

# Viscoelasticity at initial setting and gelation time of a short-term soft liner incorporated with clove, star anise, and kaffir lime essential oils

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**Objective:** In this study, a short-term denture soft liner was incorporated with *Eugenia caryophyllata*, *Illicium verum*, and *Citrus hystrix* essential oils. An influence of these essential oils on initial viscoelastic properties of the modified soft liner was investigated.

**Materials and methods:** Various concentrations of three essential oils were added into the short-term soft liner. The initial viscoelasticity of these materials was evaluated by an oscillating plate rheometer. Three parameters including a gelation time, initial elastic and viscous moduli at 360 seconds ( $G'_{360}$  and  $G''_{360}$ ) were determined. Five independent experiments were done.

**Results:** For the soft liner without additive, its gelation time was 92 seconds, approximately. The  $G'_{360}$  and  $G''_{360}$  of the material were 95 and 26 kPa, respectively. After incorporating all essential oils into the soft liner, both  $G'_{360}$  and  $G''_{360}$  were decreased in a dose-dependent manner. The addition of 10 - 20 %v/v of *E. caryophyllata* oil and 20 - 30 %v/v of *I. velum* oils did not significantly influence on the gelation time of the soft liner. Whereas, the incorporation of *C. hystrix* oil at 35 %v/v, 40 %v/v, and 45 %v/v significantly prolonged the gelation time of the soft liner to be 160, 204, and 322 seconds, respectively.

**Conclusions:** Higher concentrations of the essential oils diminished initial moduli of the short-term soft liner. *E. caryophyllata* and *I. velum* oils seemed to be more appropriate additives without an alteration in the gelation time of the soft liner. The incorporation of *C. hystrix* oil into the short-term soft liner dramatically changed both gelation and initial moduli.

**Keywords:** essential oils, gelation time, rheometer, soft lining material, viscoelastic

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## Introduction

Denture soft liners have been utilized in several indications, including ill-fitting dentures, tissue trauma, post-operative surgery, and maxillofacial defects. These materials are applied beneath denture bases to improve denture adaptation and tissue healing [1]. However, these materials are prone to be a reservoir for microbial accumulation and can also result in candida-associated denture stomatitis [2, 3]. So, there have been attempts to use several kinds of additives to improve the anti-microbial properties

of soft lining materials [4-8]. Some conventional anti-fungal drugs such as nystatin, miconazole and fluconazole were firstly incorporated into the materials to overcome such problem [4,5] Nevertheless, these drugs have been reported about toxicity issues [9]. Natural products that provide an anti-fungal effect would be, therefore, more appropriate for human health.

Recently, essential oils extracted from plants or fruits have been incorporated into soft lining materials to improve anti-microbial properties [7, 8]. Such natural derive compounds seem to be more attractive than conventional drugs due to relative biocompatibility. Herein,

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three essential oils, including *Eugenia caryophyllata*, *Illicium verum*, and *Citrus hystrix* oils were introduced as additives into a soft liner.

*E. caryophyllata* or 'clove' is an evergreen tree of the *Myrtaceae* family that is firstly originated in Indonesia on island of Muluccas and continuously cultured in the various parts of the world. Its essential oil, dominantly contains eugenol, extracted from leaves has been shown the wide range of pharmacological effects including antimicrobial, analgesic, antioxidant, and anti-inflammatory properties [10,11]. The clove oil is also commonly used in dental applications. *I. verum* or 'star anise' is an evergreen native tree from southern China and northeast Vietnam. Its essential oil is commonly used in food and drug. *Trans*-anethole, main chemical composition of the star anise oil, has been reported about anti-microbial properties against various species [10,12]. *C. hystrix* or 'kaffir lime' oils is a citrus fruit native to Southeast Asia and southern China. Its essential oil contains *D*-limonene and  $\beta$ -Pinene that has been reported about the pharmacological effects including anti-microbial, anti-inflammatory, and antioxidant activities [13,14]. Due to the anti-microbial activities of these essential oils, they could be candidates to be used as anti-fungal additives into soft lining materials.

Besides anti-fungal properties, an influence of such additives on an initial viscoelasticity of

soft lining materials should also be examined. The viscoelastic property is one crucial property of the denture soft liner [15]. It could relate to several clinical parameters, including setting time, working time, flowability, bulk rigidity, etc. Once the soft liner was incorporated by the essential oils with an effective anti-fungal concentration, the modified soft liner should not be extremely changed in viscoelastic properties.

The aim of this study is to investigate an alteration in the initial viscoelasticity of a short-term soft liner after incorporating with different essential oils. Three concentrations of each essential oil were used in the present study. Three viscoelastic parameters including gelation time, elastic modulus, and viscous modulus during initial setting phase were evaluated by using the dynamic testing in an oscillating plate rheometer [16].

## Materials and methods

### Denture soft liner and anti-fungal agents

GC Soft Liner™ (GC corporation, Bunkyo-ku, Tokyo, Japan) was used as the short-term soft liner in this study. The standard powder/liquid (P/L) ratio is 1.22 g/1 g. The density of GC soft liner liquid at 25 °C is 0.9 g/mL. Its chemical compositions of powder and liquid were shown in Table 1 [17, 18].

**Table 1** Main chemical compositions of the short-term soft liner and three essential oils in this study.

Materials	Main chemical compositions
GC soft liner powder	Poly(ethyl methacrylate) (PEMA, Molecular weight $\sim 2.34 \times 10^5$ g/mol)
GC soft liner liquid	Butylphthalylbutyl glycolate (BPBG, 80.9%); Dibutylphthalate (DBP, 4.3%); Ethanol (14.8%)
<i>E. caryophyllata</i>	Eugenol (> 50%), Benzyl alcohol (20-25%), Caryophyllene (10-20%)
<i>I. verum</i>	<i>trans</i> -Anethole (87%); Estragole (3%); 5,6,7,8-Tetrahydroquinoxaline (1%)
<i>C. hystrix</i>	<i>D</i> -Limonene (26%); $\beta$ -Pinene (24%); <i>p</i> -Cymene (12%); $\alpha$ -Terpineol (9%); Terpinen-4-ol (8%)

There were three essential oils used as additives in this study. *I. verum* and *C. hystrix* fruit oils were purchased from Thai-China Flavours and Fragrances Industry Co. Ltd., Nonthaburi, Thailand. *E. caryophyllata* leaf oil was purchased from M-Dent company (Mahidol University, Bangkok, Thailand). All essential oils were extracted by hydrodistillation technique. The main chemical compositions of three essential oils analyzed by gas chromatography-mass spectrometry (GC-MS) from the product supplier are shown in Table 1.

### Measurement of initial viscoelasticity of the modified soft liner

The viscoelastic property at the initial setting of the modified soft liner was investigated by a controlled-stress rheometer (RHEOPLUS/32 Service V3.40 21000066-36670) in a parallel plate configuration. Three concentrations of each essential oils into GC soft liner were selected. These compositions appeared to be effective in anti-candidal activity (unpublished data). The concentrations of essential oils in total liquid

were displayed as percentages by volume (Table 2). Certain amount of essential oils was freshly dispersed with the liquid of GC soft liner in a sterile beaker and then mixed with the powder of GC soft liner at room temperature. After mixing, the materials were immediately placed on the lower plate of the rheometer. The upper plate (diameter = 25 mm) was then lowered into the 1 mm gap position. The changes of viscoelasticity with a time of the materials were recorded at 37°C. The testing condition was set at constant stress (40 Pa) and constant frequency (1 Hz). A similar procedure was also done for GC soft liner without additive. Five tests were carried out for each composition.

From the dynamic viscoelastic experiment, the complex shear modulus ( $G^*$ ) is calculated from the shear stress ( $\tau$ ) and shear strain ( $\gamma$ ). The phase lag between stress represents as  $\delta$ . The shear storage modulus ( $G'$ ), shear loss modulus ( $G''$ ), and loss tangent or damping coefficient ( $\tan \delta$ ) can be defined as the following equation; [16]

**Table 2** Detail compositions of each group of GC soft liner with different essential oils.

Type of essential oil	Oil concentration in total liquid (%)	Oil content (mL)	GC soft liner <sup>a</sup>		Total liquid (mL)
			Powder (g)	Liquid (mL) <sup>b</sup>	
Control (No additive)	0	0	1.22	1.11	1.11
<i>E. caryophyllus</i> oil	10	0.123	1.22	1.11	1.23
	15	0.196	1.22	1.11	1.31
	20	0.278	1.22	1.11	1.39
<i>I. verum</i> oil	20	0.278	1.22	1.11	1.39
	25	0.370	1.22	1.11	1.48
	30	0.476	1.22	1.11	1.59
<i>C. hystrix</i> oil	35	0.598	1.22	1.11	1.71
	40	0.740	1.22	1.11	1.85
	45	0.908	1.22	1.11	2.02

<sup>a</sup> The powder-to-liquid ratio of GC soft liner is 1.22 by weight.

<sup>b</sup> The density of GC soft liner liquid at 25°C is 0.9 g/mL.

$$|G^*| = \tau/\gamma$$

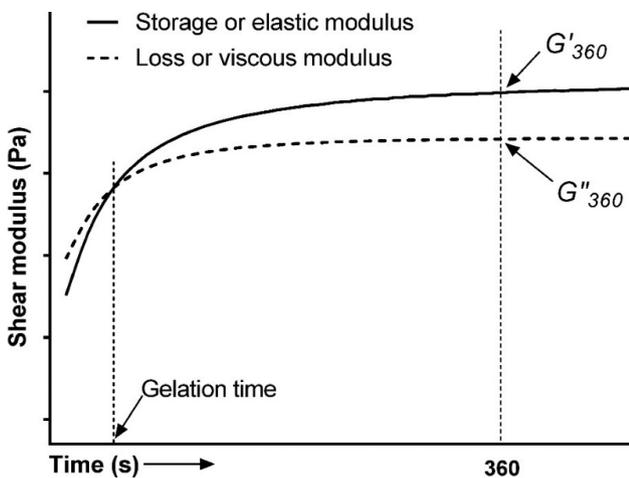
$$G^* = G' + iG'' \text{ (where } i^2 = -1)$$

$$G' = |G^*| \cdot \cos \delta$$

$$G'' = |G^*| \cdot \sin \delta$$

$$\tan \delta = G''/G'$$

Theoretically,  $G'$  and  $G''$  are so-called elastic and viscous moduli. The time point when  $G'$  and  $G''$  are equal ( $\tan \delta = 1$ ) can define as the gelation time or 'gel point' (Figure 1) [19]. The initial elastic and viscous moduli at 360 seconds, defined as  $G'_{360}$  and  $G''_{360}$ , were also recorded to evaluate the elastic and viscous properties of the soft liners after the initial setting (Figure 1).



**Figure 1** Measurement of gelation time, initial elastic and viscous moduli ( $G'_{360}$  and  $G''_{360}$ ) of the soft liner analyzed by the oscillating rheometer.

### Statistical analysis

All statistical computations were performed by SPSS software (IBM Corp. released 2013, IBM SPSS statistics for Windows, version 22.0, IBM, Armonk, NY, USA). Shapiro-Wilk test was performed to validate the normality of the data and Levene's test for equality of variances between group data. The comparisons in gelation time,  $G'_{360}$ , and  $G''_{360}$  of the soft liner incorporated with various concentrations of each essential oil were analyzed by one-way analysis of variance (ANOVA). The multiple

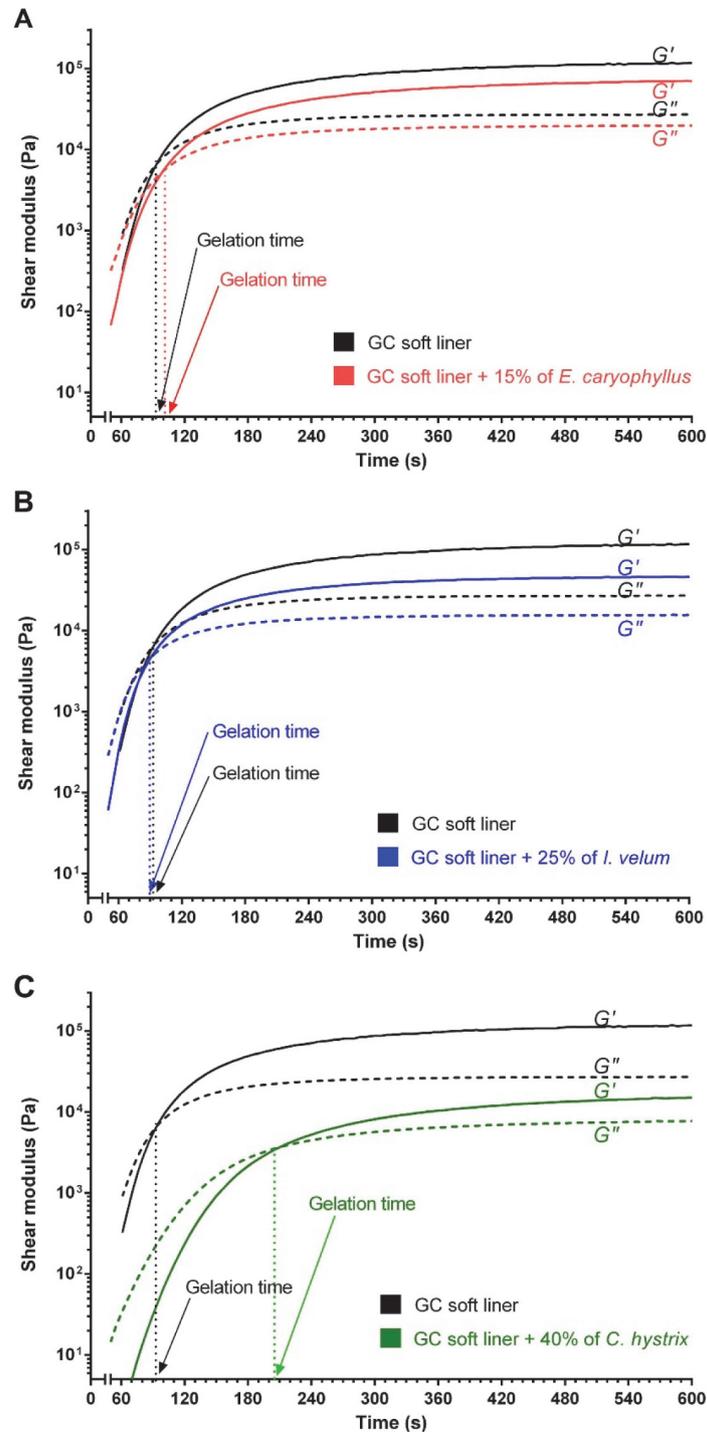
comparisons between groups were performed by Tukey's test when the data met the homogeneity of variances assumption or by Games-Howell *post-hoc* test for unequal variance situation. The significance level was set at  $\alpha = 0.05$ .

## Results

The viscoelastic properties at the initial stage of the GC soft liner control are illustrated in Figure 2 and Table 3. Both storage and loss moduli of the soft liner dramatically raised during the first two minutes and then gradually increased with almost stable values after 8 minutes approximately. At the beginning after mixing, the storage modulus was less than the loss modulus. Noticeably, the storage modulus raised sharply until reaching the intersect point ( $G' = G''$ ) and then exceeded the loss modulus. The gelation time of the GC soft liner without additive corresponding to the intersect point that was about one and a half minutes. At 6 minutes after mixing,  $G'_{360}$  and  $G''_{360}$  of the material were 95 and 26 kPa, respectively.

Incorporation of the essential oils diminished both storage and loss moduli of the GC soft liner during setting reaction (Figure 2 and Table 3). The soft liner with *C. hystrix* oil significantly had a longer gelation time than that of the control in a dose-dependent manner ( $p < 0.001$ ). The gelation times were about 160, 204, and 322 seconds for the soft liner with 35 %v/v, 40 %v/v, and 45 %v/v of *C. hystrix* oil, respectively. In contrast, the incorporation of *E. caryophyllata* and *I. verum* oils into the soft liner did not significantly affect the gelation time.

The addition of the essential oils not only influenced the gelation time, but it also decreased the elastic and viscous moduli after the initial setting (Figure 2 and Table 3). All essential oils appeared the dose-dependent effect to both  $G'_{360}$  and  $G''_{360}$  with almost statistical significance in pair comparisons.



**Figure 2** Initial viscoelasticity of GC soft liner control compared with different additives: A) 15 %v/v *E. caryophyllata* oil; B) 25 %v/v *I. verum* oil; C) 40 %v/v *C. hystrix* oil. The gelation time is defined as the time at which  $G' = G''$ . Abbreviation:  $G'$ , storage shear modulus;  $G''$ , loss shear modulus;  $\tan \delta$ , damping coefficient.

**Table 3** Gelation time and initial elastic and viscous moduli at 360 seconds after mixing of GC soft liner incorporated with different concentrations of three essential oils.

Modification of GC soft liner		Gelation time (s)	$G'_{360}$ ( $\times 10^4$ Pa)	$G''_{360}$ ( $\times 10^4$ Pa)
Essential oil	Concentration			
None (Control)	0 %v/v	91.9 $\pm$ 2.36 <sup>A</sup>	9.54 $\pm$ 0.14 <sup>A</sup>	2.59 $\pm$ 0.09 <sup>A</sup>
	10 %v/v	97.8 $\pm$ 5.07 <sup>A</sup>	6.73 $\pm$ 0.40 <sup>B</sup>	2.08 $\pm$ 0.08 <sup>B</sup>
<i>E. caryophyllata</i>	15 %v/v	102.4 $\pm$ 7.86 <sup>A</sup>	5.69 $\pm$ 0.93 <sup>C</sup>	1.86 $\pm$ 0.22 <sup>C</sup>
	20 %v/v	105.6 $\pm$ 4.45 <sup>A</sup>	4.39 $\pm$ 0.51 <sup>D</sup>	1.57 $\pm$ 0.11 <sup>D</sup>
<i>I. verum</i>	20 %v/v	88.8 $\pm$ 6.26 <sup>A</sup>	4.95 $\pm$ 0.41 <sup>C, D</sup>	1.65 $\pm$ 0.11 <sup>C, D</sup>
	25 %v/v	91.4 $\pm$ 3.85 <sup>A</sup>	4.23 $\pm$ 0.31 <sup>D</sup>	1.50 $\pm$ 0.09 <sup>D, E</sup>
	30 %v/v	87.6 $\pm$ 9.78 <sup>A</sup>	3.35 $\pm$ 0.25 <sup>E</sup>	1.32 $\pm$ 0.08 <sup>E</sup>
<i>C. hystrix</i>	35 %v/v	160.2 $\pm$ 1.92 <sup>B</sup>	1.77 $\pm$ 0.19 <sup>F</sup>	0.92 $\pm$ 0.06 <sup>F</sup>
	40 %v/v	204.0 $\pm$ 8.80 <sup>C</sup>	1.05 $\pm$ 0.09 <sup>F, G</sup>	0.66 $\pm$ 0.04 <sup>G</sup>
	45 %v/v	322.4 $\pm$ 15.03 <sup>D</sup>	0.47 $\pm$ 0.03 <sup>G</sup>	0.41 $\pm$ 0.02 <sup>H</sup>

Mean pairs with different superscripts in each column indicate statistical difference ( $p < 0.05$ ).

Abbreviation:  $G'_{360}$ , Initial elastic modulus at 360 seconds after mixing the soft liner;

$G''_{360}$ , Initial viscous modulus at 360 seconds after mixing the soft liner.

## Discussion

As mentioned previously, three essential oils were used as additives into the short-term soft liner. The essential oils were prior mixed with the liquid component of GC soft liner. Such manipulation is a feasible method that allows the homogeneity of the material. Regarding their chemical constituents, Eugenol in *E. caryophyllata* oil, *trans*-anethole in *I. verum* oil, *D*-limonene and -Pinene in *C. hystrix* oil have been reported about the anti-fungal activity against *C. albicans* [10, 20-21]. Therefore, the soft liner in combination with these additives could show an advantage in the anti-candidal property.

However, the incorporation of the essential oils might affect properties of the soft lining material. The present study has focused the effect of such additives on the initial viscoelastic properties of the soft liner. The initial viscoelasticity

is one clinically relevant property that could relate to setting time, working time, flowability, bulk rigidity, etc. The storage and loss moduli at the initial setting period can reflect material stiffness and viscosity during a chairside manipulation. While, the gelation time can relate to the setting time and working time of the soft liners [15, 22]. According to the manufacturer's instruction, the intraoral working time of GC soft liner is about 4-5 minutes. In the present study, six minutes after mixing would be therefore defined as the initial setting of this soft liner. Consequently, the initial storage and loss moduli were recorded at six minutes (Figure 1). Additionally, the change in both moduli with time can imply the crosslinked gel formation of the soft lining material [16, 22]. The rheology of the material immediately after mixing is dominated by the loss modulus ( $G''$ ) or viscous component due to fluid state. Thereafter, the storage modulus ( $G'$ ) or elastic component is

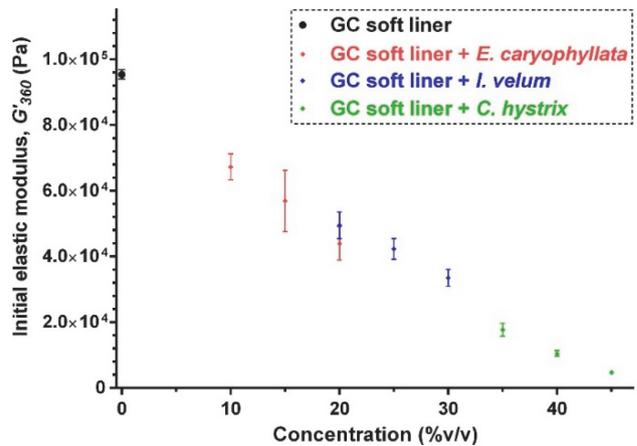
raised sharply due to the physical crosslink in the material structure and then exceeds the loss modulus. Hence, the intersection point between  $G'$  and  $G''$  curves can indicate the fluid-gel transition period defined as 'gelation time' in many studies [16, 19].

Herein, GC soft liner without additive exhibited a typical viscoelastic behavior during the dynamic testing (Figure 2). The material was dominated with the viscous component at the beginning of mixing and turned to be mainly contributed by the elastic component after the gel point at 90 seconds approximately.

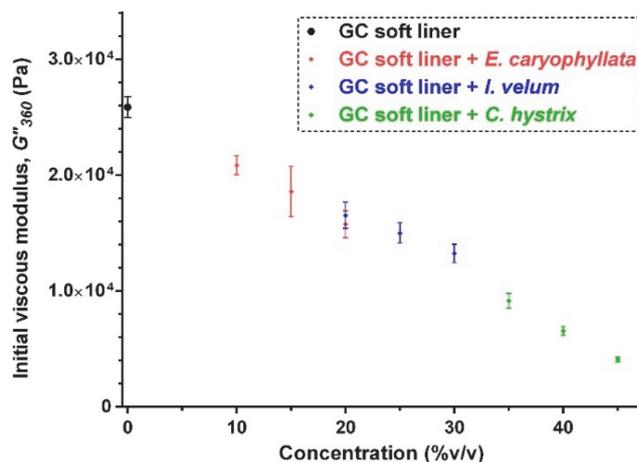
After incorporating the essential oils, the alteration in the initial viscoelasticity of the soft liner was detected. All essential oils showed a diminished effect on the storage and loss moduli at six minutes after mixing in a dose-dependent manner (Table 3). Interestingly, the plot between both moduli against several concentrations of all essential oils likely appeared to be a proportional relationship (Figure 3 and 4). This could be implied that the decrease in the moduli was mainly contributed by the oil concentrations, not by a type of essential oils. Basically, the addition of the oil content increased liquid component in the soft liner system and, therefore, decreased the P/L ratio. The lower P/L ratio of soft liners, the lesser modulus of the materials could be obtained [15, 23]. Therefore, the soft liner with a higher amount of the essential oils would be softer and easier in deformation.

On the contrary, there was a dissimilar effect on the gelation time among GC soft liner with different essential oils (Table 3). *C. hystrix* oil led to longer gelation time of the soft liner in a dose-dependent manner (Figure 5). Theoretically, the gel formation of this short-term soft liner can occur *via* physically crosslinked between polymer chains [24, 25]. When the essential oils were added into the soft liner system, the polymer chains would be surrounded by oil molecules

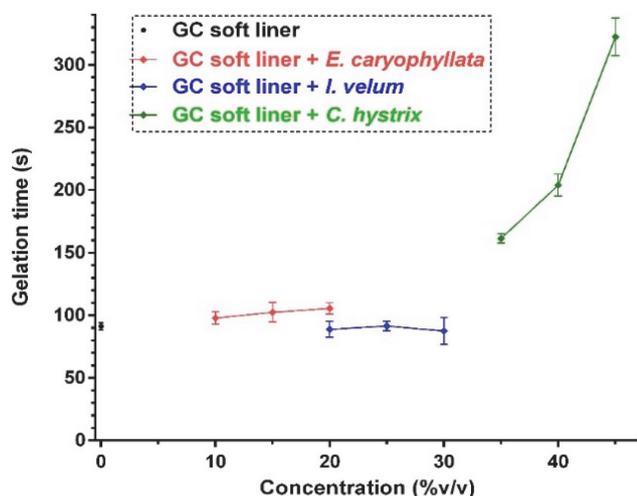
that can retard the crosslinking mechanism. In such situation, an extended period would be required to form sufficient polymer chain crosslinks. Accordingly, the compositions with higher contents of *C. hystrix* oil could result in the longer gelation time.



**Figure 3** The initial elastic modulus ( $G'_{360}$ ) of GC soft liner incorporated with several concentrations of different essential oils. The data were shown as means with standard deviations.



**Figure 4** The initial viscous modulus ( $G''_{360}$ ) of GC soft liner incorporated with several concentrations of different essential oils. The data were shown as mean with standard deviation.



**Figure 5** The gelation time of GC soft liner incorporated with several concentrations of different essential oils. The data were shown as mean with standard deviation.

Nevertheless, the incorporation of *E. caryophyllata* and *I. verum* oils did not strongly influence the gelation time of the soft liner (Table 3 and Figure 5). Besides the P/L ratio, another parameter that can affect the gelation process is

the molecular structures of the solvents or additives. The main chemical constituents of both essential oils are eugenol and *trans*-anethole for *E. caryophyllata* and *I. verum* oils, respectively. Interestingly, their molecular structures consist of an ester functional group, which is also found in PEMA and plasticizers' molecules. Moreover, their solubility parameters ( $\delta$ ) are nearly similar to those of PEMA and plasticizers (Table 4) [26-28]. This could imply that the main compounds in *E. caryophyllata* and *I. verum* oils can diffuse into the swelling polymer network in the same manner as the plasticizers. Additionally, both of them have relatively low molecular weights in comparison to such conventional plasticizers (Table 4). With the lower molecular weight of the additives, the gelation process can occur more rapidly [27]. Consequently, the prolonged effect in the gelation process due to higher contents of *E. caryophyllata* and *I. verum* oils additives appeared to be compensated by the rapid molecular diffusion of their main constituents.

**Table 4** Solubility parameters corresponding to previous references of main chemical constituents in GC soft liner and three essential oils.

Materials	Main compositions	Molecular weight (g/mol)	Solubility parameter $\delta$ (MPa <sup>1/2</sup> )	Ref.
GC soft liner powder	PEMA	$2.34 \times 10^5$	20.47	(26)
GC soft liner liquid	BPBG	336.40	20.65	(24)
	DBP	278.34	19.00	(24)
	Ethanol	46.07	26.00	(24)
<i>E. caryophyllus</i> oil	Eugenol	164.20	20.70	(27)
	Benzyl alcohol	108.14	23.79	(26)
<i>I. velum</i> oil	<i>trans</i> -anethole	148.21	19.70	(27)
<i>C. hystrix</i> oil	<i>D</i> -limonene	136.24	17.72	(28)
	$\beta$ -pinene	136.24	17.57	(28)

## Conclusion

Within the limitation in this study, all essential oils decreased both initial elastic and viscous moduli of the short-term soft liner in the dose-dependent manner. *E. caryophyllata* and *I. verum* oils would be more appropriate additives that did not alter the gelation time of the soft liner. Whereas *C. hystrix* oil led to the dramatical change in both gelation and initial moduli. Further studies about biocompatibility and other material properties should also be investigated.

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**Conflict of interest:** None

**Ethical approval:** None (Laboratory study)

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