Effect of Fermented Milk Containing Probiotics on Microhardness of Silorane- and Methacrylate-Based Resin Composites

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Abstract

Purpose: The aim of this study was to evaluate the effect of probiotic fermented milks on microhardness of methacrylate-based resins and silorane based composite resin. **Methods:** Measurements of microhardness of Filtek Z350XT (Z3); Evolu-X (EV), Charisma (CH) and Filtek P90 Silorane (P9) resins were recorded after cycling, for 15 days, in 20 mL of artificial saliva, ethanol and probiotics fermented milks (Yakult® Traditional, Actimel®, Chamyto®, Ninho® Soleil Fermented Milk). Between cycles, the resin disks were kept in artificial saliva at 37˚C $\pm$ 1˚C. The pH values of the solutions and titratable acidity with Na(OH) to reach pH 5.5 and 7.0 were recorded. Data were analyzed using ANOVA and Tukey's test (α=0,05). **Results:** All methacrylate-based resins exhibited significant reduction in microhardness when exposed to Chamyto and Ninho Soleil probiotics similar to the samples immersed in ethanol. Silorane-based resin did not undergo significant changes in microhardness values in any of the experimental solutions (p=0.139). pH values of probiotics were between 2.86 and 3.37. Chamyto and Ninho Soleil needed larger amounts of Na (OH) to achieve neutral pH. **Conclusions:** Titratable acidity of fermented milk with probiotics seemed to play a significant role on surface hardness of methacrylate-based composite resins, more than pH value. Silorane-based resin showed no significant change in the hardness after exposed to acid fermented milk tested.

Key Words: Composite resins, Silorane resin, Hardness, Dental restoration

Introduction

Generally, teeth undergo physiological attrion due to functional and parafunctional habits However, the antagonist tooth may or may not be restored, therefore, the restorative material must be able to withstand the wear from masticatory load and other parafunctional habits. Currently the composite resin is the most commonly used tooth coloured material for direct restorations, due to its ability to adhere to the tooth by micromechnical means, requiring minimal tooth preparation and possesing high wear resistance and hardness [1]. But under acidic conditions, the composite resin can degrade over a period and show surface roughness, reduction in wear resistance and hardness leading to loss of continuity and microleakage [2]. The excessive consumption of acidic beverages is the most common source of extrinsic acid to the oral environment [3]. Probiotic fermented milk is a good example of drink with acidic pH, consumed worldwide due to its beneficial properties. Probiotic fermented milk is composed of sugar, milk and *lactobacillus.* It acts by modifying the microflora, competing for nutrients with pathogens and preventing their adhesion to the intestinal epithelium [4]. Probiotic bacteria have been used in treatment of chronic inflammatory diseases and prevention of a wide variety of human diseases, acting in the neutralization of toxins, in vitamins metabolism and increasing immunity [5,6]. Probiotics have been very useful in reducing the *Streptococci mutans* count as long as they are being consumed [7]. A recent study [8] showed that *Lactobacillus rhamnosus* can inhibit the formation of dental plaque by reducing the production of glucan from *S. mutans*, and thus may be incorporated in strategies for prevention of dental caries. However, due to its acidity, fermented milk exhibits erosive potential and can promote dental erosion in severity varying from initial enamel erosion to extensive loss of tooth leading to dentinal hypersensitivity [9].

In addition to possible changes in the enamel, low pH may also influence the mechanical properties of the composite resin by accelerating biodegradation through a complex process leading to the collapse of the polymer matrix [2]. This may lead to, release of residual monomers, reduction in hardness, change in topography, increase in roughness, accumulation of biofilm, increase risk of secondary caries, sensitivity, pulpal inflammation and debonding of the restorative material [10].

Therefore, studies exploring the effect of acidic beverages on the hardness of composite resin are important for the improvement of the material and patient education in order to increase the longevity of restorations. However, we still lack the information on the effect of probiotic fermented milks on composite resins. Therefore, restorative resins marketed, particularly those that have been modified, should be evaluated and compared for providing support to the product improvement and its clinical indications. This study aimed to evaluate and compare the effect of probiotic fermented milks

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on the surface hardness of methacrylate-based resin and silorane-based resin simulating the washing effect of saliva during the consumption of that drink.

Materials and Methods

Sixty samples from each material were made with three universal methacrylate-based composites and one silorane resin. Then the samples were immersed in four brands of probiotic fermented milk: Chamyto; Ninho Soleil Fermented Milk; Yakult Traditional and Actimel, in artificial saliva and ethanol. The characteristics of materials used in this study are listed in *Table 1*.

The composites were placed in a ring-shaped metallic matrix of stainless steel (5 mm inner diameter x 2 mm high) in a single increment. Immediately after inserting the material was covered with a polyester strip, and then with a glass slide. An axial load of 500 g was applied for 1 minute to promote superficial uniformity. After removal of the load and glass slide the composites were irradiated for the time recommended by the manufacturers, using soft-start polymerization method (LED Emiter B; Schuster Com. Equip. Odontológicos Ltda., RS, Brasil - 1150 mW cm^2). The samples were kept in dry lightproof vials for 24 h.

Cycling in the solutions

Twenty-four hours after curing the samples divided into groups (n=10) were placed in a suspended mesh nylon net to allow the liquid reach all surfaces of the resin. Then the samples were immersed five times in 20 mL of each solution for 3 seconds alternating immersion in the test solution with immersion in artificial saliva for the same time. The cycling was done once a day for 15 days. At the end of each daily cycling the samples were immersed in distilled water for 3 s to wash out the solution and then stored in artificial saliva at 37°C $\pm$ 1°C for 24 hours until the next cycling. Artificial saliva and ethanol were used obeying the same cycling parameters and represent the negative and positive controls, respectively.

The protocol was based on time to drink + 80 mL of fermented milk per day during 15 days. Previous experiment was done with two individuals. They took 80 mL of fermented milk in 5 sips, each sip took an average 3 seconds. The experiment was repeated once/day during five days. Then Kappa index was applied and the result was 7.6.

Knoop hardness measurements

Hardness was measured using a Knoop microhardness indenter (HMV-2000, Shimadzu, Japan) under a load of 50 g for 10 s. Measurements were performed at three locations on the top of samples, and the mean of Knoop hardness number

Table 1. *Composition of composites and solutions used in the research.*

MATERIAL, TYPE (code)	MANUFACTURER	COMPOSITION*	BATCH/SHADE
Filtek Z350XT - Nanofilled methacrylate-based composite **(Z3)**	3M/ESPE, St. Paul, MN, EUA.	Bis-GMA, Bis-EMA, TEGDMA, BHT, pigments, SiO2 20 nm, 5.00–20.00 nm nanoclusters, 0.60–1.40 µm ZrO2/SiO2. 55.6% (v/v)	881381/A2E
Evolu-X - Nanohybrid methacrylate-based composite **(EV)**	Dentsply, Petrópolis, RJ, Brasil	Bis-GMA, Bis-Ema, TEGDMA, Ba-Al Si glass, Ba-Al-F-B-Si glass nanofiller 0.6 – 0.8 1/4 µm 10 – 20 nm. 58%(v/v)	7840F/A2
Charisma -Microhybrid methacrylate-based composite **(CH)**	Hereaus Kulzer GmbH, Gruner Wag, Hanau, Alemanha.	BIS-GMA, Ba-Al-B-F-Si Glass, Pyrogenic SiO2 (0.01–0.07 µm). 58% (v/v)	10101/A2
Filtek P90 - Microhybrid silorane-based composite **(P9)**	3M/ESPE, St. Paul, MN, EUA	Silorane, 0.01–3.50 µm (mean 0.47 µm) quartz particles, yttrium fluoride. 55% (v/v)	N571333/A2
Artificial Saliva **(SA)**	Handled weekly in Cariology Laboratory of Federal University of Alagoas	Potassium chloride, sodium chloride, magnesium chloride, dibasic potassium phosphate, calcium chloride, sorbitol, sodiumcarboxymethylcellulose, distilled water.	-
Yakult® traditional **(YT)**	Yakult S/A Indústria e Comércio, Lorena, SP, Brasil	Skimmed milk and / or skimmed milk reconstituted, sugar, glucose, milk yeast and flavour.	L1108
Actimel® **(AC)**	Danone, Poços de Caldas, MG, Brasil	Semi-skimmed milk and / or partially skimmed milk reconstituted, liquid sugar, milk powder, dextrose, vitamin C and milk yeast.	ERVPKCZT
Chamyto® **(CH)**	Nestlé, Araras, SP, Brasil	Reconstituted skimmed milk, sugar syrup, invert sugar, milk yeast, zinc sulfate, stabilizer pectin, flavor and sweetener sucralose.	L4183132315
Ninho® Soleil Fermented Milk **(NS)**	Nestlé, Araras, SP, Brasil	Reconstituted skimmed milk, syrup sugar, invert sugar, milk yeast, tricalcium phosphate, zinc sulphate, vitamin D, stabilizer pectin, citric acid and flavoring.	L4181132312
Ethanol **(ET)**	Álcool Santa Cruz Ltda, Guarulhos, SP, Brasil	Ethyl Alcohol 99.3º GL	98

*Composition of all products according the manufacturers.

Bis-GMA: bisphenol A diglycidyl methacrylate; Bis-EMA: ethoxylated bisphenol A glycol dimethacrylate; TEGDMA: triethylene glycol dimethacrylate; BHT: butylhydroxytoluene; silorane represents a mixture that is made of both siloxane and oxirane structural moieties.

(KHN) was recorded for each specimen. Knoop hardness was determined after storage for 24 h (baseline) in dry lightproof vials and after cycling in experimental solutions.

Determination of pH and titratable acidity

The acidity of each drinking fermented milks; artificial saliva and distilled water were measured three times with a digital PH meter (Digicrom Analítica Ltda., São Paulo, Brasil). Then, the neutralisable acidity of drinking fermented milks was tested by placing 30 mL of the fermented milks in a glass beaker placed. Sample was stirred continuously and sodium hydroxide solution (NaOH) 0.5 M was gradually added to the fermented milks sample and the pH rise was monitored. The volume of NaOH required to increase the pH of the sample to pH 5.5 and pH 7.0 was recorded. pH measurement of ethanol 99.3 gl haven't been done due it be a pure organic solution. The pH value of ethanol used was given by manufacturer and was between 6.0 and 8.0.

Statistical analisys

After verification of the hypothesis of equality of variances by Levene's F test data was analyzed using the F test (ANOVA) followed by Tukey's test for multiple pairwise comparisons (α=0,05) with SPSS software (Statistical Package for the Social Sciences) v.21.

Results

Table 2 shows the mean of Knoop hardness of the composite resins as a function of the solutions utilized. It is observed that the methacrylate-based resins exhibited significant reduction in hardness when exposed to Chamyto and Ninho Soleil probiotics, similar to Etanol group (p<0,05). Whereas Yakult (p=0,327) and Actimel (p=0,406) probiotics did not significantly change the surface hardness of any tested resins *(Figures 1 and 2)*. P9 resin did not undergo significant changes *(Figure 3)* in the values of surface hardness when exposed to probiotics and ethanol (*p*=0,139). All probiotic fermented milks are acidic and Yakult presented the lowest pH value, however, Yakult did not significantly change the hardness of resins *(Table 3)*.

Discussion

One of the most important properties that determines the durability of restorative materials in the oral cavity is its resistance to degradation [11] either by physical or chemical means. In the oral enviornment restorations can be exposed to the chemical agents either intermittently during ingestion of food and drinks or continuously by dental plaque/calculus or debris deposited on the tooth surface and on margins of restorations [12]. Foods and drinks with low pH values may cause erosive effect, or chemical wear without the participation of microorganisms, that affects both the tooth structure and restorative materials [11]. In the present study different probiotics produced different effects on hardness of composite resins. This could possibly be due to difference in composition of these probiotics and their interaction with composite resins. However, manufacturers do not provide a detailed composition of the probiotic fermented milks which makes it difficult to discuss their interaction.

Many studies have demonstrated the erosive activity of citric, malic, phosphoric, and other acids as ingredients of beverages on the organic matrix and filler contents

Table 2. *Means of hardness values ± standard deviation of the tested resins.*

			Probiotics				
Resins	**BA**	**AS**	**YT**	**AC**	**CH**	**NS**	**ET**
Z350	125.2 ± 6.71^{aA}	117.33 12.38^{aA}	118.37 ± 4.90^{aA}	118.80 7.64^{aA}	52.20 ± 5.67^{bA}	59.07 4.89^{bA}	55.17 ± 3.78^{bA}
Evolu-X	101.07 ± 7.64^{aB}	108.59 8.49^{aAB}	104.22 ± 10.81^{aB}	102.48 7.87^{aB}	52.53 ± 4.69^{bA}	53.87 2.78^{bAC}	50.32 5.72^{bB}
Charisma	78.48 ± 5.02^{aC}	78.69 9.42^{aB}	79.84 ± 8.78^{aC}	70.92 18.66^{aC}	53.11 ± 4.66^{bA}	39.57 6.34^{cB}	38.10 3.45^{cC}
P90	47.37 ± 2.66^{aD}	47.40 ± 2.29^{aC}	48.46 ± 3.90^{aD}	48.32 ± 2.81^{aD}	44.83 ± 3.02^{aB}	48.45 ± 4.17^{aC}	46.85 ± 2.32^{aB}

Representation of mean values of composite resins hardness as a function of solutions used. BA (Baseline: KHN 24 h after polymerization); AS (Artificial saliva); YT (Yakult); AC (Actimel); CH (Chamito); NS (Ninho Soleil); ET (Ethanol). F-test (ANOVA). Different capital letters indicate significant difference between composite resins by Tukey's paired comparisons at 5.0% (p<0.05). If all the lower case letters are different, there is a significant difference between solutions by Tukey's paired comparisons at 5.0% (p<0.05).

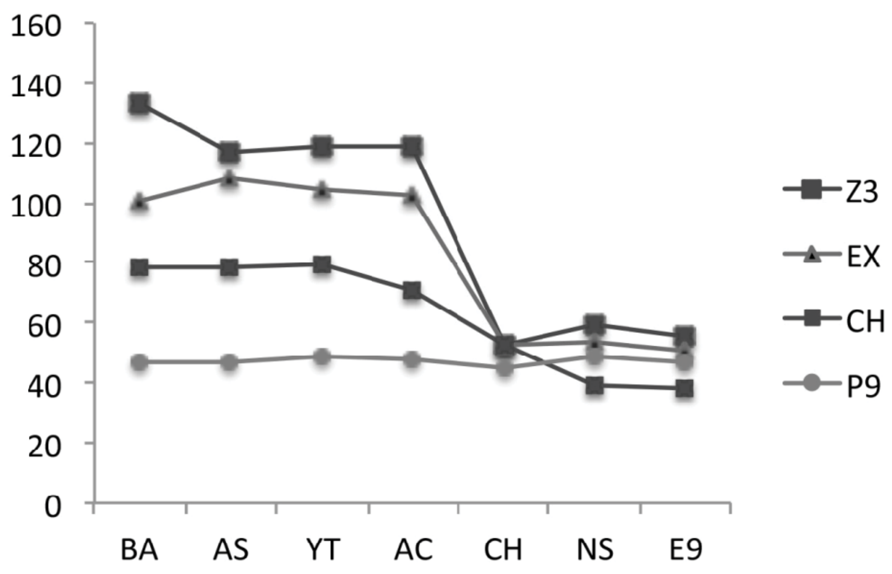

Figure 1. *Yakult and Actmel probiotics did not significantly change the hardness of any composite resins. BA (Baseline); AS (Artificial saliva); YT (Yakult); AC (Actimel); CH (Chamito); NS (Ninho Soleil); ET (Ethanol); Z3 (Z350XT); EX (Evolu-X); CH (Charisma); P9 (P90 Silorane).*

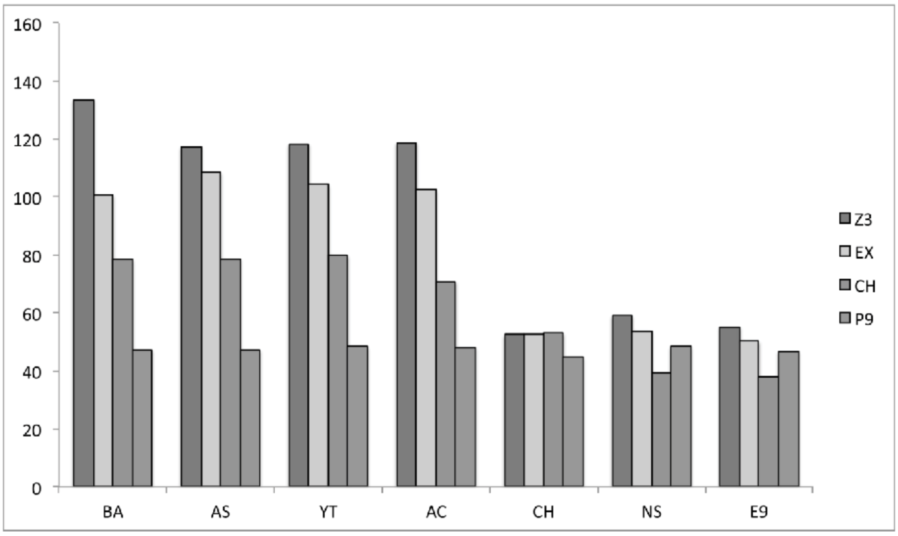

Figure 2. *Hardness Koop values of P9 resin was not significantly changed by probiotics or by ethanol. BA (Baseline); AS (Artificial saliva); YT (Yakult); AC (Actimel); CH (Chamito); NS (Ninho Soleil); ET (Ethanol); Z3 (Z350XT); EX (Evolu-X); CH (Charisma); P9 (P90 Silorane).*

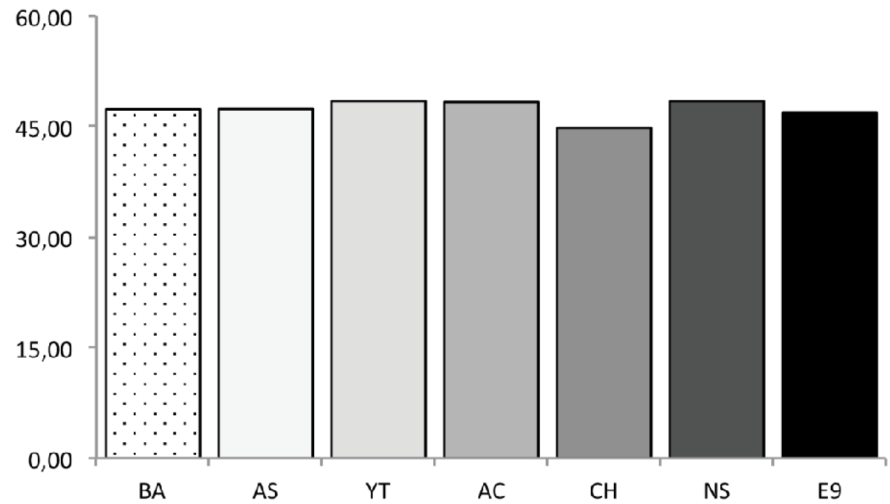

Figure 3. *P9 KHN mean values. One-way ANOVA F-test demonstrated that probiotic fermented milks had no significant influence on KHN values of silorane-based resin. BA (Baseline); AS (Artificial saliva); YT (Yakult); AC (Actimel); CH (Chamito); NS (Ninho Soleil); ET (Ethanol).*

Table 3. *pH values of solutions utilized and titratable acidity with Na(OH).*

		Titritable NaOH 0,5M			
		To reach pH 5.5		**To reach pH 7.0**	
Solution	**pH**	**Volume (mL)**	**pH**	**Volume (mL)**	**pH**
YAKULT	2.86	5.6	5.54	6.9	7.15
ACTIMEL	3.37	5.5	5.58	8.0	7.02
CHAMYTO	2.91	7.3	5.67	9.1	7.14
NINHO SOLEIL	2.96	7.5	5.63	10.0	7.24
ARTIFICIAL SALIVA	7.03	-	-	-	-
DISTILLED WATER	6.09	-	-	-	-

Mean pH values of the solutions and titratable acidity with NaOH 0.5 M to reach pH 5.5 and pH 7.0. All probiotic fermented milks are acidic. Chamyto and Ninho Soleil needed larger amounts of Na(OH) to achieve neutral pH.

of composite resins [13-16] Lactic acid is the main acid produced by the plaque microorganisms and is a product of the *Lactobacillus* fermentation process in fermented milk beverages [14]. Non-alcoholic beverages contain phosphoric acid [11] whereas both malic acid and citric acid are the main acids in many fruit drinks and juices [17]. Fermented milk Ninho and Soleil contain citric acid alomg with lactic acid in its composition and have shown higher tritable acidity compared to other fermented milks used in this research. This could be explained due to the synergic action of both lactic and citric acids, thereby, reducing the hardness values of methacrylate-based resins. Information regarding the effects of citric acid on degradation of composites is still limited, but it is known that acid causes failures in the load / matrix interface and microcracks in the organic matrix [18,19] as seen after thermal cycling [20].

The mechanical properties of a composite resin, considering the material composition are related to the polymer matrix, the inorganic filler and bonding agent of fill to resin [1]. Since resins with different matrix and filler have different physical and mechanical characteristics, it can show varied susceptibility to degradation in acidic environment [2].

The absorption of water molecules by hydrophilic monomers from matrix can result in hydrolytic degradation and breakage of the bonds between filler particles and organic matrix, which decreases the mechanical properties of the resin. Thus, even the aqueous medium of the oral cavity causes intrinsic damage to the composites, which can explains the reduction in the hardness of the methacrylate-based resins (Z3, EX and CH) when exposed only to artificial saliva. Although the oral environment produces a defense by salivary action, which acts as a protective barrier [10], the exposure to wet inviroment can causes hydrolytic degradation of the silane that overlying the load [19,21], hydrolysis of ester radicals present in methacrylate monomers such as Bis-GMA, Bis-EMA, TEGDMA and UDMA [2] or dilating the matrix [22] reducing frictional forces between the polymer chains [23].

P9 is a silorane-based resin [24], and their monomers are more hydrophobic than the methacrylate-based resins [25], which hinder the penetration of liquids into the formed polymer allowing greater resistance in wet environment [24]. The polymerization process of silorane is by cationic ring opening reaction, providing a new linear molecular configuration that off sets the shrinkage of the resin when new chemical bonds are formed [25] and produces a polymer more resistant to degradation [24,26]. Previous studies have shown that silorane has properties comparable to methacrilate-based resins such as reduced marginal microleakage [26], higher flexural strength [27], or better than methacrilate-based resins such as low solubility in water and a significant reduction of the sorption of water and biological fluids [26]. These differences between the materials, especially considering aspects such as system of monomers, type and size of fillers, and the chemical bonding between load and resinous matrix, may be responsible for differences in resistance to chemical and mechanical degradation of those materials [24].

Addition of inorganic particles filler (quartz, colloidal silica, glass) increases the mechanical properties of the resin by reducing the amount of organic matrix. It minimizes the disadvantages such as polymerization shrinkage, high coefficient of linear thermal expansion and water sorption. The highest percentual in filler volume will result in higher hardness [1,24], as observed in the present study. Among the methacrylate-based resins, Z3 had the highest hardness, which can be explained by the greater volume of inorganic filler of this material (63.3% v.v.) while both EX and CH have a percentual lower load (58.0% v.v.) and exhibited lower hardness value.

Despite EX and CH have the same volume load percentage, CH has Bis-GMA and TEGDMA as main monomers. Bis-GMA is a monomer with high molecular weight (228.29). Large molecules do not produce very efficient cross-links and probably form a less dense polymer network, facilitating the penetration and action of liquids [28] within the polymer, potentiating the action of liquid acid substances. Also, TEGDMA is a hydrophilic monomer [21] and this composition may have favored the lowest hardness values observed in CH resin.

All probiotics tested in this study and ethanol did not produce a significant reduction in hardness of silorane-based composite resin. This could possibly due to presence of silorane as resinous matrix, which is more resistant to sorption and solubility than methacrylate-based resin [24]. These results are in agreement with the study done by Mohmmed [10] who used the yogurt drink as fermented milks having pH below the critical value for enamel dissolution (5.5) and observed significant reduction in the surface hardness of the methacrylate-based resin, without significant effect on silorane-based resin. Although previous sutudies had been shown lower hardness values for P9 resin [24,27].

The greater resistance of P9 resin can not be attributed to the size and volume of the filler particles, as it has a percentage of filler (v.v) lower than Z3 resins, CH and EX. P9 has a combination of fine particles of silica and ítrium trifluoride connected to the organic matrix by a bonding agent epoxy functional silane through a silanization process that is similar to the methacrylate-based restorative materials, which filler particles are bonded to resinous matrix by methacrylate-functional silane [24,25]. The bond filler / matrix in silorane-based resins associated to the hydrophobic nature of the siloxane groups may be the responsible by the maintenance of hardness values showed on this study.

The titratable acidity is the amount of alkali (base) required to be added to an acid solution to bring it to reach a neutral pH. Therefore, it represents the amount of available acid and is an indication of the strength and erosion potential of the solution [29]. In this study, volumes of alkali required to achieve both pH 5.5 and pH 7.0 was observed because pH 5.5 is the critical pH value for dissolution of enamel and pH 7.0 is a neutral pH. Chamyto and Ninho Soleil were the fermented milks that needed the largest volumes of alkali to achieve neutral pH which may be responsible for its greater deleterious effect on methacrylate-based resins demonstrating lower values of surface hardness. While traditional Yakult that has presented the lowest pH value did not causes significant reduction in the surface hardness of the composites. This was the probiotic with lower titritable acidity. The most easily buffered drinks should present less noxious effects from its acidity on the surface of the restorative material, reducing the risk of degradation of restoration in the oral environment. Findings of Tanuta et al. [30] suggest that the titratable acidity of a beverage influences salivary pH values after drinking acidic beverages more than the beverage's pH.

The present study also took into account the buffering effect exerted by saliva in the oral cavity to balance the low pH values. It has been established that the corrosive potential of an acidic solution is related to their pH, titratable ability, buffer capacity, the solution degree of saturation [31] and high temperatures [20]. In vivo, saliva can buffer the acidity in oral inviroment and limit the softening of the surface of the composite resins [32,33]. The media to which restorative materials are exposed can exert great influence in their chemical degradation [13,16]. The methodology of this study was designed to simulate the real scenario of daily intake of probiotics for 15 days. During consumption, the drink comes in contact with the teeth and then is washed out by saliva between sips. Thus, normal flow, neutral pH, good buffer capacity of saliva and the ingestion of probiotic fermented milks at low temperatures can act simultaneously to reduce the deleterius effect of low pH of the beverage on the surface of the composite resin.

Under clinical conditions in which a beverage is continuously sipped for minutes, the effects of pH and titratable acidity on the maintenance of a low salivary pH may be different and cause different effects on composite resins and teeth [30]. However, it's important to consider that sometimes while the beverage is into the mouth, the volume of saliva present and/or secreted can be very small to induce a significant buffering in the beverage pH, especially in patients with reduced salivary flow. In most of the studies the effect of low pH beverages on composite resins and the effect of low salivary flow or xerostomia have not been considered. Therefore, further studies should be carried out to address these parameters.

Conclusions

Based on the results obtained and within the limitations it can be concluded that all probiotics tested showed pH value below the critical pH (5.5) required for demineralization of dental enamel. Titratable acidity of fermented milk with probiotics seemed to play a significant role on surface hardness of methacrylate-based composite resins, more than pH value. Silorane-based resin showed no significant change in the hardness after exposed to acid fermented milk tested.

Acknowledgments

The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq for financial support.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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