



## ***Formulation Physical Characteristic of Hard Candy Lozenge of Citrus Limon Essential Oil on Various Types of Sugar Free Candy Base (Isomalt, Mannitol, Sorbitol)***

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Citrus limon is a natural ingredient that has potential as an anti-anxiety. The dopamine-enhancing effect on the blood brain barrier due to consuming citrus limon essential oil can help relieve stress, fatigue, dizziness, and anxiety. This study aims to formulate citrus limon essential oil hard candy lozenge with various types of sugar free candy bases and evaluate its effect on the physical characteristic of the lozenge. Previously, this study was preceded by identifying the D-Limonene compounds as an active material on the citrus limon essential oil using the GC-MS method. There are three formulas used in this study, including F1 (isomalt), F2 (mannitol), and F3 (sorbitol). Physical characteristic tests carried out include organoleptic, weight variations, dimension, hardness, friability, dissolved pH, and dissolve time. The identification test of citrus limon essential oil using GC-MS showed the presence of D-Limonene compound. Thus, it can be concluded that the raw material of the oil was in accordance with the established quality. The results of the physical characteristics of the three formulas in the tablet dimension test, hardness, and the dissolved pH met the requirements. In addition, the organoleptic test of F1 and F3 yields better results than F2 (murky white color and rough texture). The evaluation of weight variation, friability, and dissolve time of F1 and F3 met specifications, while F2 did not meet specifications. Therefore, the sugar free candy base that can be developed into a hard candy lozenge in this study was by using isomalt (F1) and sorbitol (F3).

## INTRODUCTION

Citrus limon is a natural ingredient that has potential as an anti-anxiety. The dopamine-enhancing effect on the blood brain barrier due to consuming citrus limon essential oil can help relieve stress, fatigue, dizziness, and anxiety (1). The constituent components of citrus limon are D-Limonene (47,24-55,23%), geranial, and neral. The D-Limonene compound is the main content of citrus limon which can provide sedative and calming effects, making it a good choice for anxiety (2). Patients suffering from anxiety, depression, or other psychotic-related disorders often have difficulty swallowing tablets. In such cases, buccal preparations, such as lozenges, would be an effective solution to ensure that patients receive a good and efficient treatment regimen (3).

Lozenge is a solid dosage form that is intended to dissolve in the mouth or pharynx (4). Lozenge is classified into 4 types according to its composition and appearance, including chewy lozenge, compressed lozenge, soft lozenge, and hard candy lozenge (5).

Hard candy lozenge consists of a mixture of sugar and other carbohydrates that have a candy-like shape (6). The advantages of hard candy lozenge are its sweet taste, smooth texture, and it dissolves slowly in the mouth (5).

There are several methods that can be used in the process of making lozenge, including direct compression, wet granulation, heat-congealing, and melting-molding (7). Melting-molding method is carried out by melting the candy base at its melting point (145-156°C), then mixed with other excipients, and finally poured into the mold (6). These excipients such as binders, sweeteners, flavors, acidulants (8).

Based on the excipient of candy base, hard candy lozenges are divided into two types, namely sugar base and sugar-free candy base (6). In this study, sugar free candy base was used because it can be consumed by patients without experiencing side effects caused by sugar, such as tooth decay and increase blood glucose. There are 3 types of sugar free candy base used in this study,

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including isomalt (Formula 1), mannitol (Formula 2), and sorbitol (Formula 3).

Isomalt is a substitute for sucrose that has similar characteristics to sucrose. Isomalt has a sweetness level of about 0,45-0,6 times compared to sucrose (=1,0) (9). Mannitol has a sweet taste equivalent to glucose and half of sucrose (10). Sorbitol is an additive that is often used as a filler in solid dosage forms because it has a sweet taste (50-60% sweetness level of sucrose) (10). The three bases can give a cool sensation in the mouth and are proven not to increase blood glucose levels, so those are safe for diabetics. In addition, it also does not cause tooth decay because it does not increase or decrease the pH of the mouth after consumption (9). However, because all three bases still have a fairly low level of sweetness when compared to sucrose, so in this study stevia used as an additional sweetener which has a sweetness level 300 times that of sucrose (11). Othe additional excipients in this study were gum arabica as a binder, citric acid as an acidulant, and peppermint as a flavoring.

The difference in the type of bases used in this study is expected to affect the physical characteristic of the lozenge tablets. The physical characteristic of the lozenge tablets was carried out by determining the organoleptic, weight variation, dimensions (diameter), hardness, friability, dissolving pH, and dissolving time. In addition, the identification of the D-Limonene compound in the raw material of citrus limon essential oil was previously carried out using GC-MS method. The results of the 3 formulas will be analyzed statistically using the one-way Anova method followed by the post-hoc method.

## MATERIAL AND METHODS

The materials used in this study were citrus limon essential oil food grade (Rumah Atsiri Indonesia, Karanganyar), isomalt p.g (Beneo,

Germany), mannitol p.g (Qingdao Bright Moon Seaweed Group, China), sorbitol p.g (Ueno Fine Chemical Industry, Thailand), gum acacia p.g (Merck, Germany), stevia food grade (Soho Nootropics, China), citric acid p.g (Merck, Germany), Peppermint food grade (Rumah Atsiri Indonesia, Karanganyar).

The tools and instruments used in this study were GC-MS QP2010 SE (Shimadzu, Japan), friability tester (Erweka, Germany), hardness tester TBH 125 (Erweka, Germany), pH meter Laqua 1100 (Horiba, Japan), Hanson Phase One Disintegration Tester (Hanson, USA), Scout digital scales (OHAUS, USA), hotplate magnetic stirrer GuardianTM 3000 (OHAUS, USA), caliper with an accuracy of 0,05 mm (Tricle Brand, China), refrigerator, molds, and other glassware,

## Identification of Citrus Limon Essential Oil by GC-MS Method

The first test began by identifying the D-Limonene compound in citrus limon essential oil using GC-MS, a modification of the method from Hojjati *et al* (12). Helium was used as a carrier gas with a flow rate of 0,9 mL/min with a split ratio of 1:20. Furthermore, the injector and detector were set at 230°C and 280°C, respectively. Then, 2 µL of essential oil was injected and run for 27 minutes. The content of essential oil will be identified based on the retention time of GC-MS and a comparison of similarity index, comparison of mass spectra with the library WILEY7, WILEY8, NIST (NIST08, NIST08s)-national institute of standards and technology.

## Formula

There are 3 formulas (Table 1), each using a different sugar free candy base. The weight of each hard candy lozenge was made at 1,5 grams.

Table 1. Composition of different sugar free candy base

NO	COMPONENT	FUNCTION	(%)		
			F1	F2	F3
1	Citrus limon essential oil	Active pharmaceutical ingredient	4	4	4
2	Isomalt*	Sugar free candy base	90	-	-
3	Mannitol*	Sugar free candy base	-	90	-
4	Sorbitol*	Sugar free candy base	-	-	90
5	Gum acacia	Binder	5	5	5
6	Stevia	Sweetener	0,45	0,45	0,45
7	Citric acid	Acidulant	0,45	0,45	0,45
8	Peppermint	Flavor	0,1	0,1	0,1

\*Sugar free candy base was mixed with distilled water at 1/3 of its total weight.

## Lozenge specification

The hard candy lozenge that has been made was expected to meet the specifications as in Table 2 below.

Table 2. Lozenge specification

No	Testing	Specification
----	---------	---------------

1	Organoleptic	<ul style="list-style-type: none"> <li>- Color: clear yellow</li> <li>- Odor: lemon mint</li> <li>- Taste: sweet lemon mint</li> <li>- Texture: smooth</li> <li>- Shape: cube</li> </ul>
2	Weight variation	1,5 grams $\pm$ 2% (1,47-1,53 grams) (4,6)
3	Dimensions (diameter)	11,25 mm $\pm$ 5%
4	Hardness	4-8 kP (13)
5	Friability	< 1% (14)
6	Dissolving pH	3,8 – 7,0 (5)
7	Dissolving time	5-10 min (300-600 second) (5)

### Procedure for Making Hard Candy Lozenge

The manufacturing process begins by dissolving the sugar free candy base into distilled water (1/3 of the weight of the bases) and heating it while stirring evenly until it reaches the desired melting point (isomalt = 155°C; mannitol = 165°C; sorbitol 145°C). After the bases melted and the water had reduced, the other excipients, such as gum acacia, stevia, and citric acid were added and stirred until homogeneous. Citrus limon and peppermint were added when the mixture is slightly warm and stirred again until homogeneous. The mixture was then poured into molds and put into a refrigerator (2-8°C) until it becomes solid form (4,15).

### Physical Characterization

#### Organoleptic Test

Organoleptic tests were carried out by identifying color, odor, taste, texture, and shape. The aim was to determine the differences in physical organoleptic properties of hard candy lozenge in each formula.

#### Weight Variation Test

The weight variation test was conducted by weighing 10 lozenge one by one using a Scout digital scale (OHAUS, USA) and then calculating the average weight and standard deviation (15). The specifications of the tablet weight for hard candy lozenge are 1,5-4,5 grams (4,6). The weight of the lozenge used in this study was 1,5 grams  $\pm$  2% (1,47-1,53 grams).

#### Dimension (Diameter) Test

This test was conducted by measuring the diameter of 10 lozenges using a caliper (accuracy level 0,05 mm) (15). The diameter of the hard candy lozenge used in this study was 11,25 mm  $\pm$  5%.

#### Hardness Test

Hardness test was conducted on 10 hard candy lozenges using a TBH 125 Hardness tester (Erweka, Germany) (4). The hardness

results were expressed in kilopond (kP) with test requirements of 4-8 kP (13).

#### Friability Test

This test was conducted to determine the resistance of hard candy lozenge to friction and shock during the manufacturing and distribution process using friability tester (Erweka, Germany). The test was conducted in accordance with the provisions in the USP. The 10 hard candy lozenges were cleaned one by one using a soft brush and then the initial weight of the sample was weight ( $w_0$ ). Furthermore, the sample was inserted into the device at a speed of 25 rpm for 4 minutes. After that, the sample was removed and cleaned again using a soft brush and then weighed ( $w_1$ ) to determine the percentage of friability (14). This test was conducted 3 times in replication. The equation used to calculate the %friability can be seen as follows:

$$\% \text{ Friability} = \left( \frac{(w_0 - w_1)}{w_0} \right) \times 100\%$$

\* $w_0$  = initial weight;  $w_1$  = final weight

#### Dissolving pH Test

This test was carried out using a Laqua 1100 pH meter (Horiba, Japan). The test began by dissolving 1 hard candy lozenge into 200 mL of distilled water, then the pH was measured 3 times in replication (16). The pH requirements for this lozenge are 3,8-7,0 (5).

#### Dissolving Time Test

The dissolving time test was conducted by inserting 6 hard candy lozenges into each basket tube of the disintegration tester instrument. The solvent used was distilled water at 37°C. The dissolving time of the hard candy lozenge was marked by the complete dissolution of the lozenge in the solvent media (15). Then, the time was recorded in seconds. This dissolving time test was carried out 3 times in replication. The ideal dissolving time for the hard candy lozenges is 5-10 minutes (5).

## RESULTS AND DISCUSSION

### Results of Citrus Limon Essential Oil Using GC-MS Method

The results of the identification test of citrus limon essential oil using the GC-MS method can be seen in **Table 3**.

**Table 3. Identification test of citrus limon essential oil using the GC-MS method**

Peak #	Rt (min)	Compound	BasePeak (m/z)	Similarity %	Area
5	10,474	<i>IR-alpha-Pinene</i>	93	97	2671457
6	11,406	<i>L-beta--Pinene.</i>	93	96	10876375
7	12,478	<i>D-Limonene</i>	68	94	27246829

8	12,807	1,4-Cyclohexadiene.	93	96	5926192
9	13,216	Cyclohexene.	93	97	379650
10	14,617	2-Isopropyl-5-Methylcyclohexanol	71	96	220410
11	15,608	Cis-Citral	41	97	2007662
12	16,034	2,6-Octadienal,3,7-Dimethyl	69	97	2821241
13	17,194	2,6-Octadien-1-ol,3,7-Dimethyl-Acetate,(Z)-	69	97	825031
14	17,440	2,6-Octadien-1-ol,	69	97	825592
15	19,284	Beta-bisabolen	69	95	415692

According to the literature, analysis of citrus limon essential oil using GC-MS, obtained the findings of the presence of D-Limonene content which has a molecular formula of  $C_{10}H_{16}$  with a retention time of 7,01 minutes and a percentage area of 96,79% (17). **Table 3** showed that D-Limonene was the main component of citrus limon. This compound was proven by the results of the GC-MS chromatogram at peak number 7 at a retention time of 12,478 min, the resulting MS fragment pattern showed the D-Limonene compound with a base peak of  $m/z$  68 (**Figure 1**). This was in accordance with the fragment pattern in the NIST Chemistry WebBook (18). Based on these results, it showed that citrus limon essential oil in this study contains D-Limonene compounds that are in accordance with CoA and comparative literature. Thus, it could be concluded that the essential oil has met the specified quality.

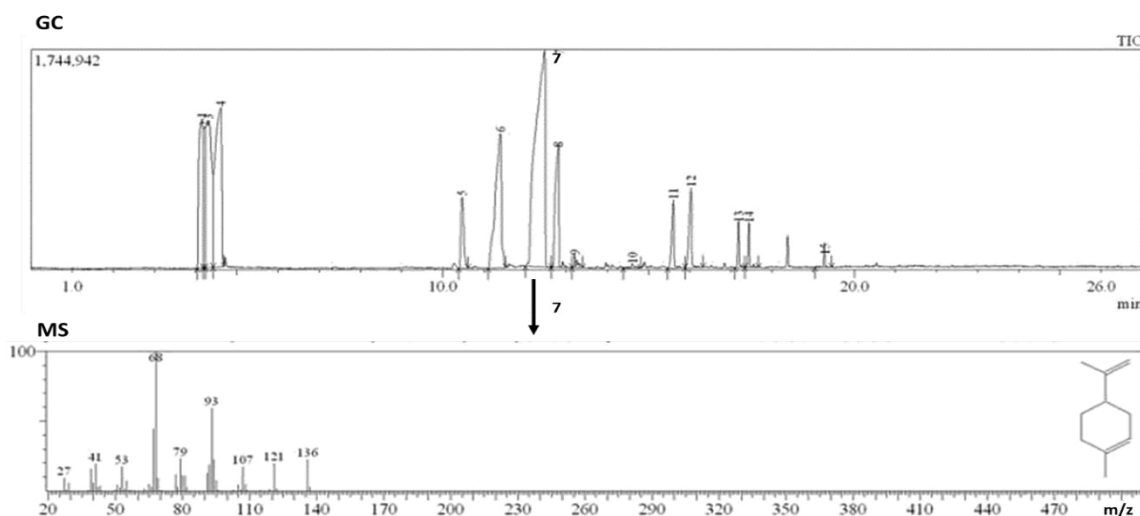


Figure 1. Chromatogram of citrus limon and MS compound D-Limonene (peak number 7)

### Results of Physical Characterization of Hard Candy Lozenge

#### Organoleptic

The results of organoleptic observations in each formula (F1, F2, F3) are shown in **Figure 2**. F1 (isomalt) and F3 (sorbitol) gave a sweeter taste compared to F2 (mannitol). This showed that the addition of stevia with the same concentration in F2 still does not provide a good enough sweet taste. In addition, this was also because the level of sweetness of mannitol was only 50% of the sweetness of sucrose (10). F1 and F3 gave a clear yellowish color, while F2 gave a cloudy white color. The cloudy white color in F2 was caused by mannitol not being able to melt completely when heated and not being able to dissolve completely in water (low water solubility of 216 mg/mL at 25°C) (19).

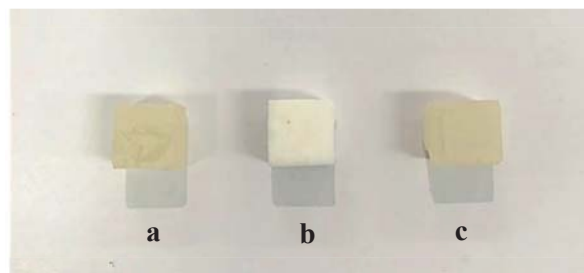


Figure 2. Organoleptic of hard candy lozenge (a) F1 isomalt, (b) F2 mannitol, (c) F3 sorbitol

#### Weight Variation

**Table 4** showed that the weight of hard candy lozenge F1 and F3 were  $1,50 \pm 0,01$  grams dan  $1,49 \pm 0,02$  grams, respectively. Both formulas have an average weight that was in accordance with the desired specifications, which was in the range of 1,47-1,53 grams. Meanwhile, F2 showed that the average weight did not meet the specification range. This was due to the difficulty

in the process of pouring the melted lozenge into the mold, mannitol could not melt perfectly during heating so that air cavities were formed in the mold during the process. Therefore, this would have an impact on the results of the hard candy lozenge which was easily fragile when removed from the mold.

#### Dimension (Diameter)

**Table 4** showed that 3 formulas have provided a lozenge diameter that has met the specifications, F1, F2, F3 were  $11,27 \pm 0,01$  mm;  $11,23 \pm 0,01$  mm; dan  $11,26 \pm 0,01$  mm, respectively. This proves that melting method has been able to provide a good and reproducible hard candy lozenge dimension result.

#### Hardness and Friability

Hardness and friability testing on hard candy lozenge aims to observe the resistance of lozenge to impacts and friction during process, storage, shipping, or before use by patients (4). **Table 4** showed that F2 has the lowest hardness compared to F1 and F3. The same thing is also shown in the result of friability that F2 has the highest friability percentage value (above 1%) (14). This can be associated with the characteristics of the sugar base used, mannitol, which is difficult to melt and dissolve in water during the heating process. So that, the hard candy lozenge has cavities or hollow shape and tends to be cloudy white because mannitol cannot mix very well with other excipients (19).

#### Dissolving pH

The pH of 3 formulas have met the specifications, F1, F2, F3 were  $4,01 \pm 0,07$ ;  $3,90 \pm 0,01$ ; and  $4,04 \pm 0,05$ ; respectively (5). In addition, the 3 formulas did not show any significant difference in pH between formulas (sig.  $p > 0,05$ ). The pH of 3 formulas tend to acidic due to the addition of acidulant (citric acid) to the formula. This citric acid is intended to add a sour taste that can balance the sweet taste of the candy (20).

#### Dissolving Time

Dissolving time testing was conducted to determine the time required for the hard candy lozenge to completely dissolve in distilled water. **Table 6** showed that F1 and F3 provide dissolving times of  $594 \text{ seconds} \pm 5$  dan  $571 \text{ seconds} \pm 13$ , respectively. These both formulas have met the desired specifications, which are in the range between 300-600 seconds (5). Meanwhile, F2 provides the longest dissolving time 1382 seconds  $\pm 226$ . This is due to the low solubility of mannitol in water (216 mg/mL at 25°C) which causes the dissolving time of mannitol as the base of F2 to be very long (19).

**Table 4. The physical characterization results of citrus limon hard candy lozenges**

Physical Characterization	Results		
	F1	F2	F3
Weight variation	$1,50 \text{ grams} \pm 0,01$	$1,15 \text{ grams} \pm 0,05$	$1,49 \text{ grams} \pm 0,02$
Dimension	$11,27 \text{ mm} \pm$	$11,23 \text{ mm} \pm$	$11,26 \text{ mm} \pm$

(diameter)*	0,01	0,01	0,01
Hardness*	$8,0 \text{ kP} \pm 0,16$	$6,0 \text{ kP} \pm 0,20$	$7,1 \text{ kP} \pm 0,25$
Friability	$0,09\% \pm 0,16$	$1,23\% \pm 0,58$	$0,43\% \pm 0,22$
Dissolving pH*	$4,01 \pm 0,07$	$3,90 \pm 0,01$	$4,04 \pm 0,05$
Dissolving time	$594 \text{ seconds} \pm 5$	$1382 \text{ seconds} \pm 226$	$571 \text{ seconds} \pm 13$

\*The physical characterization of the 3 formulas has met the requirements.

## CONCLUSION

In this study, it can be concluded that the results of the identification test of citrus limon essential oil using GC-MS showed the presence of D-Limonene compound, so this essential oil was in accordance with the specified quality. In addition, the difference in the type of sugar free candy base can affect the physical characteristics of citrus limon lozenge. Isomalt (F1) and sorbitol (F3) can be developed into hard candy lozenge because they provide good physical quality.

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## CONFLICT OF INTEREST

The data published in this manuscript has not constitute a conflict of interest to any party.

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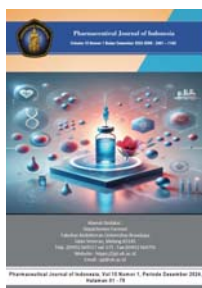
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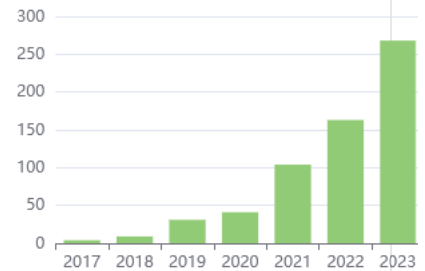
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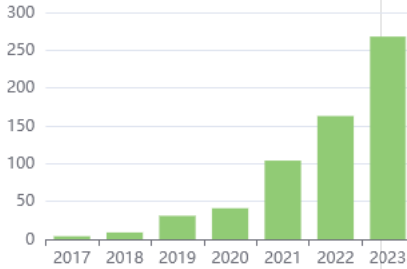
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