

Flexural Strength, Antimicrobial Activity and Color Stability of Ginger (*zingiber officinale*) Modified Heat Cured Denture Base Material

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Abstract Objective: This study evaluated ginger modified heat cured poly methyl methacrylate (PMMA) denture base material regarding; surface roughness, flexural strength, antimicrobial activity, and color stability. **Materials and methods:** Ginger was used in powder and oil forms to modify the polymer and monomer. Seven experimental groups were formulated; Group (I) control; Group (II) 3 wt% ginger-powder modified polymer; Group (III) 5 wt% ginger-powder modified polymer; Group (IV) 3 v/v% ginger-oil modified monomer; Group (V) 5 v/v% ginger-oil modified monomer; Group (VI) 3 wt% ginger-powder modified polymer mixed with 5 v/v% ginger-oil modified monomer; Group (VII) 5 wt% ginger-powder modified polymer mixed with 3 v/v% ginger-oil modified monomer. Specimens were tested for surface topography, surface roughness, flexural strength, antimicrobial activity, and color stability. The data were statistically analyzed by one-way ANOVA and post-hoc LSD analysis with a significance factor of $\alpha = 0.05$. **Results:** The PMMA polymer modified with 3 v/v% ginger-oil (Group IV) exhibited a significant reduction in surface roughness, a significant increase in flexural strength with clinically acceptable color change. No antimicrobial activity was detected for any of the evaluated groups. **Conclusions:** 3 v/v% ginger-oil was able to improve the mechanical properties of heat cured PMMA denture base resin with a tolerable color change.

Keywords: flexural strength, ginger, PMMA, reinforced, surface roughness

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1. Introduction

Poly methyl methacrylate (PMMA) resins have been used for the manufacture of denture bases for over 50 years. In spite of PMMA extraordinarily advantages (like its easiness of fabrication with simple equipment), various limitations have been previously documented. Denture bases based on PMMA resin are prone to water sorption, which can adversely affect their mechanical properties [1]. High water sorption and solubility of such resins can have a serious impact by diminishing their flexural strength and fatigue limit [2,3].

Acrylic resin denture fracture is an unresolved trouble in removable prosthodontics [4], it may occur in-use as a consequence of its unsatisfactory transverse strength, impact strength or fatigue resistance. Attempts have been made to enhance the mechanical properties of acrylic resin by increasing the material bulk in the most heavily stressed area, through copolymerization, cross-linking,

and reinforcement with different fibers [5].

Another significant drawback of the PMMA resins is their liability to support the biofilm formation attributed to their surface roughness and free energy that may encourage microbial adherence [6]. The surface properties studies of the denture base material have revealed a direct link between surface roughness, plaque accumulation and adherence of *Candida albicans* [6,7]. In denture related stomatitis cases, an increased amount of *Candida* species was reported [8]. In prosthetic and restorative materials, the acceptable surface roughness (Ra) value is 0.2 μm , where no further reduction in plaque accumulation is expected [9,10]. An increase in prosthesis roughness might be a source of oral tissues' micro traumas [11].

The esthetic appearance of prosthesis is indeed a significant mandatory feature that must satisfy patients' expectations. Moreover, color stability is a critical clinical property, and color changes may be a sign of material aging or damaging [12,13]. Many factors can cause discoloration of acrylic resin such as; the dissolution of ingredients, stain accumulation, surface roughness, water

sorption and degradation of intrinsic pigments. The resistance of resins to color changes can be influenced by the inorganic fillers' structure, resins' physical properties and their chemical characteristics [14].

Ginger essential oil is a valuable product because of its biological and antimicrobial (antifungal, and antibacterial) properties [15-18]. Since it has highly volatile components (α -pinene, β -pinene) that may be missed during storage and use, it is very essential to enhance oil stability, in order to expand its range of applications. An effective way to increase the stability of essential oils is by encapsulation in polymer matrixes to protect the oil from its degradation [19].

Accordingly, this study was conducted to evaluate PMMA denture base resin' properties after its modification with ginger, considering surface topography, surface roughness, flexural strength, antimicrobial activity and color stability. The null-hypothesis was that the addition of ginger would not improve mechanical properties, antibacterial activity and esthetic properties of heat cured PMMA denture base resin.

2. Materials and Methods

A commercial heat cured PMMA denture base material (IQ-15; IMICRYL, Turkey) was blended in various proportions with ginger (powder and oil forms) that were purchased from the available local markets. Unmodified powder and monomer were used as control. Experimental powder was made by hand mixing PMMA powder and 3 and 5% (w/w) ginger powder using a glass mortar and pestle for 10 min. Experimental monomers were made by the addition of 3 and 5% (v/v) ginger-oil with the resin monomer. The mixture was stirred for 4 h using a magnetic stirrer. Seven experimental groups were considered for this study; group (I) control (unmodified PMMA); group (II) 3 wt% ginger modified PMMA powder with unmodified monomer; group (III) 5 wt% ginger modified PMMA powder with unmodified monomer; group (IV) unmodified PMMA powder with 3 v/v% ginger oil modified monomer; group (V) unmodified PMMA powder with 5 v/v% ginger oil modified monomer; group (VI) 3 wt% ginger modified PMMA powder with 5 v/v% ginger oil modified monomer; group (VII) 5 wt% ginger modified PMMA powder with 3 v/v% ginger oil modified monomer.

2.1. Specimens Preparation

Specimens were processed inside a custom made stainless steel mold according to the manufacturer's instructions. The mold was coated with a thin layer of petroleum jelly, then the base plate wax (Cavex, Haarlem, Holland) was softened, pressed into the mold, left to cool to room temperature, and later the wax pattern was carefully removed.

The wax specimens were embedded in type III dental stone in a dental flask. The flask was immersed in boiling water for 5 min, and the mold cavity was then rinsed with boiling water to eliminate all wax remnants. PMMA powder and liquid monomer were mixed with a ratio of (25 g/10 ml) in a ceramic jar for 1 min. The paste was kneaded and packed into the mold space then, trial closure was done at 1500 Psi, flashes were removed and final

closure was done at 3500 Psi under hydraulic bench press (Carlo De Giorgi S.R.L., Italy) for 30 min. Curing was done in a water bath at 100°C for 20 min as recommended by the manufacturer. After polymerization, the flasks were bench cooled at room temperature for 30 min and then water cooling was done for 15 min before opening the flask. Specimens were finished with 400-grit silicon carbide paper (Norton; Saint-Gobain Abrasivos, Brazil) to remove irregularities and then polished by a wet rag wheel with aslurry pumice. The specimens were stored in distilled water at room temperature for 48 h prior to testing.

2.2. Transmission Electron Microscopy (TEM)

Ginger powder (0.1 gm) and PMMA powder were ultra-sonicated for 30 min in distilled water and loaded on (200-mesh) carbon coated copper grid to be examined by TEM (JOEL, JEM-2100, Japan) to determine particle shape, size and distribution of both ginger powder and PMMA powder.

2.3. Scanning Electron Microscopy (SEM)

The surface topography of the resin specimens was studied using SEM. Specimens were first mounted on aluminum stubs and gold sputtered to obtain better image resolution and avoid electrostatic charging prior to analysis by SEM (JEOL, JSM-6510LV, Japan) operated at an accelerating voltage of 15 kV. The examination of all groups was done at magnification X 500 and 2000.

2.4. Surface Roughness

Thirty-Five disc shaped specimens (10 mm in diameter and 2 mm in thickness) were prepared as previously described. Surface roughness was measured by a profilometer (Surftest SJ210, Mitutoyo Corp., Kawasaki, Japan) according to the ISO 4287-1997. Each specimen was scanned 5 times and the mean roughness parameter (Ra) was calculated in (μ m). The tracing length was 8 mm, at a scanning speed 0.5 mm/s. The resolution of the record data was 0.01 μ m.

2.5. Flexural Strength

Thirty-five bar shaped (64 mm \times 10 mm \times 3.3 mm) specimens (five for each group) were prepared. Flexural strength test was performed according to the ISO standard 1567: 1999. Preceding the test, each specimen was measured for its; length, width and thickness with a digital caliper (Mitutoyo, Kawasaki, Japan) with a measuring accuracy of \pm 0.1 mm. Specimens were subjected to three-point loading with a universal testing machine (Model 3345; Lloyd Testing Machine, England) at crosshead speed of 5 mm/min and the data were recorded using computer software (Nexygen 4.6 Lloyd Instruments Ltd. 2002, UK). The flexural testing device consisted of a central loading plunger and two supports. The distance between the supports was 50 mm (represents the space between the maxillary molars in a complete denture). The load was applied perpendicular to the center of the specimen until fracture occurred. Flexural strength was calculated using the equation:

$$FS = 3FL / 2bd^2$$

where; FS is the flexural strength in (MPa), F is the load or force at fracture in (N), L is the span length of specimen between two supports (50 mm), b is the width, and d is the thickness.

2.6. Antimicrobial Activity

The ginger powder and oil were first solitary tested for their antimicrobial activity. Antimicrobial activity was determined using agar diffusion method. The diameter of the inhibition zones surrounding specimens were measured in (mm) at three different points. A total number of 140 disc shaped specimens of 5 mm diameter and 3 mm thickness were prepared (20 specimens per each group; 5 specimens for each microorganism). The *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus* microorganisms were cultured from clinical samples and kept overnight in a specific culture media at 37°C. *Streptococcus mutans* was a reference strain (UA 159). A base layer containing 15 mL of agar mixed with 100 µl of inoculum was prepared in a sterilized Petri dish (100 mm diameter) at pH of 7.5. After the solidification of culture medium, discs were transferred to the plates and incubated at 37°C for 48 h. Positive control was included in each plate. Such controls was composed of a sterile cellulose paper (8 mm) that was impregnated with either ampicillin (200 µg/disc) as antibacterial, or fluconazole (5 µg/disc) as antifungal.

2.7. Color Measurement

Thirty-five disc shaped specimens with dimensions 40 mm x 3 mm (five specimens for each group) were processed, finished and polished as previously mentioned. The color changes were evaluated using a spectrophotometer (BYK-Gardner, GmbH, Geretsried, Germany) which was calibrated before testing according to the manufacturer's instructions. The spectrophotometer was adjusted for multi-measure mode in which three readings of each specimen were obtained, and the mean value of these readings was recorded. A constant lighting condition of the measurement area was implemented. Measurements were performed according to the CIE (Commission Internationale de l' Eclairage) L*a*b* system covers all colors visible to the human eye and allows studies of color difference in dental materials. The L* value is a measure of lightness. The a* value represents positions on a red-green axis. The color becomes more red, with more positive a* value; and more green, with more negative a* value. The b* value denotes positions on a yellow-blue axis. The color becomes more yellow, as b* becomes more positive in value; and more blue, as b* becomes more negative in value. The equation utilized for calculating color differences is:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

where; ΔE is the color difference of the specimen, ΔL*, Δa* and Δb* are the differences in the L*, a* and b* values of each specimen from that of control. In order to better relate ΔE to the clinical implications according to the National Bureau of Standards (NBS), the ΔE were converted by the following formula to NBS units:

$$NBS \text{ unit} = \Delta E \times 0.92.$$

A value of (0-0.5) of NBS units is considered an extremely slight color change, (0.5-1.5) is a slight change, (1.5-3.0) is a perceivable change, (3.0-6.0) is an appreciable change, (6.0-12.0) is much appreciable and above 12.0 is considered a change to another color [20].

2.8. Statistical Analysis

The statistical analysis was done using statistical package for the social sciences (SPSS) version 20. All data were analyzed using one-way ANOVA and Post hoc LSD test for pairwise comparison with a significance factor of α = 0.05.

3. Results

3.1. TEM

Figure 1 shows the TEM results for the ginger and PMMA powder used in the study. For the ginger powder, TEM displays irregular particles' shape distribution in globular and spindle form, the powder seems to have some tendency to be arranged in dense asymmetric clusters (Figures 1a, 1b and 1c). For the PMMA powder, TEM validates spherical particles distribution with multimodal size ranging from 49.38- 328.19 nm (Figure 1d). The PMMA powder when aggregates in clusters show a nano-sized spherical regular arrangement (Figures 1e and 1f).

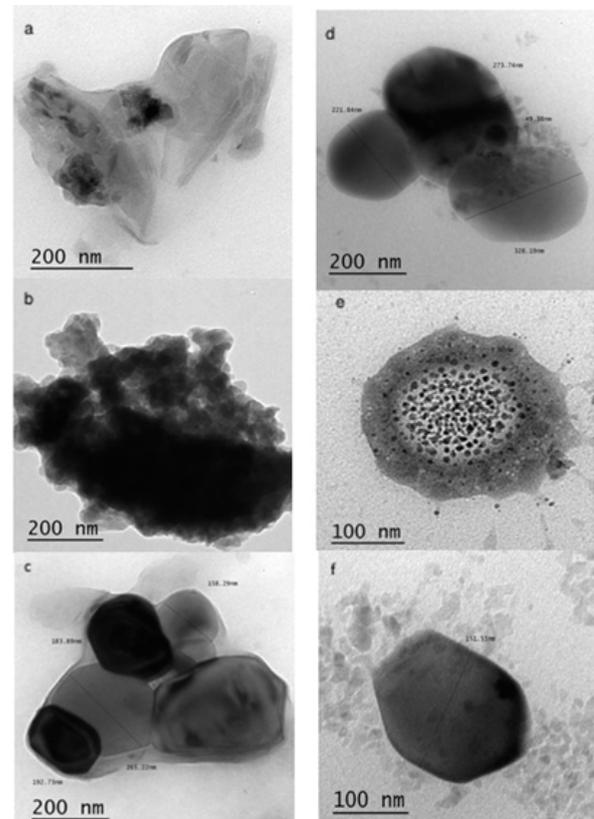


Figure 1. Transmission Electron photomicrograph of ginger and PMMA powder showing; a) Ginger's irregular particle shape distribution of globular and spindle forms; b) ginger particles' tendency for cluster formation; c) ginger powder particle size distribution; d) PMMA spherical multimodal particle size distribution; e) PMMA cluster of nano-sized spherical particles; f) PMMA discrete particle

3.2. SEM

SEM results for all groups at two magnifications are shown in Figure 2 to Figure 5. Figure 2 shows control PMMA heat cured resin, which reveals unmodified polymer matrix. Figure 3 shows group (II) and (III) ginger powder modified resin. Figure 3a and Figure 3b represent group (II); show thinned resin matrix, separated with large intervening spaces, and dispersed spherical shaped particles of different sizes. Figure 3c and Figure 3d represent group (III); they show resin matrix scattered with spheroidal and spindle shaped particles. Figure 4 shows group (IV) and

(V) ginger oil modified resin. Figure 4a and Figure 4b represent group (IV); they show compacted resin matrix with little spaces and many dispersed particles. Figure 4c and Figure 4d represent group (V); they reveal dense globular resin matrix with some connected spaces. Figure 5 shows group (VI) and (VII) powder and oil modified resin. Figure 5a and Figure 5b represent group (VI); they display spherical and irregular-shaped particles of different sizes scattered over the resin matrix. Figure 5c and Figure 5d represent group (VII); they demonstrate large spheroidal particles embedded in a thinned woven matrix with large intervening spaces.

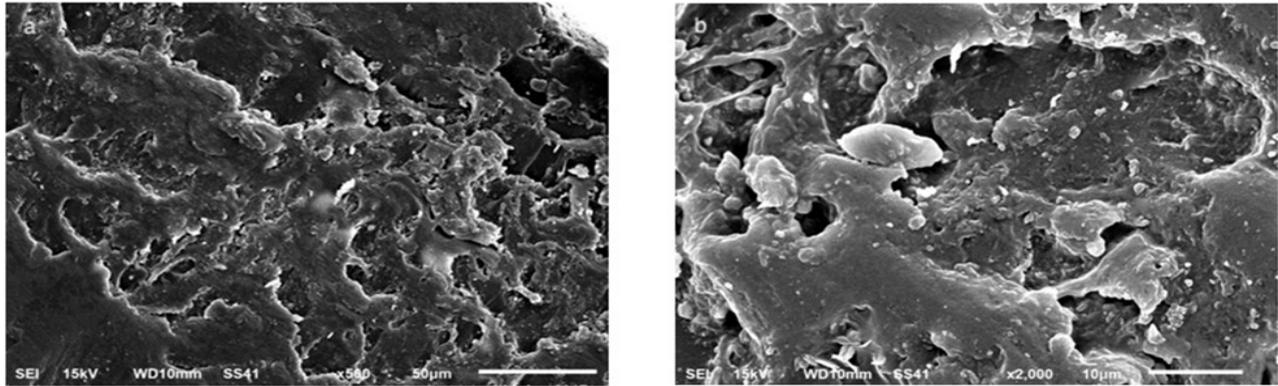


Figure 2. a) Scanning electron photomicrograph of control PMMA heat cured resin showing polymer matrix; b) higher magnification showing interconnected polymer matrix

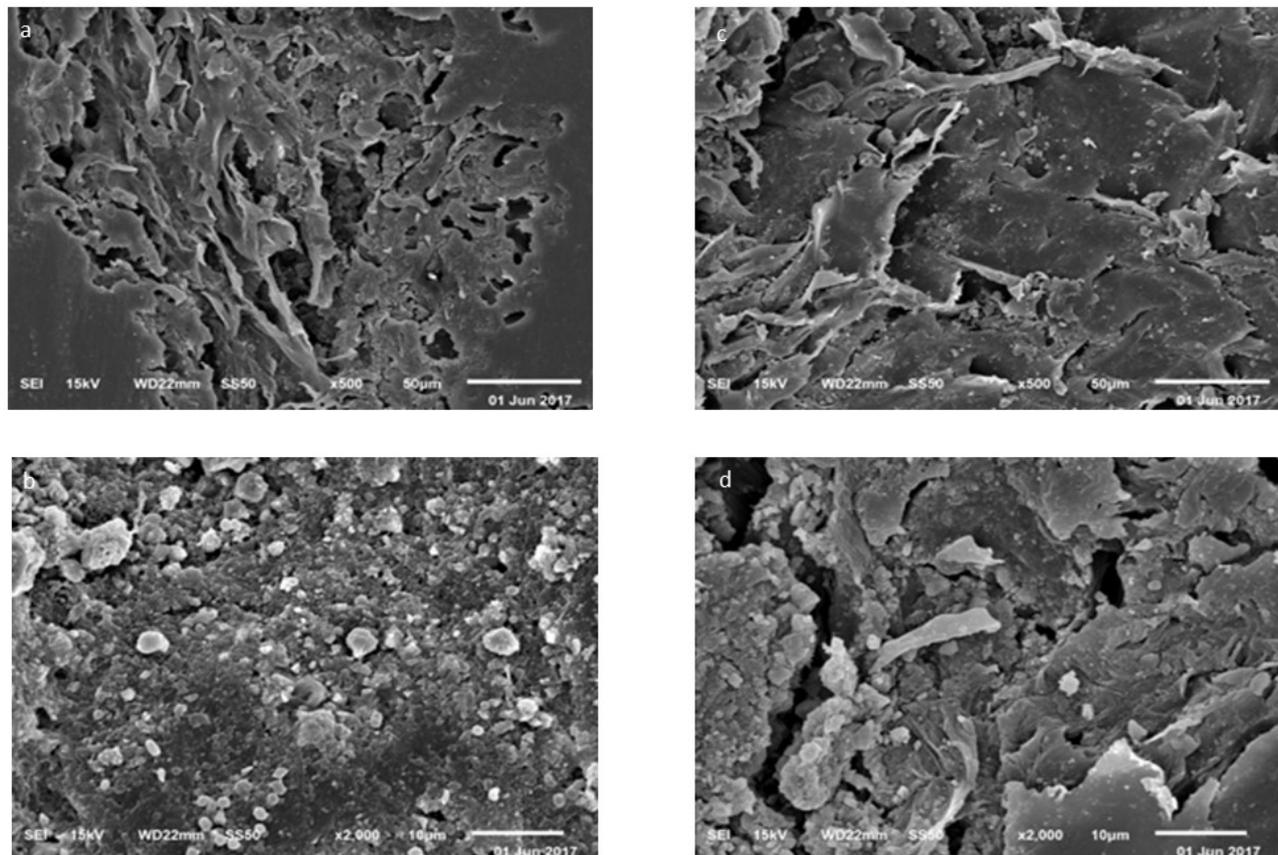


Figure 3. a) Scanning electron photomicrographs of group (II) resin modified with 3 wt% ginger powder showing thinned resin matrix separated with large intervening spaces; b) higher magnification of group (II) showing dispersed spheroidal particles of different sizes; c) Scanning electron photomicrograph of group (III) resin modified with 5 wt% ginger powder showing thinned irregular resin matrix; d) higher magnification of group (III) showing dispersed spheroidal and spindle shaped particles

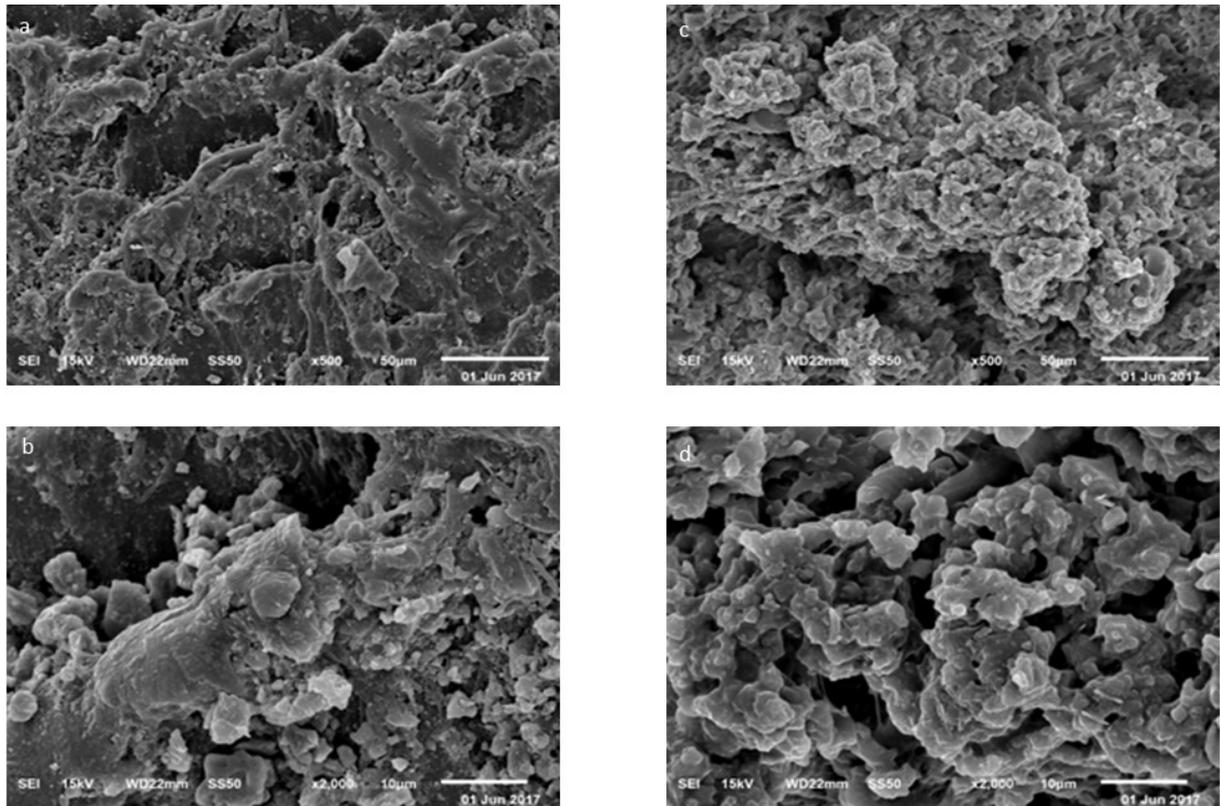


Figure 4. a) Scanning electron photomicrograph of group (IV) resin modified with 3 v/v% ginger oil showing compacted resin matrix with little spaces and minute scattered particles; b) higher magnification of group (IV) showing condensed resin matrix with many dispersed particles; c) Scanning electron photomicrograph of group (V) resin modified with 5 v/v% ginger oil showing compacted resin matrix in globular like form; d) higher magnification of group (V) showing thick connected globular resin matrix with some spaces

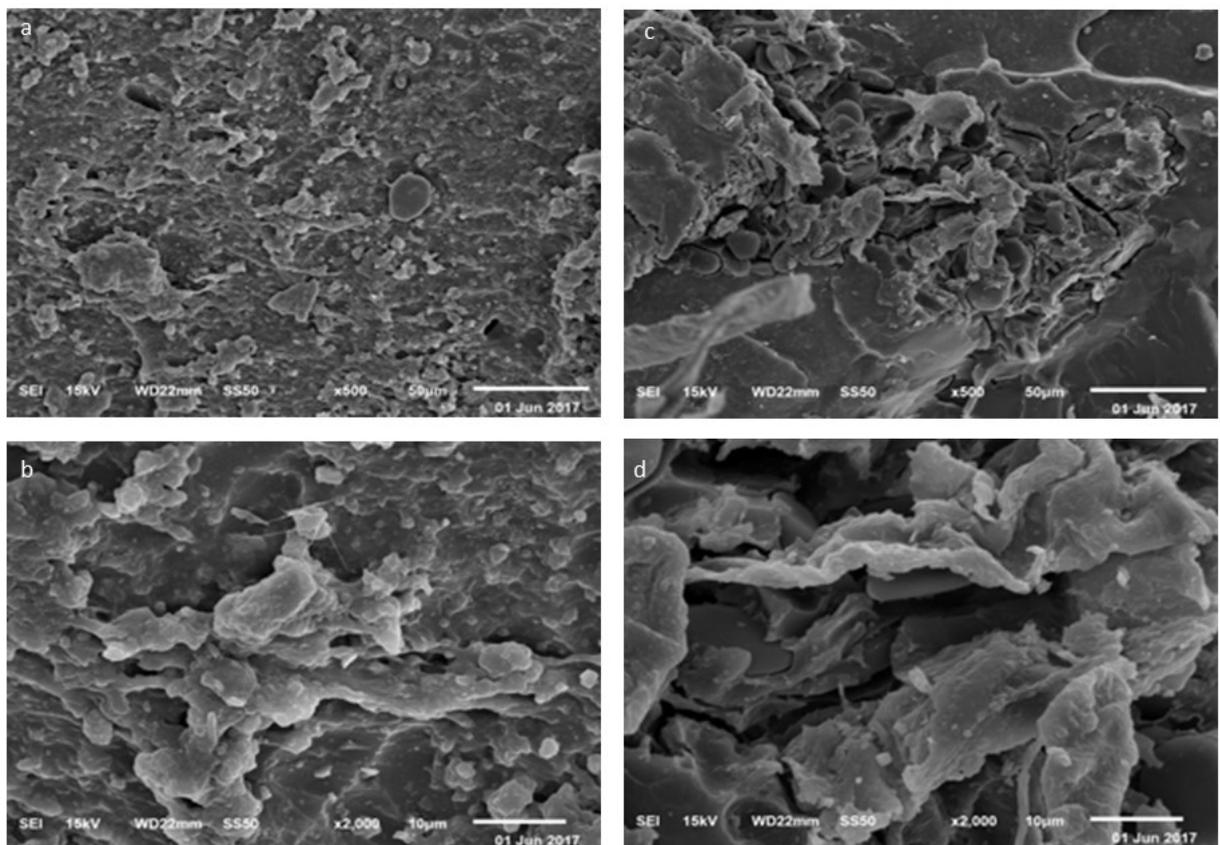


Figure 5. a) Scanning electron photomicrograph of group (VI) resin modified with 3wt% powder and 5 v/v% oil showing spherical and irregular shaped particles of different sizes scattered over the resin matrix; b) higher magnification of group (VI) showing resin matrix with spherical and globular shaped particles of different sizes; c) Scanning electron photomicrograph of group (VII) resin modified with 5wt% powder and 3 v/v% oil showing large spheroidal particles embedded in a woven matrix; d) higher magnification of group (VII) showing thinned matrix with large intervening spaces

3.3. Surface Roughness

Surface roughness means and standard deviations in (μm) are shown in Table 1. The results indicated that group (VII) had the highest roughness value (6.88 ± 0.5) while group exhibited the lowest roughness value (4.22 ± 0.89). One-way ANOVA showed that there was a significant difference among the studied groups ($P < 0.05$). The least significant difference (LSD) test results showed that the difference was statistically significant. There was no significant difference between groups I, and II, and between groups III, V and VI.

Table 1. Means and standard deviations of surface roughness in (μm) and flexural strength in (MPa)

Group	Surface roughness Mean \pm SD	Flexural strength Mean \pm SD
I	5.30 ± 0.93^b	110.88 ± 11.60^b
II	5.36 ± 0.99^b	77.53 ± 4.28^c
III	4.45 ± 0.23^{bc}	74.30 ± 12.30^c
IV	4.22 ± 0.89^c	129.07 ± 9.90^a
V	5.22 ± 0.88^{bc}	123.62 ± 5.00^a
VI	4.96 ± 0.71^{bc}	77.07 ± 6.13^c
VII	6.88 ± 0.50^a	76.09 ± 1.58^c
<i>p</i> value	0.0003	<0.0001
<i>f</i> value	6.14	45.21
LSD value	1.01	10.58

Means values with same superscripted letters are not significantly different at ($P > 0.05$).

3.4. Flexural Strength

Flexural strength means and standard deviations in (MPa) are shown in Table 1. The results indicated that the group (IV) had the highest mean value (129.07 ± 9.9) while group (III) had the lowest value (74.3 ± 12.3). One-way ANOVA showed a significant difference among the studied groups ($P < 0.05$). LSD showed that groups; IV and V had a higher significant increase in flexural strength over control and all other experimental groups. Groups II, III, VI and VII had a significant decrease in flexural strength compared to control, group IV and V. No significant difference was detected neither between groups II, III, VI and VII, nor between groups IV and V.

3.5. Antibacterial Activity

An inhibition zone of 15 mm was recognized with the ginger oil specimen against *Streptococcus* mutans only. Moreover, the results of testing ginger powder specimen presented an inhibition zone of 13.5 mm against *Streptococcus* mutans only, as well. On the other hand, no inhibition zone was detected with control and other modified groups' specimens against the different microbes used in the study.

3.6. Color Difference

Table 2 displays means and standard deviations of ΔE and NBS units between modified and control groups. One-way ANOVA showed significant differences among

the studied groups at ($P < 0.05$). The LSD test results showed that all groups were significantly different from each other. According to NBS classification, group (IV) exhibited only a perceivable change in comparison with the other formulations which produced changes ranging from marked appreciable (group V) to another color changes (groups II, III, VI and VII).

Table 2. Means and standard deviations of the color difference ΔE

Groups	ΔE	NBS	<i>p</i> value	<i>f</i> value
II & control	10.7 ± 0.82^d	9.84	<0.0001	426.11
III & control	14.71 ± 0.58^e	13.52		
IV & control	3.21 ± 0.27^f	2.95		
V & control	4.94 ± 0.59^e	4.54		
VI & control	15.7 ± 0.13^b	14.4		
VII & control	20.36 ± 0.61^a	18.73		
LSD value		0.94		

Mean values with dissimilar superscripted letters are significantly different at ($P < 0.05$).

4. Discussion

Dentures fracture in clinical service is a great concern and several attempts have been made to enhance flexural strength of PMMA. Researches in this area intended to alter the composition or to reinforce PMMA with stronger agents and develop new formulations with better properties without compromising the esthetics [21].

In the present study, the null hypothesis was partially rejected, as the PMMA resin modified with 3 and 5 v/v% ginger-oil have significantly improved the flexural strength as compared to both control and powder-modified formulation. This can be attributed to the uniform distribution of the ginger oil within the monomer which was achieved through a prolonged stirring procedure. The ginger chemical structure contains reactive double bonds that are capable of interacting with the monomer free radicals [22]. The chemical formula of ginger oil also contains a hydroxyl reactive group which could enter the polymerization reaction, increasing the cross-linking and subsequently improving the polymer strength [23]. Moreover, during the early stage of chemical polymerization reaction; the initiator decomposes leaving porosity between the particles; the ginger-oil may be packed within these pores producing stronger structure. Therefore, the free oil acts as an adhesive between the formed polymer particles enhancing their cohesion [22,24]. These interpretations are in accordance with the SE micrographs that showed a thick globular resin matrix with little intervening spaces. Therefore, the ginger-oil incorporated resin; groups (IV & V) gave a higher cross linked and consequently a higher flexure strength resin. On the contrary, the irregular particle shape of the ginger powder and their tendency to aggregate in clusters; as confirmed by the transmission electron microscopy; interfered with an even homogenous distribution of the polymer chains and hindered their cross-linking. This was noticed in the examination of resins' surface morphology and whenever ginger powder was added, the resin matrix appeared thin and wavy (Figure 3 and Figure 5).

Numerous factors can affect the resin surface roughness such as; physical properties; the cooling rate; and the polishing technique parameters (abrasive materials speed and pressure) [25,26]. Achieving a smooth mold cavity would be helpful to enhance the resins surface qualities. In the present study, the surface roughness of both control and experimental groups are rather high, which may be related to the specific resin material properties and its particle size. The lowest roughness values of group IV which is modified by the addition of 3 v/v% ginger-oil may be related to the oil' ability to seal porosity created by initiator decomposition and minimizing the chance of a rough surface production. On the other hand, group V which is modified by 5 v/v% ginger oil exhibited a higher non-significant roughness value than group IV, this could be related to the PMMA particles size increase that occurs after being encapsulated with 5 v/v% ginger-oil as noticed by SEM results and was also concluded by Racoti *et al.* in 2016 [24]. Moreover, the highest roughness value of group VII may be due to the incorporated ginger-powder with its irregular particle shape; spheroidal and spindle shaped; together with its tendency to aggregate in clusters of non-homogenous distribution that interfered with proper finishing and polishing, this might have predominated the ginger-oil sealing effect.

The antimicrobial properties of both powder and oil ginger were confirmed by using the agar diffusion assay. The ginger specimens showed markedly recognized inhibition zones against *Streptococcus* mutans in both powder and oil forms. The variation in the diameter of the inhibition zone between powder and oil ginger may be related to the different concentration of the ginger' active ingredients per unit volume plus the superior diffusion of the oil form. The absence of inhibition zones against *Staphylococcus aureus* and *Escherichia coli* may be due to the presence of a thick layer of peptidoglycan that protects those micro-organisms against antibacterial agents such as antibiotics, toxins, chemicals, and degradative enzymes [27,28].

The effect of ginger against *Candida albicans* is not well-established. The fungus is dissimilar from the bacteria, having a rigid skeleton cell wall consisting of approximately 80 to 90% carbohydrate and microfibrillar polymers (b-glucans and chitin), that provides the cell with strong physical properties. Accordingly, it is anticipated that this rigid skeleton protects *Candida albicans* against ginger [29]. A previous study investigated the antibacterial activity of ginger against *Escherichia coli* and *Staphylococcus aureus* and confirmed its antibacterial activity with ethanolic, methanolic and hexanic extracts [30]. The present study did not reveal any inhibition zone against all used microbes. This may be interrelated to the high temperature used for resin processing. This is in agreement with another study which concluded that ginger-extract lost its antimicrobial activity against *Klebsiella pneumoniae*, *Escherichia coli*, and *Staphylococcus aureus* when it was boiled [31].

One of the most satisfactory properties of the acrylic resins denture base material is their acceptable esthetic color, so it is important to be upheld when modifying resins by either reinforcing or antimicrobial agents. The ginger essential oil color ranges from pale yellow to a darker amber color. In the current study, modification of

the resins with both ginger forms produced a significant change in color. The higher the ginger concentration incorporated within the resin, the greater the color changes detected which ranges from marked appreciable to another color changes. The minimum change recognized was for group IV (3 v/v% ginger oil modified monomer), which was a perceivable change of NBS 2.95. According to the National Bureau of standard the color differences of 3.3 NBS units are acceptable in clinical dentistry [20].

Previous studies indicated that the trained human eye cannot visually detect color differences with ΔE values lower than 1.0, while untrained eye cannot detect color differences of ΔE values lower than 2.0 [32,33,34]. In other words, the color changes are subjective and dependent on the patient acceptance of the prosthesis. The color changes produced supposed to be greatly influenced not only by the ginger concentration but also by its ability to be packed within the structure without leaching out by its comparable polymeric nature.

5. Conclusions and Recommendations

Although 3 v/v % ginger oil-modified heat cured PMMA denture base material seems to be very promising formulation that exhibited diminished surface roughness and enhanced flexural strength with an acceptable color change, further studies should be implemented to evaluate the color stability of heat cured acrylic resin modified with 3 v/v% ginger oil after aging, as well as its impact strength. Furthermore, the effect of ginger-oil' addition to chemical cured resin on the antibacterial properties should be considered to analyze the influence of temperature changes on ginger efficacy. Supplementary studies should evaluate the antimicrobial effects of ginger-modified acrylics against common oral pathogens, using clinically relevant methodologies (such as adhesion, biofilm formation, percent of killing, etc). Also, mechanical properties such as elastic modulus, hardness, wear resistance and impact strength (after tooth brushing simulation, i.e.) should be determined.

Clinical Significance

3 v/v% ginger-oil modified heat cured PMMA resin may be a considerable reinforced formulation of a denture base material without compromised esthetic.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Authors' Contributions

Both Aref NS and El-Wassefy NA were responsible for conceptualization, formal analysis, funding, methodology, investigation, resources, statistical analysis, writing original draft, and reviewing it critically for important intellectual content. El-Mahdy RH carried out the

microbiological investigation. Final editing of the draft was done by Said MM for important intellectual content. All authors read and approved the final manuscript.

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