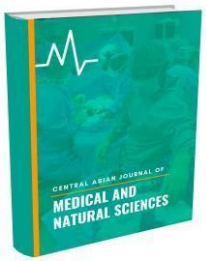

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Article

Effect of Chia Seed Oil Addition on Bacterial Adhesion, Tensile Strength, and Hardness in Soft Denture Liners (An in-vitro study)

Ali Saad Ahmed[1*], Safwan Abdul-Hammed[2], Rusul Saad Ahmed[3], Luma Nasrat Arab[4]

1,2,4. Department of Prosthodontics, College of dentistry, Tikrit University, Tikrit, Iraq
3. Department of Pedodontics, College of dentistry, Tikrit University, Tikrit, Iraq
* Correspondence: ali.s.ahmed@tu.edu.iq

Abstract: Denture soft liners play a critical role in enhancing patient comfort and preventing tissue irritation, especially in individuals with resorbed ridges. However, their long-term use is hindered by microbial colonization, particularly Candida albicans, leading to conditions such as denture-induced stomatitis. This study aimed to evaluate the mechanical properties and antibacterial effectiveness of heat-cured soft liners incorporating Chia seed oil at 0.5% and 2% concentrations. A control group was compared to these formulations, and Staphylococcus epidermidis was used to assess bacterial adherence. Hardness and tensile strength were measured using a durometer and a universal testing machine, respectively, while statistical analysis employed one-way ANOVA and Tukey tests. Results showed that both concentrations of Chia seed oil significantly reduced bacterial adherence ($P < 0.01$) but also led to a decrease in hardness and tensile strength ($P < 0.05$). These findings highlight the potential of Chia seed oil as an antimicrobial additive in soft liners, offering improvements in oral health and patient care outcomes.

Keywords: Antimicrobial, Chia Seed Oil, Dental Materials, Oral Health, Soft Liners.

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1. Introduction

The denture base's improper fitting to the supporting tissues can be fixed by denture relining. Resilience is provided by soft liners, which lessen tissue irritation and improve patient comfort when wearing dentures [1, 2]. To promote the healing of inflamed oral tissues and restore the patient's ability to bite and masticate, these liners are essential in absorbing and redistributing masticatory forces [3]. Soft-liners provide benefits in reducing mucosal irritation, but they have drawbacks as well, particularly in terms of microbial colonization. Fungi and other pathogens that can enter soft liners can cause stomatitis associated with dentures. Patients with this prevalent oral mucosal infection can identify it by its redness and pain [4, 5]. Consequently, patients continue to have a great deal of difficulty with candida albicans.

This colonization and the ensuing risk of stomatitis must be prevented by thoroughly washing the denture and the soft liner that goes with it. However, conventional cleaning methods are not always effective, particularly for those with cognitive or physical impairments [6, 7]. Therefore, a different strategy is needed to prevent microbial colonization. A medicinal cost plant could hold the key to finding a novel antimicrobial agent in light of the global use of antimicrobial medications, which has resulted in the

evolution of resistant bacteria species [8]. Many plant extracts can serve as natural alternatives to manufactured medications since medicinal plants are affordable, safe, and efficacious [9].

Plant-based biologic drugs are a relatively new development in medicine [10, 11]. Comparing herbal remedies to synthetic ones, the former are less microbially resistant, more affordable, safer, and more readily available [12]. Some species of salvia, including Salvia hispa nica L., an annual herbaceous plant in the Lamiaceae family, are commonly referred as chia. Chia seeds are utilized nowadays for their nutritional and therapeutic qualities [13]. Chia seeds are a healthy addition to gluten-free diets because they don't contain any harmful substances or gluten [14]. Several investigations on the advantages of chia seed extract for general human health have been carried out in the past [15].

Because of their high fibre content, they are utilized as an appetite suppressant for intestinal modulation, blood glucose control, and weight reduction [16]. Vitamins, phenolic acids, and linolenic acid (C18:3 n-3, ALA) are abundant in chia seeds [17]. Fatty acids have a significant and vital role in the health, antibacterial, and antioxidant properties of chia seeds. Its vast diversity of secondary metabolic products, including flavonoids, phenols, and terpenes, confers antibacterial properties [18]. In recent years, there has been a burgeoning interest in natural and herbal alternatives for disinfecting medical devices, including soft-liner prostheses. Therefore, this study aims to evaluate the antibacterial efficacy of different concentrations of natural cold-pressed chia seed oil incorporated into soft-lining denture materials.

2. Materials and Methods

Study design and grouping

Three separate tests, including the investigation of three different groups, were carried out throughout this in-vitro experimental study. Based on preset percentages of Chia seed oil obtained from early pilot investigations, these groupings were established. The groupings were specifically categorized as follows: Group A represented the control group, which consisted of specimens with a weight percentage of 0.0 oil added. On the other hand, Group B was the experimental group that included 0.5 % oil powder, while Group C was another experimental cohort that contained 2 % oil powder. Remarkably, throughout the experimental period, each of these groups consisted of 10 samples.

Staphylococcus epidermidis Isolation and identification

At Ghazi al-Hariri Hospital, patients wearing maxillofacial prostheses had their diseased regions swabbed to isolate S. epidermidis. Carefully administered swabs (AFCO, Jordan) were used on the affected area, taking care not to touch the necrotic tissue. The specimens were incubated in an incubator (Mermat, Germany) at 37°C for 48 hours before being cultivated on blood agar and mannitol salt agar. After an overnight incubation on blood agar, colonies of S. epidermidis were identified by their 1-2 mm diameter, elevated, round, smooth, and glistening texture that ranged from faintly transparent to opaque. On the blood agar medium, they displayed a non-hemolytic pattern [19] (Figure 1).

Figure 1. S. epidermidis on blood agar

Their staphylococcal nature was established by a positive catalase test, whilst streptococci were ruled out by a negative result. The manufacturer's instructions were then followed while using the VITEK 2 compact identification system. After transferring the isolates to glass tubes with 3 mL of distilled water, the turbidity was measured and adjusted to guarantee a bacterial cell count that met the 0.5 Macfarlane criterion. Then, employing negative pressure, these bacterial suspensions were put into cassettes and placed within the VITEK 2 compact system to undergo a 12-hour biochemical reaction. The VITEK 2 compact system's specialist software was used to analyze the data, making it easier to precisely identify the different strains and species of bacteria [20].

Soft liner Sample preparation

Using AutoCAD software and a laser cutting machine (JL-1612, Jinan Link Manufacture and Trading Co., Ltd., China), plastic disc models were created. 30 plastic discs with a diameter of 10 mm and a thickness of 3.0 mm were used for the bacterial adherence test [21], 30 Type 2 dumbbell-shaped plates measuring 75 mm in length and a thickness of 2 mm for the tensile strength test, and finally 30 disc-shaped plastics with a diameter of 35 mm and a thickness of 6 mm were used for the Shore A hardness test [22]. The plastic designs were filled with extra silicone, once the silicone was set, they were covered with separating media (Shanghai New Century Dental Material Co., Ltd., China) and the lower flask half was filled with dental stone. It was covered once more after hardening, then the upper flask half was inserted. After giving the flask an hour to solidify, the designs were taken out to make mold gaps.

The manufacturer's instructions state that the volume/parts by weight mixing ratio for the heat-cured acrylic soft liner is 1 mL liquid to 1.2 g of powder for the control samples. Soft liner liquid was mixed with two different concentrations of Chia seed oil (0.5% and 2.0% by volume) to create the experimental samples. To establish an exact P/L ratio, the volume of added oil was deducted from the volume of soft-liner liquid [23]. The necessary quantity of the oil was poured into a dry, clean glass beaker along with soft liner liquid. The combination was then stirred in a sonication apparatus (Soniprep-150, England) for 20 seconds at 120 W and 60 KHz to achieve total homogeneity. Then, the mixture was promptly mixed with soft liner powder. The soft liner was put into silicone molds and pushed to guarantee uniform distribution once it reached the dough stage.

After that, the molds were subjected to hydraulic pressure (BegoHydrofix, Germany) to eliminate surplus material and attain consistency. After removing extra material, the molds were allowed to dry before the flask was sealed with a hydraulic press and submerged in water to cure. The flasks were heated in a digital water bath (Memmert,

Germany) for 90 minutes at 70°C and then for 30 minutes at 100°C as part of the curing procedure. Following curing, the specimens were carefully removed from the flasks and finished using a silicon polishing bur and sharp blades (Figure 2). The flasks were then gently cooled. The specimens were conditioned for 48 hours at 37°C in distilled water following ADA standard No. 12 (1999).

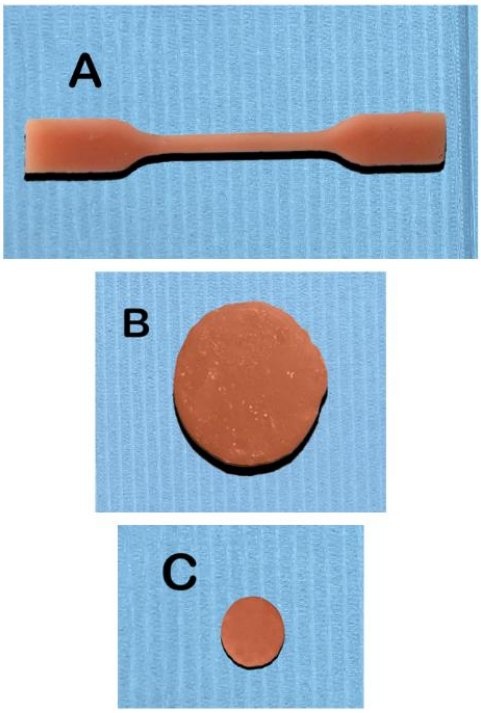

Figure 2. Test specimens; A, Tensile strength.
B, Shore A hardness. C, Bacterial adherence.

Bacterial adherence test

The medium used to create the bacterial suspension—Miller broth HIMEDIA, India—was crucial for determining S. epidermidis' capacity to adhere to the soft-liner specimens. To make this broth, 25 grams of the medium were dissolved in 1000 millilitres of distilled water, and the mixture was then autoclaved for 15 minutes at 121 degrees Celsius with 15 pounds of pressure. Next, a McFarland densitometer was used to create a bacterial solution with 10^7 colony-forming units per millilitres (CFU/ml), following 0.5 McFarland standards [24].

The soft-liner specimens were autoclaved for 20 minutes at 121°C to sterilize them [25]. Following their immersion in sterile plastic containers holding the prepared bacterial solution, these sterile specimens were allowed to incubate at room temperature for one hour. After the incubation period was over, the specimens were taken out of the suspension and gently shaken twice for a minute each time in phosphate-buffered saline solution (PBS) to get rid of any non-adherent bacterial cells. They were then dried using filter paper [26].

Methanol (CARLO ERBA, France) was used to fix the adhering bacterial cells on the soft-liner specimens. After fixing, the samples were stained for 60 seconds with Crystal Violet HIMEDIA, India, washed again with PBS solution for 30 seconds, and then dried with filter paper. An inverted light microscope (Karl Kolb, Germany) was used for the examination [27]. After counting the adhering cells, the mean was determined.

Tensile strength

The testing protocol followed ISO 37:2011. The specimen was positioned in a computerized universal testing machine (Laryee Technology Co., Ltd., China) such that only the centre portion of the specimen was visible (Figure 3). Every specimen in the universal testing machine was stretched until it cut, with the bottom member moving at a steady crosshead speed of 500 mm/min while the top member stayed fixed. The computer program then reported the highest force at the break. When a specimen broke outside of the narrow section, it was not taken into consideration, and the test was repeated on another specimen until ten correct readings were obtained.

Using the following formula:

Tensile strength = F / A

Where:

F: The highest force noted during the break (N).

A: The specimen's initial cross-sectional area (mm2).

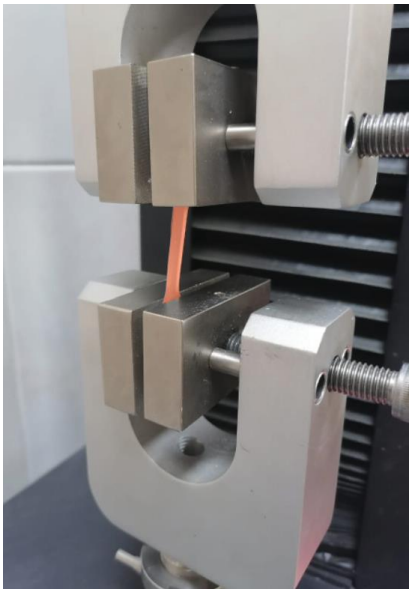

Figure 3. Tensile strength test.

Shore A hardness

Hardness testing was carried out utilizing a digital shore A durometer (TIME Group Inc., China) and a 1.25mm blunt indenter following ISO 7619-1 (2010) standards. The hardness value was determined by taking the average of the five measured spots, which were spaced six millimetres apart from the lateral boundaries of each specimen.

Statistical analysis

The statistical analysis used SPSS version 26. The study employed One Way ANOVA and Bonferroni post hoc test with a significance threshold of P=0.05 to ascertain the group differences. $P<0.01$ was regarded as extremely significant, and $P<0.05$ was significant.

3. Results

Bacterial adherence test

Adherent cells were measured for every specimen using an inverted light microscope, using the recommended device scale for accurate measurements. Then, following the device's recommended scale calibration for precise data processing, the mean cell count was ascertained (Figure 4). In comparison to group A (Control), the mean counts of adhering bacterial cells in both experimental groups, B and C, were considerably lower. With a mean count of 13.49, group C showed the lowest number, followed by group B with a value of 21.41. At 80.71, the control group, on the other hand, had the highest mean count of adhering bacterial cells (Table 1).

Table 1. Statistical test results for bacterial adherence test.

Bacterial adherence test			ANOVA		Bonferroni post hoc	
Group	Mean	±SD	F	P value	Groups	P value
Group A	102.2000	2.20101	6341.921	.000	A B	.000
Group B	30.6100	2.22583			A C	.000
Group C	11.5800	1.00089			B C	.000
Levene statistics=3.142, p value=0. .059						

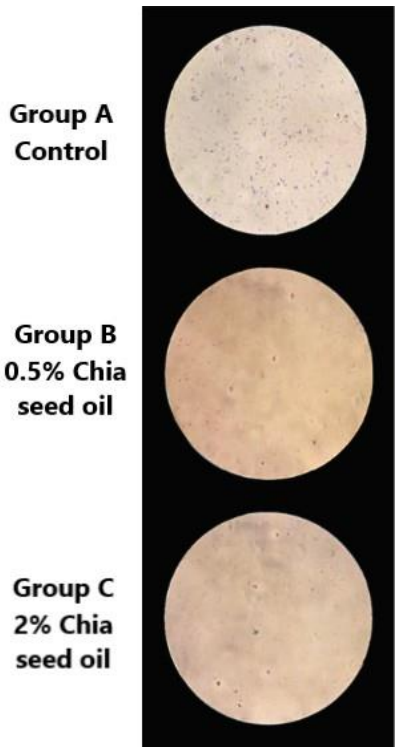

Figure 4. Inverted light microscope for the three groups. S. epidermidis appear as violet dots.

Tensile strength

The experimental group A had the highest value of tear strength (3.54) followed by group B (3.22) and group C (2.73). One-way ANOVA revealed significant differences between the groups, however, the post hoc test revealed a significant difference (P<0.05) between group A and group C only (Table 2).

Table 2. Statistical test results for tensile strength test.

Tensile strength test			ANOVA		Bonferroni post hoc	
Group	Mean	±SD	F	P value	Groups	P value
Group A	3.54	.59479	6.891	.004	A B	.471
Group B	3.22	.32931			A C	.003
Group C	2.73	.51218			B C	.103
Levene statistics=2.003, p value=0.154						

Shore A hardness

Surface hardness was decreased however, the decrease was non-significant statistically (P>0.05) as revealed in Table 3.

Table 3. Statistical test results for shore A hardness test.

Shore A hardness			ANOVA	
Group	Mean	±SD	F	P value
Group A	51.1500	.70907	2.830	.077
Group B	50.6500	1.04907		
Group C	50.3000	.57542		
Levene statistics=2.003, p value=0. .154				

4. Discussion

Chia seed oil is a rich source of omega-3 fatty acids such as alpha linolenic acid (ALA). These fatty acids are known for their ability to integrate into bacterial cell membranes due to their amphipathic nature as they have both hydrophobic water repelling parts and hydrophilic water attracting parts[28]. The unique structure of omega-3 fatty acids characterized by long chains and multiple double bonds can induce greater fluidity into the membrane. This increased fluidity alters the tight packing of phospholipids, leading to a more permeable membrane which allows the leakage of essential cellular components like ions and proteins [29].

Omega-3 fatty acids contain cis double bonds that can cause damage to the bacterial cell membrane by bending. This affects the stability of the membrane and make it more prone to damage. In addition, the non-bilayer reactions and vesicles can also be caused by the presence of omega-3 fatty acids which further contribute to the bacterial cell membrane

damage [30]. Not only omega-3, but phenolic acids are also present in Chia seed oil. These are Caffeic acid and chlorogenic acid which can inhibit bacterial proteases and glycosides by blocking the active sites of these enzymes, or by interfering with their functions [28, 31].

Chia seed oil's phenolic acids also interfere with energy production mechanisms by blocking citric acid cycles and glycolytic pathway enzymes, thus affecting ATP synthesis, which is vital for bacterial functions [32]. Furthermore, phenolic acids can generate oxygen-reactive species by changing the redox state. These ROS species can damage the bacterial DNA, ultimately leading to cell death [33]. Moreover, phenolic acids bind to liposomal subunits and disrupt the translation process leading to the production of defective proteins or the inhibition of essential ones. By altering signal transduction pathways, these phenolic acids can alter gene expression including those related to virulence and stress responses [34].

The mechanism of bacterial biofilm formation can also be affected by the phenolic complexes. These mechanisms are vital for bacterial pathogenicity. The acids act by preventing the initiation of biofilms by altering the bacterial surfaces and soft-liner matrix. In certain instances, Caffeic and chlorogenic acids can dissolve the already established biofilms by damaging the bacterial extracellular matrix components and interfering with quorum systems which are responsible for biofilm production [35]. Finally, phenolic caffeic and chlorogenic acids can induce apoptosis by producing oxidative stress and interrupting critical cellular processes. These acids can cause programmed cell death which results in the bacterial cell being eliminated [36]. The decrease in tensile strength and surface hardness after the addition of the oil can be explained by the fact that Chia seed oil functions as a plasticizer inside the polymer matrix.

Plasticizers are added to polymers to improve flexibility, but this generally comes at the experience of the polymer's strength. In the case of Chai seed oil, its role as a plasticizer disrupts the interactions between the polymer chains as the oil molecules integrate themselves into the polymer network causing the chains to become more mobile and less tightly connected [37]. This increased mobility decreases the rigidity of the material and decreases the hardness. Additionally, chia seed oil induces swelling in the polymer matrix. The oil interacts with the polymer chains causing the matrix to absorb the oil and expand. Such swelling alters the mechanical properties of the material by increasing its flexibility but also decreases its tensile strength and hardness [38, 39]. The extent of swelling depends on how well chia seed oil mixes with the polymer and the oil's ability to penetrate the polymer structure.

As the polymer becomes less dense and expanded, further reduction in tensile strength and hardness occurs. Moreover, the crosslinking network of the polymer can be altered by the added oil. Soft liners are typically made from cross-linked polymers in which the cross-links provide structural stability. Chia seed oil interferes with the formation and maintenance of these crosslinks, either by disrupting the existing bonds or by preventing new crosslink formation, thus weakening the material and decreasing its mechanical strength [40].

Finally, the presence of chia seed oil inside the polymer matrix led to inhomogeneity inside the soft-liner material. If the oil is not uniformly distributed throughout the matrix, it can create weak areas and inconsistencies inside the material. Such an effect can further reduce the tensile strength and hardness as the material properties are altered.

5. Conclusion

Within the limits of this study, chia seed oil, when used as an additive in denture soft liner, can inhibit S. epidermidis biofilms and improve antimicrobial effectiveness. However, a decrease in tensile strength and hardness is an indication that consideration is needed to balance the antimicrobial effects with the potential impact on mechanical properties.

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