAF for NaF600, AmF500, NaF+Xyl500 and NaF500 was significantly higher than MFP600 ($p<0.05$); while the % increase in 1-min PAF was the highest with AmF500 > MFP600 = NaF500 > NaF600 and NaF+Xyl500 ($p<0.05$).

Figure 3b shows the % increase in PAF and 1-min PAF with incorporation of L-Arg in CFDs. The % increase in PAF for AmF500 and NaF+Xyl500 > NaF600 > NaF500 = MFP600 ($p<0.05$). The % increase in 1-min PAF was the highest for AmF500 ($p<0.05$), followed by NaF500 > MFP600 > NaF600 = NaF+Xyl500 ($p<0.05$) (Figure 3b).

Overall, the acid-diffusion method results demonstrated that incorporating L-Arg and L-Arg.HCl significantly improved the F bioavailability in CFDs, ranging from 1-13%;

Table 3: 1-min potentially available fluorides (n=3) in the experimental and control groups (small alphabets indicate differences between the experimental and control groups identified by 1-way ANOVA with Tukey's HSD test in each row).

1-min PAF in µg/g F (Mean ± SD)			
Groups	**Control**	**+ L-Arg. HCl**	**+ L-Arg**
NaF600	401.39 ± 5.32^{a}	414.22 ± 0.77^{b}	413.83 ± 0.88^{b}
MFP600	368.24 ± 2.08^{a}	400.80 ± 1.72^{b}	396.67 ± 2.23^{c}
AmF500	215.41 ± 0.85^{a}	239.03 ± 1.83^{b}	242.31 ± 0.51^{c}
NaF+Xyl500	295.28 ± 0.63^{a}	301.32 ± 1.18^{b}	301.89 ± 0.67^{b}
NaF500	302.88 ± 0.64^{a}	327.08 ± 5.57^{b}	336.09 ± 1.32^{c}

Principal Component Analysis

The analysis depicted that Cl in L-Arg.HCl significantly reduced the pH of the toothpaste slurries, showing strong inverse correlations between pH and element-Cl (Figure 4). Calcium remained undetermined in the tested samples and it was not included in the analysis. The eigenvalues as demonstrated in the scree plot reduced the dimensions of the variable to 3 components. The magnitude of the variables in the individual components was identified by the varimax rotation method as presented in Figure 4. The 3 factors/reduced components were namely toothpaste elements/molecules, variables affecting pH, and added arginine.

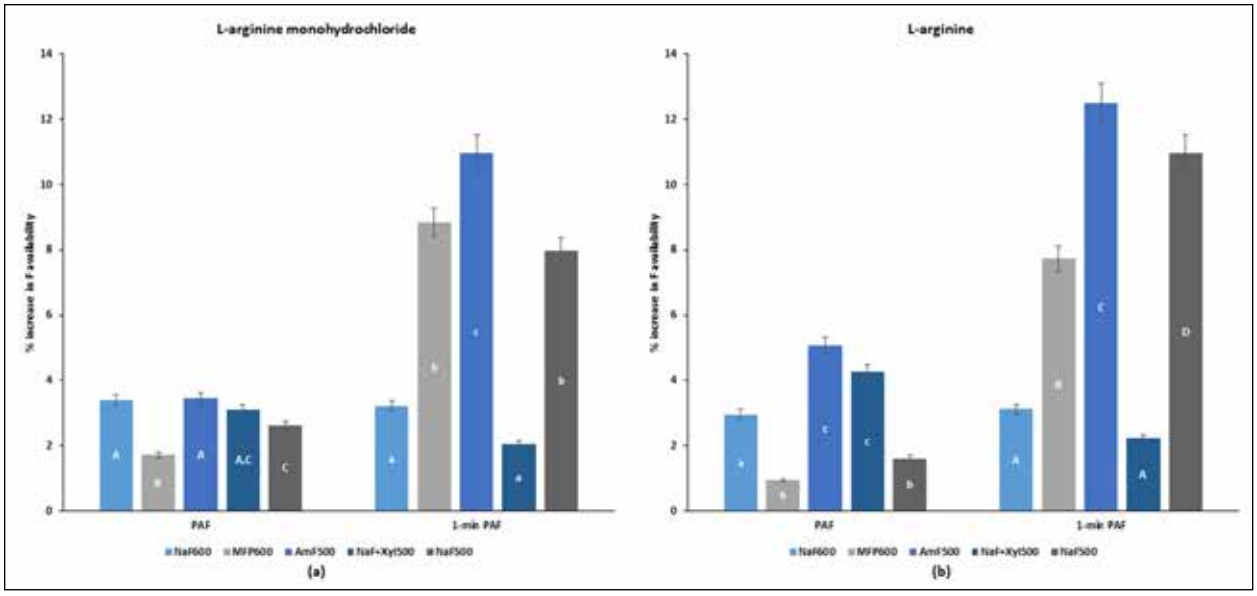


Figure 3: % increase in fluoride bioavailability in child formula dentifrices with – (a) L-arginine monohydrochloride (n=3); and (b) L-arginine (n=3) (the differences for each PAF/1-min PAF are indicated using small/capital alphabets identified by 1-way ANOVA with Tukey's HSD test).

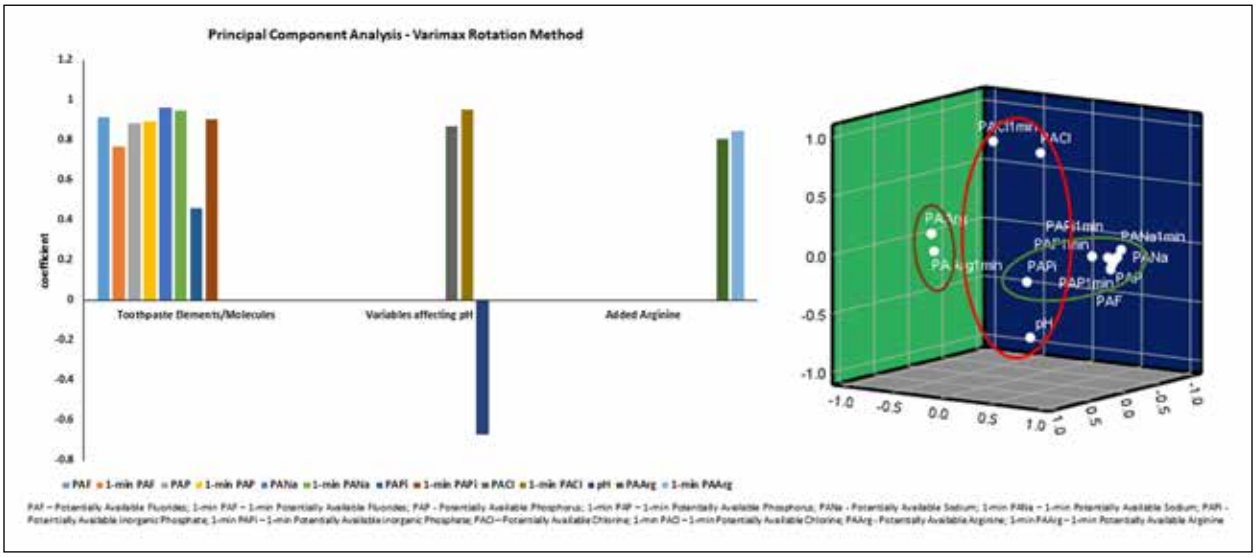


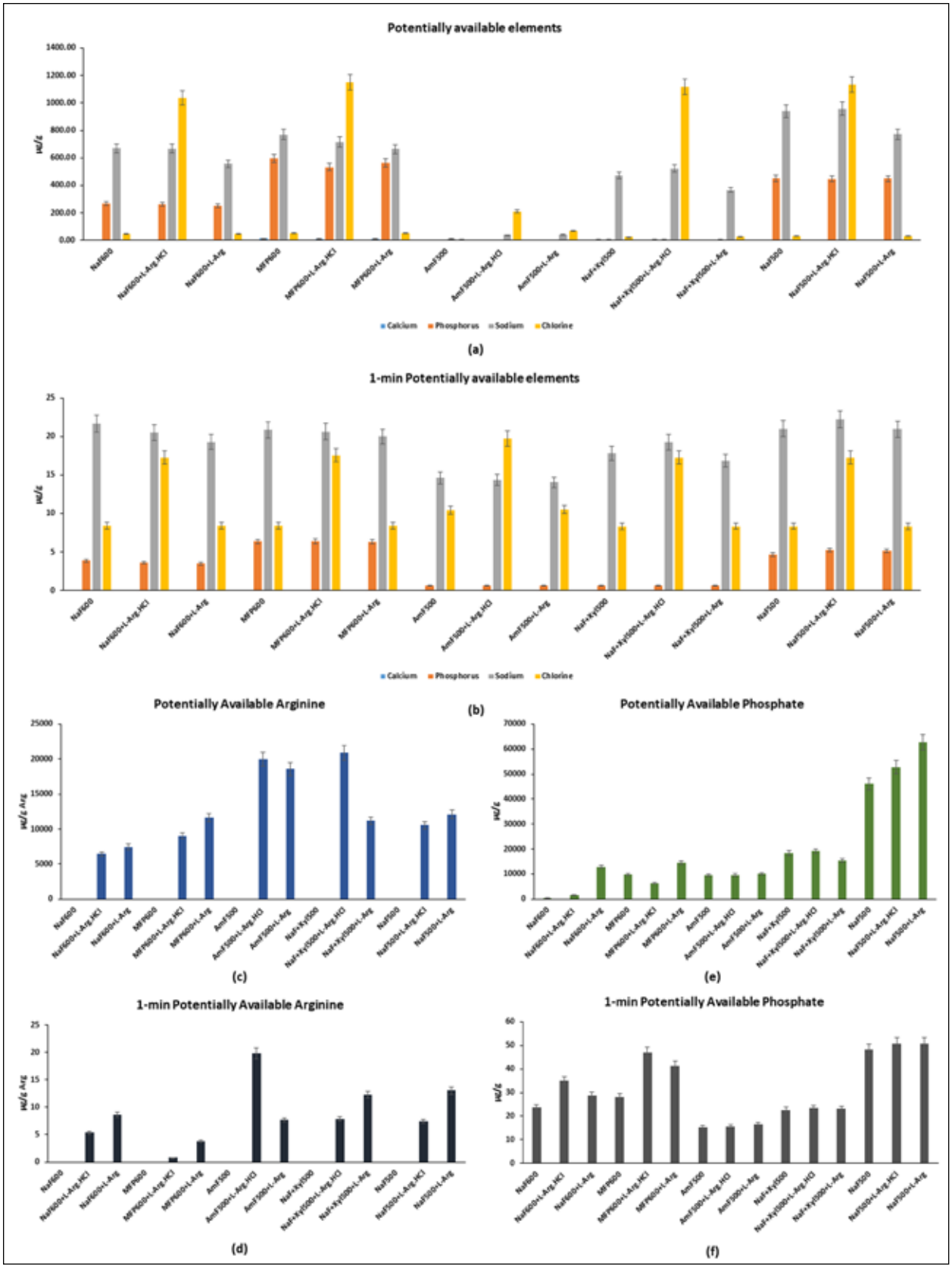
Figure 4: Principal Component Factor Analysis

As the added arginine was detected as an external variable, they were presented with individuality; whereas elements F, P, Pi, Na at different levels were reduced together. Supplemental Figure S1 shows the detected molecular and elemental analysis which was included in the PCA analysis.

Overall, PCA analysis demonstrated that L-Arg.HCl explicitly affected the pH of toothpaste slurries due to the presence of Cl in the form of HCl; whereas the inherent elements/molecules – Na, P, Pi, F at different levels remain distinct with unidentified influence on the variables (Figure 4).

DISCUSSION

The present study represents the first report which showed that the incorporation of arginine or its hydrochloride salt at 2% w/w in CFDs significantly increased fluoride bioavailability. Hence, the null hypothesis that incorporation of arginine in CFDs has no influence on fluoride bioavailability was rejected. There is a general agreement that the continuous availability of low levels of fluoride in the oral cavity help in reducing dissolution of calcified tissues and remineralization of early enamel caries.[26] This can be achieved by prolonged and persistent use of fluorides toothpastes with maximum possible bioavailability, which have been considered the most effective delivery mode of fluorides worldwide.[19]and therefore fluoride exposure, and prevalence and severity of dental fluorosis and dental caries. After successful trials, programs for water fluoridation were introduced, and industry developed effective fluoride-containing toothpastes and other fluoride vehicles. Reductions in caries experience were recorded in many countries, attributable to the widespread use of fluoride. This is a considerable success story; oral health for many was radically improved. The present study represents the first report which showed that the incorporation of arginine or its



Supplemental Figure S1: Elemental (Ca, P, Na, Cl) and molecular (arginine and inorganic phosphate) analysis for control and experimental toothpaste slurries. (a) Potentially available calcium, phosphorus, sodium, and chlorine; (b) 1-min potentially available calcium, phosphorus, sodium, and chlorine; (c) potentially available arginine; and (d) 1-min potentially available arginine; (e) potentially available inorganic phosphate; (f) 1-min potentially available inorganic phosphate in control and experimental child formula dentifrice

hydrochloride salt at 2% w/w in CFDs significantly increased fluoride bioavailability. Hence, the null hypothesis that incorporation of arginine in CFDs has no influence on fluoride bioavailability was rejected. There is a general agreement that the continuous availability of low levels of fluoride in the oral cavity help in reducing dissolution of calcified tissues and remineralization of early enamel caries.[26] This can be achieved by prolonged and persistent use of fluorides toothpastes with maximum possible bioavailability, which have been considered the most effective delivery mode of fluorides worldwide.[19]

The estimated TF for all the dentifrices (except NaF500) was less than the labelled F. For 600 ppm CFDs, the TF was 558–570 ppm, and for 500 ppm CFDs, the TF was 459–514 ppm. The TSF (bioavailable F) for NaF (only)-containing dentifrices was higher than other CFDs as the inorganic F (NaF) has limited reactivity with silica base. Still, the % insoluble F in the tested CFDs ranged between 4-32%, which could be due to the incompatibility between fluoride and the abrasive agents.[27] The estimated TF for all the dentifrices (except NaF500) was less than the labelled F. For 600 ppm CFDs, the TF was 558 - 570 ppm, and for 500 ppm CFDs, the TF was 459 - 514 ppm. The TSF (bioavailable F) for NaF (only)-containing dentifrices was higher than other CFDs as the inorganic F (NaF) has limited reactivity with silica base. Still, the % insoluble F in the tested CFDs ranged between 4-32%, which could be due to the incompatibility between fluoride and the abrasive agents.[27] The range (4-32%) of inactive or insoluble F estimated is within the permissible range as per US FDA, which allows toothpaste formulations to contain at least 60% of labelled F content as TSF.[28] However, the suggested range might be significant for CFDs, as the evident caries control is reported with >1000 ppm F dentifrices.5,[29] It should be noted that the ionic F content of the NaF (only)-containing dentifrices was significantly higher than other CFDs, enhancing the bioavailability of free F ions in the oral cavity.[26]

The pH of toothpaste slurries with L-Arg was > 9 for all the CFDs, this could be attributed to the acid dissociation constant of arginine (pKa = 12.48) that renders the molecule its basic properties. The PCA analysis further showed that the Cl in L-Arg.HCl significantly reduced the pH of CFDs. The decrease in pH might negatively influence the oral environment as the proportion of cariogenic bacteria is known to increase with low pH. Sequentially, the proportional growth of health-associated bacteria is inhibited with the decrease in base production from salivary components metabolism.[30] On the contrary, the low pH may assist in greater deposition of calcium fluoride-like complex on enamel surfaces compared to pH above critical pH (>5.5).[31] Thus, the transient presence of low pH toothpaste within the oral cavity could be beneficial to the high-risk patients. Also, it is quite possible that the inherent buffer potential of salivary components might prevent the sustenance of low pH in the oral cavity; hence, further studies are required to clarify the effect of such pH change on oral bacteria, remineralization potential and enamel fluoride uptake within the incipient caries lesion.

The buffer capacity of the dentifrices depicts its efficiency in resisting pH changes. In the present study, the buffer capacity of added L-Arg was significantly higher than the added L-Arg.HCl, suggesting that the addition of L-Arg might minimally affect the pH of the dentifrices as all CFDs (except AmF500) had approximately neutral baseline pH. It also elucidates that the incorporation of L-Arg is admissible by the CFDs with a pH change above neutral due to inherent basic properties of the molecule. Conversely, the Cl component of L-Arg.HCl influences the buffer capacity of the CFDs and significantly reduces pH of the toothpaste slurries as shown by PCA.

Potentially or 1-min potentially available F indicates the fraction of bioavailable F (free ionic or ionizable F) in the dentifrices that can intervene the re-/demineralization dynamics by enamel interaction. In the present study it was observed that arginine (L-Arg/L-Arg. HCl) significantly improved the fluoride bioavailability of CFDs, possibly by generation of organic fluorides (arginine-fluoride), which is an easily accessible and stable form of fluoride ion source.[32] Also, the positively charged arginine are able to block the ion-pairs of excipients from forming with fluoride.[33] This could be due to the strong affinity of smaller halides (F) for positively charged groups like guanidinium.[33–35] The results are in agreement with our previous study, which showed that the incorporation of L-Arg.HCl (2% w/w) in 1100 μg/g F NaF/SiO_2 toothpaste significantly increased the remineralization potential and fluoride uptake of the NaF toothpaste, due to either arginine-fluoride complex formation or NaCl interaction that increased the fluoride uptake and enhanced remineralization.[20] Besides, in the present study it was observed that AmF500 toothpaste had the highest increase in 1-min PAF which is attributed to higher concentration of insoluble F. Thus, Arg incorporation affects the F bioavailability of toothpaste matrix that retains excessive insoluble F.

Apart from the bioavailability of fluoride, the study also evaluated the bioavailability of arginine in the samples prepared for PAF and 1-min PAF. Irrespective of the arginine variant, all 1-min PAF samples showed the presence of arginine, ranging 1-20 μg/g. The bioavailability of arginine maintains biofilm pH homeostasis due to its known prebiotic effect[36], inhibiting the biofilm biomass of *S. mutans*-containing multi-species biofilm. The L-Arg.HCl moderated the beneficial order of poly-microbial biofilms, affecting its development and community composition.[37] Arginine also affects the adhesion properties of cariogenic bacteria *S. mutans.* [38] So far, evidence suggests that the presence of arginine with fluorides demonstrates a synergistic anti-microbial effect on cariogenic biofilms and helps in maintaining oral biofilms homeostasis.[21,39] Furthermore, arginine is also known to promote fluoride uptake in artificial enamel caries-like lesion, increasing the resistance of enamel to carious demineralization.[40]

Although this study identifies that arginine at 2% w/w (irrespective of the variant) improves the fluoride bioavailability of CFDs, the results needs to be interpreted with caution. The present study estimates the F bioavailability changes with only one concentration of L-Arg/L-Arg.HCl incorporated in the CFDs. Studies are needed to further discern if higher Arg (either variant) concentrations can effectuate increase in the fluoride bioavailability of CFDs. However, the present study demonstrates the first evident possibility of a biotic intervention (arginine) with fluorides that improves F bioavailability in CFDs. Further *in vitro* studies are also needed to investigate the effect of Arg containing CFDs on the enamel fluoride uptake and remineralization potential.

CONCLUSION

Under the conditions of the present study, we conclude that -

1. The child formula dentifrices containing NaF only have higher concentrations of bioavailable fluoride.

2. Insoluble fluorides retained in child formula dentifrices limits fluoride bioavailability.
3. Incorporating arginine (L-arginine or L-arginine monohydrochloride) at 2% w/w improves fluoride bioavailability of the child formula dentifrices.

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DISCLOSURE STATEMENT

All authors declare no conflict of interest

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