

Effect of frozen storage on preservation of a silicone-based test food material

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Aim: This study aimed to evaluate the effect of frozen storage on the physical properties of a silicone-based test food material, highly used to evaluate the masticatory performance in research settings. **Methods:** A total of 1,666 silicone cubes of Optosil Comfort® with 5.6-mm edges were shaped and stored at -18°C. The cubes were subsequently tested for flexural strength (maximum force, displacement, stress, and strain) before breaking ($n = 136$), changes in weight and size ($n = 170$), and masticatory performance ($n = 1360$) at eight timepoints: immediately after cube preparation (baseline, no freezing), and 1, 2, 3 and 4 weeks, and 2, 4 and 6 months after frozen storage. The cubes were thawed 8 h before each assessment. **Results:** The maximum force, stress, maximum displacement, and deformation values for the cubes were not affected by freezing ($P > 0.05$). At all of the time points, the cubes exhibited similar weight ($P = 0.366$) and size (identical values). The masticatory performance for the cubes also showed no differences from baseline through 6 months ($P = 0.061$). **Conclusion:** Freezing Optosil Comfort® silicone cubes did not alter the physical and mechanical properties of the material, being suitable to optimize the assessment of masticatory parameters for research purposes.

Keywords: Mastication. Flexural strength. Freezing. Silicone elastomers.



Introduction

A proper masticatory function is fundamental to individuals' nutrition, being related to long-term health and quality of life. Thus, studies have been developed to quantify this aspect in different population groups and to test the impact of oral rehabilitation, by using an objective masticatory performance test¹. This test determines the comminuted median particle size (X_{50}) of a test food after a certain number of masticatory cycles². Several artificial and natural materials have been used to measure the crushing and grinding capacity of teeth². However, the traditional, reliable and validated option for evaluating masticatory performance in dentate employs a portion with 17 cubes of Optosil[®] silicone (Optosil[®] 1980, Optosil P Plus[®], and Optosil-Comfort[®] versions) each with an edge length of 5.6 mm, and the total amount of ~ 3.4 g/3 cm³ (Heraeus Kulzer GmbH & Co. KG, Hanau, Germany)³.

Optosil[®] 1980 version has traditionally been described as presenting a texture quality similar to apples, raw carrots, peanuts, chocolate, or coconut⁴. The current version, Optosil-Comfort[®] (Optosil-C[®]), shows higher fracture force (18% N), degree of deformation (21% mm), and fracture work (42% N.mm) than the classic version¹. These cubes have been used to evaluate the masticatory function of both dentate subjects and fixed prostheses wearers³. The weight of 17 cubes corresponds to $\sim 7\%$ of the mean weight of a freely chosen mouthful by a dentate subject. They also represent $\sim 30\%$ of a test food maximum weight which a subject can store in their mouth⁵.

The average time needed to form Optosil-C[®] cubes in a metallic mold and trim them is approximately 30 min. To achieve complete polymerization of the material, the cubes are then incubated in an oven at 65° for 16 h⁶, and an additional 24 h is needed to disinfect the cubes⁷. When Optosil-C[®] cubes are used in masticatory performance tests, sieving, fractioning, and weighing of comminuted particles are usually completed within 45 min. Thus, the processing of Optosil-C[®] cubes involves a time-consuming and systematic laboratory sequence³. Moreover, to guarantee dimensional stability, it is recommended that Optosil[®] test food is used or stored within 7 days after polymerization⁸. To date, only one study has been reported for long-term preservation of Optosil[®] materials, where Optosil[®] 1980 version and its rarely-used modified versions, Optoweak (by the use of another catalyst) and Optosoft (by heating the silicone base), were stored in a freezer at -20 °C and did not show alterations in their properties after 26 weeks¹.

The physical characteristics of a test food determine the probability of the teeth grind the particle, the size reduction when a selected particle breaks, and the distribution of all food particles in mouth compartments during mastication⁹. Therefore, the type and quality of materials used to measure the masticatory function of individuals are critical for reliable results. Given the time-consuming process which is needed to prepare Optosil-C[®] cubes for clinical research, storing these materials without affecting their comminution attributes would be of great value. Thus, this study aimed to analyze the flexural strength, weight, size, and masticatory performance of Optosil-C[®] cubes after their storage at -18 °C for up to 6 months.

Materials and Methods

Study Design

A total of 1,666 specimens of OptosilC[®] cubes, each with an edge length of 5.6 mm, were prepared for this cross-sectional study. OptosilC[®] Comfort silicone elastomer and Activator Universal Plus (Heraeus Kulzer GmbH & Co. KG, Hanau, Germany) were manipulated according to the manufacturer's recommendations³. OptosilC[®] cubes were shaped and initially polymerized into steel matrices by a single calibrated examiner. To achieve complete polymerization, all of the specimens were incubated in an electrical stove (SP-400, Splabor, Presidente Prudente, SP, Brazil) at 60°C for 16 h⁴. After confection, the OptosilC[®] cubes were randomly divided into experimental groups to undergo a flexural strength test ($n = 136$), weight and size paired comparisons ($n = 170$), and a clinical assessment of masticatory performance ($n = 1360$).

Specimens in each experimental group were subsequently divided into eight groups to undergo testing at specific time points: immediately after confection (baseline), and then 1-week, 2-weeks, 3-weeks, 4-weeks, 2-months, 4-months, and 6-months after freezing of the cubes at -18°C in a FE 22 freezer (Electrolux, Pinhais, PR, Brazil). The freezer was maintained at an average temperature and humidity of -18°C and 63%⁸, respectively, according to a digital hygro-thermometer (Cotronic Technology Ltd, Bao'an, Shenzhen, China) with precisions of $\pm 1^\circ\text{C}$ and $\pm 5\%$, respectively. During storage, specimens were kept inside closed plastic containers. Before each evaluation, the cubes were removed from the fridge and thawed for 8 h at room temperature. The OptosilC[®] cubes used for weight and size comparisons were frozen again after each evaluation.

The sample size was determined by using the MedCalc[®] (version 18.2.1, MedCalc Software, Ostend, Belgium). A type I error $\alpha=0.05$ (significance) and a power of 0.8 were considered. In a pilot study, five OptosilC[®] cubes were evaluated in two flexural strength tests which were performed with an interval of one week between the tests. The difference of means (4.02) and standard deviation for each test (3.20 and 3.49) were used in a comparison of means test. It was calculated that a minimum of 12 observations per group was needed. Thus, a sample size of 17 OptosilC[®] cubes for each of the eight timepoints was determined ($n = 136$ cubes). For masticatory performance, five dentate volunteers were evaluated twice with an interval of one week between the evaluations. The mean difference (0.21) and standard deviation of differences (0.14) from these data were used in a paired samples t-test. Thus, a sample size of 10 portions of test food (17 OptosilC[®] cubes each) for each timepoint was needed for a total of 1,360 cubes. This sample size was also considered for weight and size paired evaluations ($10 \times 17 = 170$ cubes).

Flexural strength

A three-point flexural test was applied to 17 OptosilC[®] cubes at each of the eight timepoints evaluated ($n = 136$). The test was performed to calculate the maximum force (N), maximum displacement (mm), maximum stress (N/mm²), and maximum strain (%) before breaking the material. The assay was performed with a universal testing machine (AG-I, Shimadzu, Tokyo, Japan) with a 10 kN-load cell capacity. The speed of

the probe was 30 mm/min. The cubes were placed on two parallel supporting cylindrical pins separated by 4.2 mm. A loading force was applied to the middle of cubes by using a cylindrical loading pin¹⁰. To guarantee uniform loading of the specimen and prevent friction between the specimen and the supporting pins, supporting and loading pins were mounted to achieve free rotation around the axis parallel to the pin axis, or around the axis parallel to the specimen axis (Figure 1).

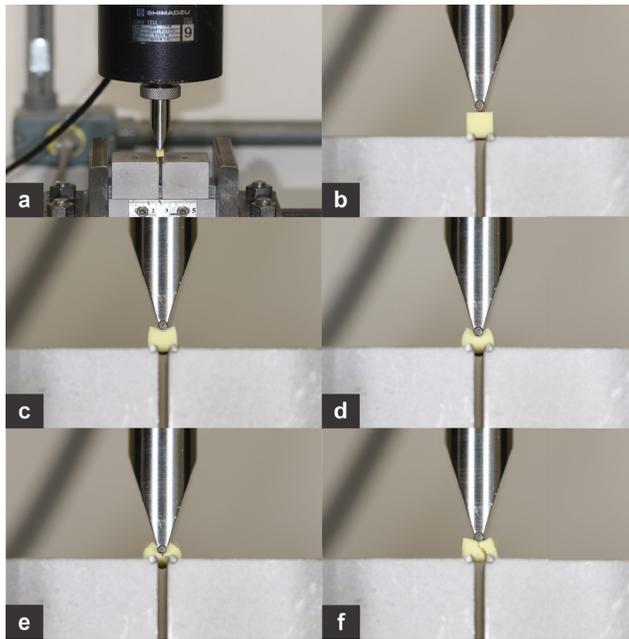


Figure 1. a: OptosilC[®] cube placed on the universal testing machine; b-e: Sequence of flexion and breakage; f: Recovery of the cube after the breakage.

Weight and size

At each of the eight timepoints evaluated, 10 portions of 17 OptosilC[®] cubes were weighed on a 0.001-g analytical balance (Mark 2060, BEL Engineering, Monza, Italy). The cubes were also sized with a sieving method by using a mesh of 5.6 mm in a shaker (Bertel Indústria Metalúrgica Ltd., Caieiras, SP, Brazil) for 20 min³. Thus, the weight and size of the cubes were evaluated by paired comparisons at each timepoint.

Masticatory performance

Inclusion criteria for participation in the clinical phase of this study were: (1) complete healthy natural dentition, (2) mesofacial biotype, (3) convex anteroposterior profile, (4) normodivergent vertical pattern, and (5) natural normocclusion, showing Angle Class I molar and canine relationships, 3 mm overjet and overbite, slight dental crowding with a midline deviation < 1 mm, and mutually protected articulation. The volunteer must have also been free of any systemic disease which could interfere with motricity, and not be under any drug which would alter saliva secretion⁹. Thus, a 37-year-old male volunteer met these criteria and was included in the study. Before enrolment, the

research was approved by the local Ethics Committee (CAAE: 15107313.8.0000.0105), and the single volunteer read and signed a consent form, which was following the Helsinki Declaration and its later amendments.

The masticatory performance test was performed by asking the volunteer to chew ten portions of 17 OptosilC® cubes ($\sim 3.4 \text{ g} / 3 \text{ cm}^3$) in a habitual manner for 20 chewing cycles, counted by the examiner. This procedure was repeated at each of the eight timepoints evaluated, with a 5-min interval between the mastication of each additional portion of cubes^{3,6,11}.

To recover all of the material, the volunteer expelled the particles onto a paper filter placed on a beaker and rinsed the oral cavity with 200 mL water. After the water was drained through the filter, it was dried in an oven at 80°C for 25 min. The particles were then sieved through a stack of nine sieves with $\sqrt{2}$ -progression mesh sizes (8.0–0.5 mm) in a shaker (Bertel Indústria Metalúrgica Ltd., Caieiras, SP, Brazil). After 20 min, the particles retained in each sieve were weighed on a 0.001-g analytical balance (Mark 2060, BEL Engineering, Monza, Italy). Masticatory performance was calculated according to Rosin-Rammler equation: $Q_w^-(X) = 1 - 2^{-\left(\frac{X}{X_{50}}\right)^b}$, with Q_w^- representing the cumulative weight percentage of the particles smaller than X (certain sieve aperture), X_{50} representing the aperture of a theoretical sieve through which 50% of the weight can pass, and b representing a broadness of size particle distribution. Then, it was verified if comminution was altered after each freezing timepoint^{3,6}.

Statistical Analysis

Data were examined with Prism (version 7; GraphPad Software, Inc., CA, USA). All inferences were based on two-tailed tests performed with a significance level of 95% and power of 80%. Parametric assumptions were discarded according to the D'Agostino-Pearson normality test, Bartlett's and Brown-Forsythe tests for homogeneity of variances, and Mauchly's test of sphericity. The results for maximum displacement and strain were compared by using one-way analysis of variance (ANOVA) on ranks and Dunn's multiple comparisons tests, respectively. Maximum force and stress data were compared by employing one-way ANOVA and Tukey post hoc tests. Meanwhile, Friedman and Dunn's multiple comparisons tests were applied to comparisons of weight and masticatory performance paired values.

When non-significant p-values were close to the level of significance ($P = 0.05$), the observed power was calculated by using the SPSS Statistics® software (v. 25, IBM Corporation, Amonk, NY, USA) with a 95% confidence level.

Results

Flexural strength data for the OptosilC® cubes are presented in Table 1. Outcomes related to resistance, as maximum force and stress achieved until breaking, presented no significant differences over time ($P = 0.071$ and $P = 0.069$, respectively). Regarding cube deformation during flexion, displacement and strain values were statistically significant for ANOVA on ranks test ($P = 0.030$ and $P = 0.030$, respectively), but the post-hoc analyses did not identify significant differences among time points ($P > 0.05$).

Table 1. Mean (SD) from the flexural strength test results ($n = 17$ Optosil® cubes).

Follow-up	Variables *			
	Force (N) [†]	Displacement (mm) [‡]	Stress (N/mm ²) [†]	Strain (%) [‡]
Baseline	63.30 ± 6.38 A	3.32 ± 0.45 A	2.27 ± 0.23 A	63.24 ± 8.62 A
One week	63.06 ± 8.96 A	3.18 ± 0.52 A	2.26 ± 0.32 A	60.63 ± 9.99 A
Two weeks	58.97 ± 4.97 A	3.36 ± 0.24 A	2.12 ± 0.18 A	64.02 ± 4.49 A
Three weeks	58.83 ± 5.89 A	3.47 ± 0.32 A	2.11 ± 0.21 A	66.14 ± 6.01 A
Four weeks	61.19 ± 4.66 A	3.49 ± 0.22 A	2.20 ± 0.17 A	66.42 ± 4.11 A
Two months	63.09 ± 4.50 A	3.55 ± 0.24 A	2.26 ± 0.16 A	67.67 ± 4.58 A
Four months	58.91 ± 5.70 A	3.43 ± 0.32 A	2.11 ± 0.20 A	65.28 ± 6.19 A
Six months	61.10 ± 4.00 A	3.51 ± 0.22 A	2.19 ± 0.14 A	66.80 ± 4.20 A

[†]One-way ANOVA and Tukey's post hoc tests ($\alpha = 0.05$);

[‡]One-way ANOVA on ranks and Dunn's multiple comparisons tests ($\alpha = 0.05$);

* Power observed (1- β error probability): Force = 0.741, Displacement = 0.804, Stress = 0.744, Strain = 0.802; Different letters indicate statistically significant differences among time points.

The weights of the OptosilC® cubes (range: 3.388–3.391 g) remained unchanged ($P = 0.366$) between the baseline and all time points. Furthermore, the cubes preserved their original dimensions and were retained in a 5.6-mm sieve.

There were no differences among the X_{50} mean values obtained at the eight time-points examined ($P = 0.061$). Similarly, no differences in the broadness of particle size distribution were observed ($P = 0.054$) (Table 2).

Table 2. Mean (SD) from the masticatory performance results ($n = 10$ portions 17 Optosil® cubes).

Follow-up time	Variables *	
	X_{50} (mm)	b
Baseline	3.10 ± 0.14 A	3.38 ± 0.25 A
One week	3.11 ± 0.13 A	3.20 ± 0.29 A
Two weeks	3.15 ± 0.22 A	2.98 ± 0.32 A
Three weeks	3.12 ± 0.25 A	2.97 ± 0.44 A
Four weeks	2.97 ± 0.33 A	3.07 ± 0.50 A
Two months	3.06 ± 0.13 A	3.29 ± 0.24 A
Four months	3.03 ± 0.09 A	3.11 ± 0.35 A
Six months	2.86 ± 0.16 A	3.09 ± 0.34 A

Friedman and Dunn's multiple comparisons tests ($\alpha = 0.05$);

* Power observed (1- β error probability): $X_{50} = 0.801$, $e b = 0.652$;

Different letters indicate statistically significant differences among time points.

Discussion

The results of this study demonstrate the stability of OptosilC® cubes after being stored at -18°C for up to six months. This long-term behavior may be explained by the chemical and physical characteristics of silicone material. Polydimethylsiloxanes are organosilicon polymers that are composed of a Si–O– linked backbone, repeating units of $-\text{[(CH}_3\text{)}_2\text{Si–O-]}_n\text{-}$ and silanol end groups¹². As a result, silicone exhibits ther-

mal stability, minimal temperature effects, and low-temperature performance¹³. This molecular configuration also provides excellent release features and surface activity, antifriction and lubricity, good damping behavior, shear stability, hydrophobic and physiological inertness, and weak intermolecular forces^{12,13}.

It is hypothesized that continuous degradation of OptosilC[®] is inhibited or at least retarded by storage at -18°C, and partly by complementary polymerization which occurs over 16 h at 60°C during the preparation of OptosilC[®] cubes. However, there is no scientific proof to support such hypotheses. Only one research indirectly detected a slight influence of freezing at -18°C on elastomer stability through impressions of a single steel gauge block to cast stone dies after 24h¹⁴.

Our laboratory findings from a 3-pint flexural strength test simulating dental occlusion also show that similar maximum force and maximum stress were needed to break the OptosilC[®] cubes at each timepoint. The low-temperature performance of silicone is due to its highly stable chemical structure. OptosilC[®] is composed of a polymeric matrix of polydimethylsiloxane and a 24.74% volumetric fraction of inorganic particles measuring 11.66 µm [e.g., Zn (6.39%), Mg (15.30%), Si (72.89%), and Na (5.42%)]¹². Moreover, the catalyst paste contains alkylsilicate and a tin-based activator (stannous octoate). A condensation reaction is driven by cross-linking (Van der Waals forces) between the hydroxyl groups (from silanol ends) and the alkyl, which produces alcohol as a byproduct^{12,15}. Silicone is then polymerized by irreversible formation of a three-dimensional network which prevents the silicone chains from sliding over each other, while still maintaining the flexibility of the material¹⁵.

The U.S. Food and Drug Administration recommends -18°C as an ideal temperature for food preservation (<https://www.fda.gov/consumers/consumer-updates>). The thermal behavior of OptosilC[®] cubes allows them to be stored at this temperature without alteration of their properties. Moreover, lower temperatures slow down the natural degradation of the polymer by decreasing the activation energy for these reactions and also slowing the propagation of microorganisms. However, no uniform reduction in reaction rate has been observed as the temperature is lowered, although there is a certain extent of adherence to Van't-Hoff's rule (the velocity of a chemical reaction increases two-fold or more for each 10°C increase in temperature)¹³. The latter is generally true when a temperature approximates that at which a reaction normally occurs.

The combination of very high siloxane chain flexibility and very few methyl/methyl interchain interactions produces polydimethylsiloxanes which have extremely low glass (-123.15°C) and low melting (-45.15 – -41.15°C and -37.15°C) transitions^{16,17}. The freezing point of polydimethylsiloxanes may also play a relevant role in defining the low-temperature use limit of this material because its mechanical properties undergo changes that are similar to a harder rubber within just a few degrees of temperature change. The freezing temperature for a material is highly dependent on the rate of cooling of that material. For example, a rapid cooling rate of 10°C per minute can result in a freezing temperature between -70°C and -80°C. In contrast, a slower cooling rate of 1°C per minute can result in a freezing temperature between -60°C and -65°C¹⁸. The freezing temperature for the Optosil[®]

material in the present study was far from Optosil®'s critical temperature. Besides, the hydrophobic nature of Optosil® (even at -18°C)¹⁹, may have prevented it from absorbing or adsorbing water.

Regarding the weight and size of the OptosilC® cubes tested, no differences were observed among all time points. These results are consistent with the long-term behavior of silicone materials. When silicone is stored at room temperature, its dimensional stability depends directly on its properties of elastic recovery, polymerization shrinkage, and evaporation of volatile components from the material^{15,20}. All condensation silicones exhibit a slight volume reduction due to cross-linking, bond rearrangements in their polymer chains, and alcohol evaporation¹⁵. Moreover, as alcohol is produced, silicone material is distorted as it is released¹². The mechanical properties of silicone have been improved with low polydispersity, long molecular chains between crosslinking points, and a faultless network with fewer dangling ends^{20,21}. After 48 h at room temperature, polymer degradation is accentuated, thereby resulting in increased shear and greater Young's moduli and dynamic viscosity²².

Degradation of polydimethylsiloxanes has been found to depend on the physical magnitude to be evaluated, failure time, temperature (e.g., at the lowest temperature, at room temperature, or highest temperature), apparent activation energy, and gas constancy. When polydimethylsiloxanes undergo artificial aging (thermal), the polymer network and the chemical structure of the backbone will be altered significantly. The degradation course mainly involves depolymerization and chain scission reactions which lead to cleavage of the main chain and the production of dangling ends (cyclic oligomeric siloxanes, higher oligomeric siloxane residues, and a smaller proportion of other components)^{20,23}. Overall, degradation can lead to "backbiting" of hydroxyl-terminated polydimethylsiloxanes and intramolecular depolymerization of end blocking polymers²⁰.

Meanwhile, clinical evaluations of masticatory performance support the hypothesis that OptosilC® cubes can be frozen since no significant differences were found. This result is supported by the mechanical properties of this material, which did not alter over time. The single volunteer in the present study acted as a "chewing device"⁹. Considering that the masticatory process involves muscle activity to generate mandibular movements and exert bite forces for food comminution by teeth¹, standardizing the masticatory performance test by one volunteer reduces possible bias related to muscle strength.

Although the variables flexural strength and broadness of particle size distribution (b) showed a significance level near 0.05, the power of the tests presented appropriate values ($1-\beta$). In general, an 80% power or higher is considered statistically powerful. In the present study, the power achieved was around 75%, which could be considered enough to avoid a type II error.

It is important to emphasize that the present study combined *in vitro* and *in vivo* assessment of mastication. The loading geometry for OptosilC® cubes can be explained by a crack in the surface, which may start adjacent to a cusp tip as the particle is indented, or more remotely from cusps through bending of the cubes

against a three- or greater-point cuspal support⁹. Thus, an eventual cusp-fossa or cusp-embayment occlusion can be simulated by the *in vitro* flexural strength test and the masticatory performance test allowed an *in vivo* evaluation, evidencing the reproducibility of the present results. Studies have demonstrated that the test food portion may change its presentation by decreasing the number and size of cubes (two half cubes of 9.6x9.6x4.8mm)²⁴, to avoid a functional high-test load (number of chewing cycles and bite strength) and increase the comminution degree²⁵. However, cubes with a 5.6 mm edge are still the most used method and the gold standard in masticatory research in dentistry^{1,26}. It is worthy to highlight that, despite our attempt to standardize all steps of this study, it would have been relevant to have control groups without freezing to investigate cubes' degradation over time. Thus, it could be considered a limitation of this study and considered in future researches.

In conclusion, the storage of OptosilC[®] cubes at -18°C did not modify its physical and mechanical properties. Thus, freezing this silicone-based test food material may reduce time-consuming laboratory processes during clinical research.

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Data availability

Datasets related to this article will be available upon request to the corresponding author.

Conflict of Interest

None.

Please add, before references:

Author Contributions

This research is part of the Undergraduate Coursework of Garcia Dutra de Castro, who made most of the experimental methodology, obtained the data, and contributed with paper drafting. Olívia Maria Costa de Figueredo, Mariana Barbosa Câmara de Souza and Camilla Fraga do Amaral significantly contributed to execution of the methodology and paper draft. Giancarlo de la Torre Canales contributed with interpretation of the results, manuscript writing, and revision. Alfonso Sánchez-Ayala and Renata Cunha Matheus Rodrigues Garcia conceived the idea for the study and study design. These authors were also responsible for the main data interpretation and manuscript writing. All authors have actively participated in the discussion of the manuscript's findings and have revised and approved the final version of the manuscript.

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