

## Tamarindus indica increases the stability of jamu kunyit-asam herbal suspension over Citrus aurantifolia

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### ABSTRACT

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Rapid shelf-life of traditional supplementary drink still becomes major issue for herbal production in household industries. In this study the stability formula of an herbal suspension, so called as *jamu kunyit-asam*, was investigated. The *jamu kunyit-asam* Formula-1 (F1) contains combination of *Citrus aurantifolia* and *Curcuma longa*. While the Formula-2 (F2) is consisted by mixture of *Tamarindus indica* and *C. longa*. The two formulas were compared to justify better stability between preparations. No preservatives were added, in order to comply with the local regulation. To simulate the real situation, all samples were stored in 4 different conditions: opened bottle (room temperature), closed bottle (room temperature), closed bottle (refrigerator), and freeze-thaw condition. To evaluate the stability profile, an organoleptic testing and pH alteration were frequently checked for 12 consecutive days. Several parameters including color changing, sting odor, and microscopic appearance were looked over. As the results, mold, precipitation and pungent odor were formed faster from the F1 (*C. aurantifolia* combined with *C. longa*). These findings indicates that *jamu kunyit-asam* herbal drink suspensions containing *C. longa* has less stability in combination with the *C. aurantifolia* (F1) compared to the *T. indica* (F2).

### ABSTRAK

Masa simpan (*expired date*) yang singkat minuman suplemen tradisional masih menjadi kendala utama industri kecil skala rumah tangga di Indonesia. Dalam penelitian ini penyelidikan terhadap stabilitas 2 formula suspensi herbal jamu kunyit-asam dilakukan. Jamu kunyit-asam Formula-1 (F1) mengandung perpaduan jeruk nipis (*Citrus aurantifolia*) dan kunyit (*Curcuma longa*). Sementara Formula-2 (F2) terdiri atas campuran asam jawa (*Tamarindus indica*) dan kunyit. Kedua formula dibandingkan untuk mengetahui profil stabilitas yang lebih baik diantara kedua sediaan. Tidak ada bahan pengawet ditambahkan guna mematuhi peraturan yang berlaku terkait minuman kesehatan produksi rumah tangga. Untuk mensimulasikan keadaan sesungguhnya, seluruh sampel disimpan dalam 4 kondisi berbeda: botol terbuka (suhu ruangan), botol tertutup (suhu ruangan), botol tertutup (lemari es), dan kondisi beku-cair (*freeze thaw*). Untuk mengevaluasi profil stabilitas sediaan, pengujian organoleptik dan perubahan pH dilakukan secara rutin selama 12 hari berturut-turut. Beberapa parameter terkait perubahan warna, munculnya bau khas/menyengat, dan penampakan mikroskopis diinvestigasi dalam penelitian ini. Sebagai hasil, terbentuknya jamur, terjadinya pengendapan, dan munculnya bau menyengat, terjadi lebih cepat pada F1 (kombinasi jeruk nipis dan kunyit). Temuan ini menunjukkan bahwa suspensi minuman herbal jamu kunyit-asam yang mengandung kunyit memiliki stabilitas lebih rendah ketika dikombinasikan dengan jeruk nipis (F1) ketimbang kombinasi dengan asam jawa (F2).

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## INTRODUCTION

*Jamu kunyit-asam* is one of the most attractive supplementary herbal suspensions in Indonesian. Empirically, *jamu kunyit-asam* herbal drink is made of turmeric rhizomes (*Curcuma longa*) juice and tamarind fruit (*Tamarindus indica*) pulp with small amount of sugar. This formula originally invented in Indonesia and has been used for generations. Apart from being a body freshener, turmeric-tamarind herbal drink is also widely consumed to relieve pain during women's menstruation. Curcumin, a major compound within turmeric, is an active substance with strong evidence in reducing oxidative stress in endometriosis.<sup>1</sup> Whereas, women affected by endometriosis show a higher level of oxidative stress markers than the normal one.<sup>2</sup>

Besides curcumin, another active substance such as demethoxycurcumin, bisdemethoxycurcumin and various essential oils in turmeric show ability in reducing inflammation.<sup>3</sup> It was also found that those substances are potential reducing the growth of new blood vessels (angiogenesis).<sup>4</sup> Therefore *jamu kunyit-asam* is believed to give health benefits due to its high potential antioxidants' activity.<sup>5</sup>

On the other hand, some factors may affect the potential antioxidant activity. At least two most striking factors such as 1) boiling time duration of during processing, and 2) the ingredient composition of the mixture.<sup>6</sup> The active composition of *C. longa* (curcumin and its derivatives) is easily damaged in the alkaline condition.<sup>7</sup> Therefore, keeping the suspension in acidic conditions (pH 2.5-7.0) is very important to keep its stability. Moreover, curcumin and its derivative is essential substances that responsible for the biological activity.

Pharmaceutical stability is important parameter to determine whether a suspension still good to be consumed or not. The precipitation, the color change, and the unpleasant odor are several indicators to evaluate its stability.<sup>8</sup> A study shows that storing at cold temperature (5±2 °C) will prominently

maintain antioxidant properties.<sup>9</sup> Furthermore, Rashati *et al.*<sup>10</sup> reported that variation in storage temperature may also affect the pH value of a suspension and its organoleptic properties. While the tamarind has been used for generations as mixture ingredient in the *jamu kunyit-asam*. However, lime (*Citrus aurantifolia*), a citric fruit which containing antioxidant potential to stabilize free radicals, prominent substance with antimicrobial potential to inhibit the growth of pathogenic and spoilage microorganisms, has never taken into account to substitute tamarind ingredient. In the present study, for the first time the effectiveness tamarind fruits (*T. indica*) and lime (*C. aurantifolia*) were compared in providing better stability condition for *jamu kunyit-asam* herbal drink suspension.

## MATERIAL AND METHODS

### Herbal drink formulation and preparation

Turmeric (*C. longa*), tamarind (*T. indica*) and lime (*C. aurantifolia*) were provided by small household industry Kelompok Wanita Tani (KWT) Maju Makmur from the Singosaren Subdistric, Bantul Distric, Yogyakarta, Indonesia. Formula-1 (F1) is generally composed by a mixture of *C. longa* and *Citrus aurantifolia* (turmeric and lime). To prepare the mixture, 250 g of turmeric powder, 150 g galangal and 800 g sugar were added to boiling water and boiled for 15 min. The liquid was then cooled at room temperature and poured with squeezed lemon juice (*C. aurantifolia*). Formula-2 (F2) is consisted of combination *C. longa* and *T. indica* (turmeric and tamarind). Both F1 and F2 were prepared similarly, however, the squeezed lemon juice (*C. aurantifolia*) replaced with *T. indica* in F2.

### Experimental

All suspensions were packaged in clean bottles. Microscope XSZ 107BN binocular used to evaluate the microscopic condition. However, pH

meter used to measure day-to-day acidity alteration.

Both formulas were stored in 4 different conditions. The F1 and F2 were stored in an opened bottle (room temperature), closed bottle in room temperature (25-30°C), closed bottle in refrigerator (8°C), and freeze-thaw. Freeze thaw is a notable method to evaluate the stability preparation. It was conducted by freezing the samples under -18 °C continued with defreezing at room temperature before examination. This cycle was repeated for 3 times.

Organoleptic observation and pH examination were carried out for 12 consecutive days. The organoleptic observations were carried out by observing the change in color, the change in aroma and the change in microscopic particles precipitation by using light microscope with 10 times magnification. Observation to pH alteration carried out using calibrated pH meter.

### Data analysis

In this study, the sample of each formula was taken from a single batch production. There is no replication due to limited number of samples available. A statistical analysis was not included in this study.

This study was approved by the Health Medical and Research Ethic Committee, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta (number KE/FK/1595/EC/2023).

## RESULT

### Organoleptic observation

Basically, the addition of weak acid components is widely used in food and drinks to prevent spoilage due to microbes. In this study, *C. longa* mixed with *C. aurantifolia* (F1) produce foam faster than the other mixture (F2) in open bottle room temperature storage (TABLE 1). Prominent distinguishable mold appears in F1 starting at the day-

10 when it kept under closed bottle storage at room temperature. However, no mold was detected from F2 for the same storage condition. Interestingly, any samples stored in the closed bottle in low temperature do not overgrow with mold but still causes a pungent odor on day-10 in F1.

To confirm the result, microscopic evaluation conducted. Two storage conditions i.e. under refrigerator storage and under freeze thaw condition observed frequently, day-to-day (FIGURE 1). The trend of precipitation among the samples started from the day-7 to day-12 (FIGURE 2). Both samples, either stored under refrigerator or under freeze thaw condition showed no presence of mold under microscope. This confirms the observational result of organoleptic measurement (TABLE 1). Even though a pungent odor smells in F1 (under refrigerator condition, TABLE 1) on day 10, no foam nor mold was observed under microscopic examination (FIGURE 3).

In an open bottle storage, precipitating particles of F1 occurred more quickly than F2 (FIGURE 3). This finding strongly indicated combination of *C. longa* and *T. indica* has a better particle stability profile than the other formula. Since *T. indica* has a fleshy, juicy, acidic pulp, it is logic that the homogeneity of *C. longa* suspension more consistent than the other. Under these conditions, the yellow compounds from turmeric (curcuminoids) which are not soluble in water would easily be suspended with the help of *T. indica*. These properties support its stability.

### Evaluation of pH alteration

A sudden drops of pH level began at day-8 for the sample kept under refrigerator (FIGURE 4). Even both storage conditions under room temperature and in refrigerator have similar trend, however, the pH elevation pattern is prominently different between two samples. None of the treatments reached pH under 2.5 nor over 5.

TABLE 1. The organoleptic evaluation of ‘Jamu kunyit-asam’ herbal drink in various storage conditions at particular days

Day	Room temperature				Refrigerator		Freeze-thaw	
	Opened bottle		Closed bottle		Closed bottle			
	<i>C. longa</i> mixed with							
	<i>C. aurantifolia</i>	<i>T. indica</i>	<i>C. aurantifolia</i>	<i>T. indica</i>	<i>C. aurantifolia</i>	<i>T. indica</i>	<i>C. aurantifolia</i>	<i>T. indica</i>
F-1	F-2	F-1	F-2	F-1	F-2	F-1	F-2	
1	All samples exhibit normal condition (homogenous, no foam, no mold, no pungent odor detected)							
6	Day-6: Foam appear							
7	Day-7: A lot of foam noticed	Day-7: Normal	Day-7: Normal	Day-7: Normal	Day-7: Normal	Day-7: Normal	Day-7: Normal	Day-7: Normal
8	Day-8: Pungent odor wafted.							
10	Further observation was not necessarily performed		Day-10: Mold formed		Day-10: Pungent odor wafted		Day-10: Further observation was not necessarily performed	
11			Day-12: Suspension full with Mold		Day-12: Normal		Day-12: Normal	
12			Day-12: Normal		Day-12: Normal		Day-12: Normal	

F-1: Formula-1 (*C. longa* mixed *C. aurantifolia*); F-2: Formula-2 (*C. longa* mixed *T. indica*); \*Normal: homogeneous suspension, without foam, without pungent odor, without mold appearance

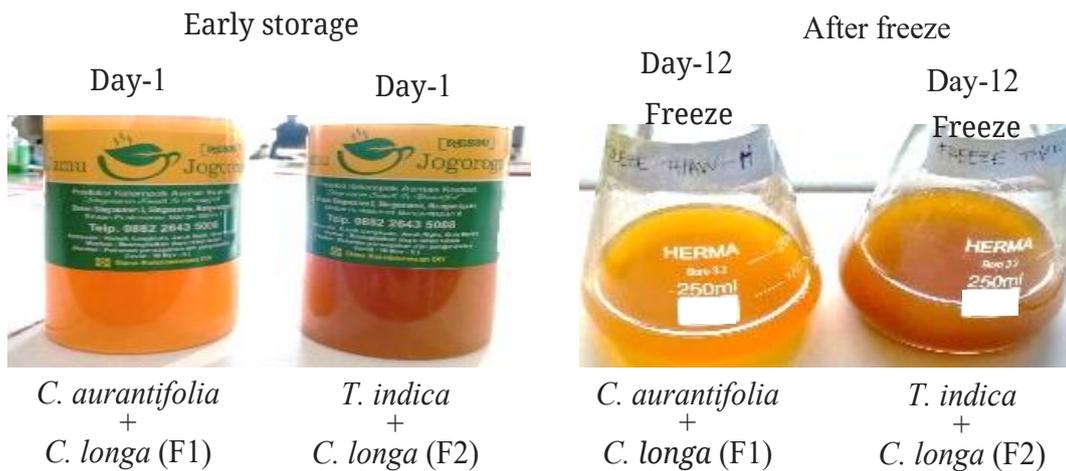


FIGURE 1. Macroscopic observation showed homogenous suspension from both formulas

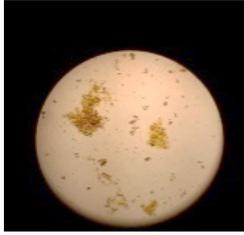
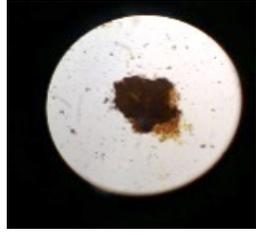
Day	Refrigerator (8°C)		Freeze thaw	
	<i>C. aurantifolia</i> + <i>C. longa</i> (F1)	<i>T. indica</i> + <i>C. longa</i> (F2)	<i>C. aurantifolia</i> + <i>C.</i> <i>longa</i> (F1)	<i>T. indica</i> + <i>C. longa</i> (F2)
7				
12				

FIGURE 2. Microscopic observation of both formula in closed bottle (in refrigerator 8 °C and freeze thaw) showed precipitation trend started between day-7 to day 12

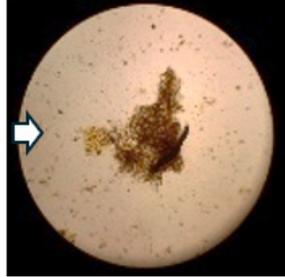
Day	Room temperature	
	<i>C. aurantifolia</i> + <i>C. longa</i> (F1)	<i>T. indica</i> + <i>C. longa</i> (F2)
1		
7		
8 -12	No observation	

FIGURE 3. Microscopic observation of both formula in open bottle (room temperature) showed that the longer the storage period, the greater of sediment particles resulted.

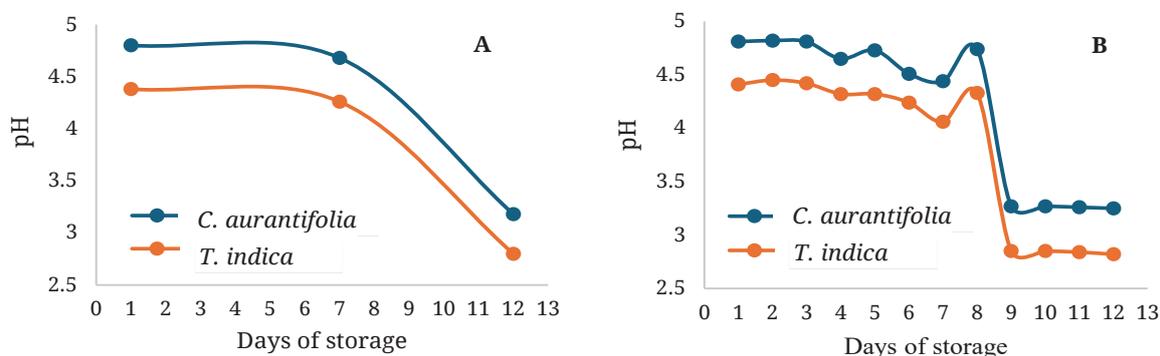


FIGURE 4. pH alteration of F1 and F2 in closed bottle. A) at room temperature and (B) in refrigerator. Decreasing pH begins from day-7 (in room temperature) and day-8 (in refrigerator) storages.

## DISCUSSION

Preservatives made from weak acids will not kill microorganisms but inhibit their growth, causing a very long lag phase.<sup>11</sup> This is beneficial, because apart from curcuminoids, acidic condition is suitable for many substances in turmeric, sometimes even improving its stability.

Generally, *C. aurantifolia* contains around 0.7 g of proteins and 1.7 g of sugar per 100 g of its fruit. *Tamarindus indica* contains at least 2 g of protein and 57.4 g of sugar per 100 g of its fruit. Protein and sugar contents may affect stability of the preparation.<sup>12</sup> With higher level of protein and sugar content of *T. indica* fruit, it was previously assumed that the stability of F2 will be lesser than F1. Surprisingly, the strong odor, mold and foam instead appear more quickly in F1 (TABLE 1). Several active compounds in tamarind which pose strong antifungal activity, such as 1-(2-furanyl)-ethanone, 5-methyl-2(5H)-furanone, 1-(3-thienyl)-ethanone, 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3, and methyl 2-furoate<sup>13</sup> might take role to this result. Besides having antifungal activity, the methyl 2-furoate is also a good biofilm inhibitor that prevents bacteria from surviving in a liquid medium.<sup>13</sup> On the other

hand, 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3 has antibacterial, antifungal, and flavoring activities.<sup>14</sup> While 1-(2-furanyl)-ethanone poses antioxidant and antifungal activity.<sup>15</sup>

Based on day-to-day pH measurements, it was found that the pH level of both formula were continuously decreasing from day-7 - for those stored at room temperature, and from day-8 for those stored in a refrigerator. Declining pH might occur due to enzymatic production that was developed by bacteria that resulting unfavorable by products.<sup>16</sup> Besides pH alteration and pungent odor, mold formation is also an important indicator toward product quality. Those, could lead to serious problems because food deterioration either by microbiological, chemical, or physical changes will potentially risk the safety used and the consumer's acceptance.

It has been observed that the mold does not become visible under freeze storage or refrigerator until day-12. However, the mold has prominently appeared and foam has been produced even before day-7 under room temperature storage. Hence, it is recommended to consume it before day-7 when it kept at room temperature.

However, a closed bottle under the refrigerator will keep the product quality longer.

The addition of weak acids in turmeric-tamarind herbal medicine also functions to extend the shelf life of the product.<sup>17</sup> In fact, it was shown that pH suspensions of both preparations decrease after the day-7 or the day-8 even though both formula has been added with additional acidic source materials. *Citrus aurantifolia* contains terpenoids by which 94% of them are including limonene, sesquiterpenes, aldehydes (citral and citronellal) and esters.<sup>18</sup> Those compounds are very volatile. Because most antimicrobial ingredients of *Citrus aurantifolia* are volatile compounds, we believe they easily damaged or oxidated and evaporated quickly. Thus, those substances are not able to maintain the stability of suspension from microbe. On the other hand, the phenolic compounds contained in *T. indica* are more stable than antimicrobial substances in *C. aurantifolia*. Moreover, they also tend to distributed more evenly throughout the solution.

These results are in line with previous study by Septiana, et al.<sup>19</sup> who found that *C. longa*-tamarind extract was able to last for 2-10 days. The addition of *T. indica* was able to maintain the stability<sup>11</sup> and to prevent from biofilm formation.<sup>20</sup> Curcumin as the active substance of turmeric is also more stable under acidic conditions.<sup>7</sup> The enolic form of curcumin was able to avoid suspension from hydrolysis and rapid degradation.<sup>17</sup> Good stability properties of curcumin and other active substances in 'Jamu Kunyit- asam' herbal suspension is expected to maintain its pharmacological effect.

Apart from the stability formula evaluation, turmeric (*C. longa*) and curcumin have been studied to have several beneficial effects. The use of *C. longa* is able to maintain healthy skin both in oral and topical preparations.<sup>21</sup> Turmeric has been studied to improve lipid profiles by reducing LDL<sup>22</sup> and triglycerides.<sup>23</sup> A randomized and

placebo-controlled trial also found that the systolic blood pressure and the hematuria were decreased significantly after pre- and post-turmeric consumption (which contains 22.1 mg curcumin, 3 capsules/day).<sup>24</sup>

The ability to control systolic blood pressure by curcumin supplementations had been reported in a meta-analysis of 11 studies.<sup>25</sup> The antidiabetic effect of *C. longa* to reduce the fasting glucose, the HbA1c, the BMI, and the suppressing oxidative stress activity and in the inflammation due to diabetic mellitus has been reported.<sup>26</sup> In a meta-analysis turmeric and curcumin also found to improve patient's condition due to joint pain and inflammation by osteoarthritis, however, its combination consumption with NSAIDs did not report to increase its side effects.<sup>27</sup> Turmeric is also reported to be able to prevent angiogenesis.<sup>4</sup> Several studies show that consuming *C. longa* is widely known to be able to maintain women's health. For example, curcumin is able to relieve pain due to premenstrual syndrome. A significant reduction in the severity of PMS syndrome was found after 3 cycles of curcumin administration compared to placebo in an RCT study.<sup>28</sup> Apart from that, turmeric is also able to suppress oxidative stress and improve inflammation in endometriosis.<sup>1</sup> However, tamarind (*T. indica*) also has beneficial pharmacological effects to the body. It contains various active compounds including alkaloid, flavonoid, tannin, phenolic, saponin and several steroid classes. Several substances in the *T. indica* have been proven to be able to produce anti-inflammatory and analgesic effects through inhibiting inflammatory mediators such as COX-2, iNOS, LOX, and TNF- $\alpha$ .<sup>29</sup> Administration of *T. indica* is also play an important role in the regulation of cartilage and bone degeneration mediators thus potentially reduce the oxidative stress in arthritis conditions.<sup>30</sup> To sum up, combination between *C. longa* and *T. indica* is not only potentially improve its stability but also its beneficial pharmacological effect.

## CONCLUSION

The herbal drink suspension *jamu turmeric-asam* consisting of *C. longa* has lower stability when mixed with *C. aurantifolia* compared to *T. indica*.

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