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Relationship of turmeric and tamarind leaf extract ratio with induction time and antioxidant activity synergism

S. Mulyani[12*] and B.A. Harsojuwono[1]

[1]Department of Agriculture Industrial Technology, Faculty of Agriculture Technology, Udayana University, Bali, Indonesia.
[2]Faculty of Agriculture Technology, Udayana University, Bukit Jimbaran, South Kuta, Badung, Bali (80362), Indonesia.
**E-mail: srimulyani@unud.ac.id*

Abstract

This study aims to determine the ratio of turmeric rhizome extract (TE) and tamarind leaf extract (TLE), which is suitable for the time of induction and the highest antioxidant synergism. The TE:TLE ratio examined was 10:1; 10:1.5 and 10:2. The chosen variable was the amount of peroxide. Observations carried out for 7 hours at one-hour intervals using ferric thiocyanate (FTC) method and thiobarbituric acid (TBA) method. Relationship between the time and absorbance were depicted to show the induction time and synergism in antioxidants. The results showed that all TE and TLE ratios had antioxidants that inhibited lipid oxidation reactions at the stage of initiation and propagation. TE and TLE ratios 10:1, 10:1.5 and 10:2 had induction time, respectively 2.9577; 3.0206 and 3.1882 hours with FTC method, 3.6116; 3.706 and 3.8722 h with the TBA method. Synergism in antioxidants was 103.534; 106.924 and 110.705 % with the FTC method, and 102.9393; 109.522 and 115.969 % with TBA. The highest antioxidant synergism in both methods was shown by the ratio of TE:TLE = 10:2.

Key word: Turmeric, tamarind leaves, induction time, synergism antioxidant

Introduction

Turmeric (*Curcuma domestica* Val.) rhizomes and tamarind (*Tamarindus indica* L.) leaves are potential sources of antioxidants. Curcumin in turmeric has shown to have antioxidant activity, (Anand *et al.*, 2008 and Lee *et al.,* 2010). The antioxidant properties in tamarind leaves are derived from the content of vitamin C (Mulyani *et al.,* 2014) and phenolic compounds (De Caluwe *et al.*, 2010 and Bhadoriya *et al.,* 2011). Combining two of antioxidants can increase their effectiveness or have synergism (Smet *et al.*, 2008; Noguer *et al.,* 2014). Suwarini and Suhendra (2008) reported that turmeric-tamarind extract had a very strong synergism of antioxidants. Satriawan and Mulyani (2007) proved that tamarind leaves and turmeric extract contain antioxidants that can inhibit lipid oxidation, and its ability is close to butylated hydroxytoluene (BHT) activities. Synergism occurs when a mixture of antioxidants produces a higher activity than the amount of antioxidant when they are used separately (Santosa, 2016).

Phenolic compounds in turmeric rhizomes and tamarind leaves are primary antioxidants that belong to the chain breaker antioxidants while ascorbic acid in tamarind leaves has the function as a secondary antioxidant / chelating agent (Santosa, 2016). Therefore, it is important to mix the two components at the right ratio to increase the effectiveness of the antioxidants. Increasing the concentration of ascorbic acid in a fixed amount of d-tocopherol produces a synergistic effect that increases sigmoidal (Ock *et al.,* 1991). It takes one to two parts of ascorbic acid to obtain a synergistic effect with 10 parts d-tocopherol to stabilize fish oil.

The synergistic effect of antioxidants can be studied in various ways, one of which is by measuring in the linoleic system. This measurement was chosen because this method is based on the measurement of the induction period of the sample because, during this period, there is rapid absorption of oxygen. Above mentioned literature indicate that there is a need to conduct research on the effect of antioxidant synergism in the mixture of turmeric and tamarind leaves. During the synergism mechanism process, the antioxidant mixture of free radicals receives two antioxidants, one that reacts with peroxyl radicals and a second antioxidant that regenerates the first, so that both antioxidants become effective (Smet, 2008). However, the extract proportion of turmeric and tamarind leaves mixture which has the highest synergism of antioxidants in the linoleic system is still unknown. Therefore, it is important to find out the synergism of the antioxidants of these two ingredients, because the occurrence of synergy will increase their effectiveness and prevent deterioration in quality.

Materials and method

Plant and chemical materials: The turmeric (*Curcuma domestica* Val.) used in this research was one of the local varieties cultivated in Petang, Badung- Bali and were harvested in November 2018. The tamarind leaves (*Tamarindus indica* L.) for this research were taken from the buds of local plantation in Jimbaran, Badung-Bali. Analysis materials used as part of this research were: sodium carbonate, thiobarbituric acid, folin ciocalteu phenol (Merck), phosphate buffer, ethanol, TBHQ (Brathaco Chem), DPPH, gallic acid (Sigma), and soybean oil (linoleic acid). The tools used were UV-Vis Biochrom Libra S60 SN 133647, spectrophotometer, centrifuge (EC HN-S II 0-9000 rpm), oven (Blue M), and incubator (Memmert, 500 model).

Preparation of turmeric and tamarind leaves extract: Turmeric rhizome were harvested at the age of eleven months and washed, and drained overnight. Each rhizome was sliced into disks approximately 0.1cm thick and then dried in the oven at 55 °C until the water content reached 10 %. The tamarind leaves that were harvested late in the afternoon, washed, and drained overnight. The leaves were put into an oven until the water content reached 10 %. The turmeric and tamarind leaves were mashed into powder and sieved at 60 mesh. The turmeric powder was weighed (500 g) and extracted with ethanol 96 % at ratio of material and solvent 1:6. The materials were macerated for 24 hours and stirred twice. The filtrate was separated and the pulp was re-macerated. Both filtrates were mixed and steamed using an evaporator at 100 rpm, 40 °C, 50 m Bar. The same extraction process was repeated on the tamarind leaves for getting two extraction.

Treatment for synergism antioxidant of turmeric and tamarind leaves extract: This study was experimental. The different ratio used to treat the turmeric and tamarind leaves extract are shown in Table 1.

Table 1. Treatment of the ratio of turmeric and tamarind leaves extract (10:1; 10:1.5; 10:2)

Treatment		Turmeric extract (TE)	Tamarind leaves extract (TLE)
TE : TLE =	C (Control)	0	0
10 : 1	A (TE ratio 10)	10 (200 ppm)	0
	B1 (TLE ratio 1)	0	1 (20 ppm)
	AB1 (TE :TLE = 10:1)	10 (200 ppm)	1 (20 ppm)
TE : TLE =	C (Control)	0	0
10 : 1.5	A (TE ratio10)	10 (200 ppm)	0
	B2 (TLE ratio 1.5)	0	1.5 (30 ppm)
	AB2 (TE :TLE = 10:1.5)	10 (200 ppm)	1.5 (30 ppm)
TE : TLE =	C (Control)	0	0
10 : 2	A (TE ratio 10)	10 (200 ppm)	0
	B3 (TLE ratio 2)	0	2 (40 ppm)
	AB3 (TE :TLE = 10:2)	10 (200 ppm)	2 (40 ppm)

Research procedure: The linoleate system consists of 2 mL of 0.1 M phosphate buffer solution, 1 mL of 50 mM linoleic acid in 96% ethanol, and 1 mL of ion-free solution. The sample solution was mixed with 1 mL of extract according to the treatment. The mixture was then put in a tightly closed container wrapped in aluminium foil and then incubated for 7 hours at 100 °C. The sample was taken to be tested every 60 min. The absorbance of samples was measured by FTC and TBA test; the measurement results of each treatment were recorded and plotted on the graph so that the equation could be obtained. From this equation, the induction period of each sample was obtained. Induction time/induction period / Oil Stability Index (OSI) was the time taken until the reaction product occurred in the form of peroxide as measured by a spectrophotometer. The value of the induction period of each mixture was then calculated to determine the antioxidant synergism of each treatment. Synergism values were calculated based on the following formula (Ock *et al.,* 1991):

$$LQ = \frac{(AB-C)-[(A-C)+(B-C)]}{[(A-C)+(B-C)]} x\ 100$$

A: Period for induction of linoleic control given turmeric extract.
B: Period for induction of linoleic control given tamarind leaves extract
C: Period for induction control.
AB: Period for induction of linoleic control with given combination of turmeric and tamarind leaves extract

The observation procedures: the procedure used is the modified TBA method and the modified thiocyanate ferry (FTC) method (Kikuzaki and Nakatami, 1993). Testing of antioxidant activity by thiobarbituric acid (TBA) method was done by taking 1 mL of the incubated sample solution and mixing it with 2 mL of 20 % trichloroacetic acid and 2 mL 0.02 M TBA solution. The mixture, then boiled for 10 min, before being cooled and centrifuged at 3000 rpm for 20 minutes. The absorbance of supernatant was measured at a wavelength of 532 nm. Testing the antioxidant activity using peroxide number method (ferric thiocyanate) was done by taking 0.1 mL of incubated sample solution and then adding 9.7 mL of 75 % ethanol and 0.1 mL of 30 % ammonium thiocyanate. After three minutes, 0.1 mL 0.02 M ferro chloride in 3.5 % HCl was added to the mixture. The red absorbance was monitored at λ 500 nm.

Results

Synergism of antioxidant turmeric and tamarind leaves by FTC method: The graph of the relationship between the time of observation and absorbance of the sample of the turmeric and tamarind leaves extract ratio (10:1, 10:1.5 and 10:2) is presented in Fig. 1. Based on the graph in Fig. 1, two equations were obtained and the induction time of each sample is shown in Table 2.

Table 2. Induction time and synergism of antioxidants TE:TLE (10:1, 10:1.5 and 10:2) by the FTC method

	Treatment	Equation 1	Equation 2	Induction time (h)
TE: TLE = 10: 1	C (Control)	y=0.007x+0.064	y=0.0396x-0.0013	2.0031
	A (TE ratio 10)	y=0.008x+0.0167	y=0.0315x-0.007	2.3277
	B1 (TLE ratio 1)	y=0.0085x+0.0653	y=0.0363x+0.0056	2.1475
	AB1 (TE:TLE = 10:1)	y=0.008x+0.0637	y=0.0387x-0.0271	2.9577
		Synergism (%)		103.5339
TE: TLE = 10: 1.5	C (Control)	y=0.007x+0.064	y=0.0396x-0.0013	2.0031
	A (TE ratio 10)	y=0.008x+0.0167	y=0.0315x-0.007	2.3277
	B1 (TLE ratio 1.5)	y=0.0125x+0.0609	y=0.0313x+0.0201	2.1702
	AB1 (TE:TLE = 10:1.5)	y=0.005x+0.0737	y=0.039x-0.029	3.0206
		Synergism (%)		106.9236
TE: TLE =	C (Control)	y=0,007x+0.064	y=0.0396x-0.0013	2.0031
10: 2	A (TE ratio 10)	y=0,008x+0.0167	y=0,0315x-0.007	2.3277
	B1 (TLE ratio 2)	y=0,0015x+0,0857	y=0,0318x+0,0178	2.2409
	AB1 (TE:TLE = 10:2)	y=0,0095x+0.0685	y=0.0281x+0,0092	3.1882
		Synergism (%)		110.7045

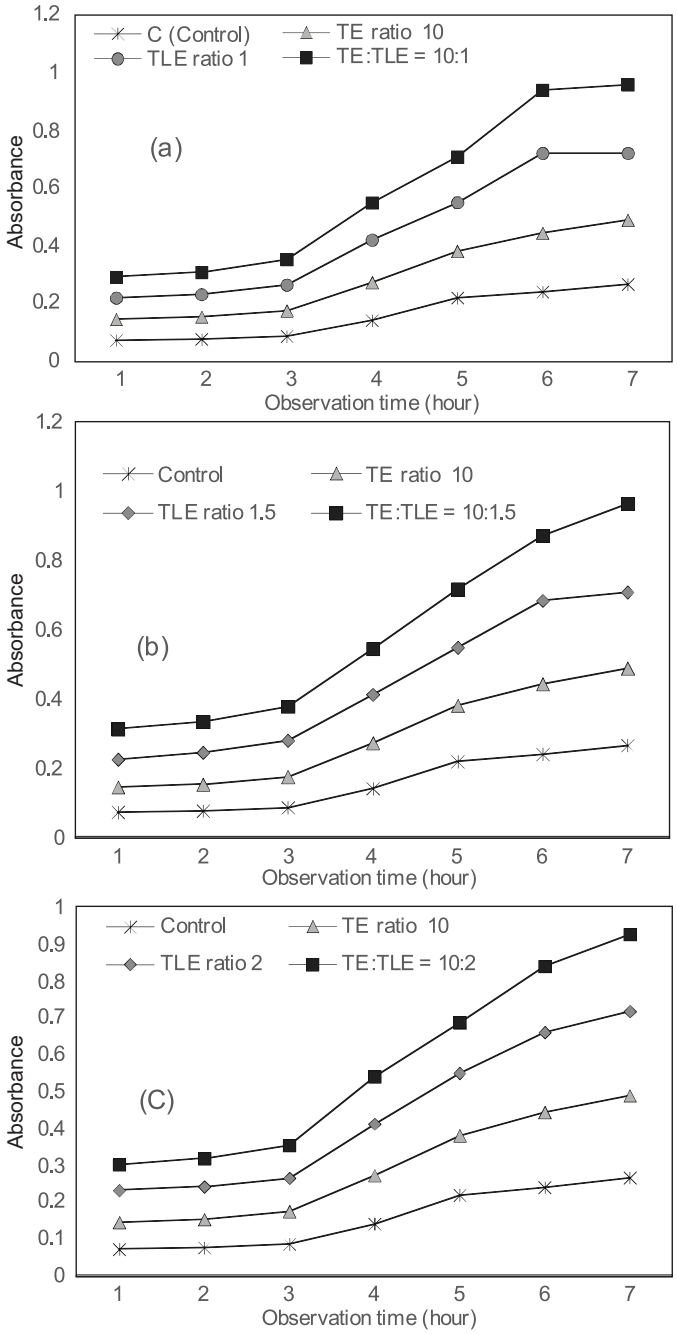

Fig. 1. Absorbance of turmeric and tamarind leaves extract in ratio 10: 1 (a), 10: 1.5 (b) and 10: 2 (c) with FTC method

The results of the measurement of induction time and synergism for each treatment are presented in Table 2. Table 2 shows that each treatment has a different induction time, which proves that each ingredient needs different time to produce a reaction by-product in the form of peroxide. The control treatment produced peroxide between the hours of 2.0031, while the treatment given antioxidants the induction time was between hours 2.1475 (TE: TLE = 10: 1) to 3.1882 (TE: TLE = 10: 2).

Synergism antioxidants of turmeric and tamarind leaves with TBA method: The graph of the relationship between the time of observation and absorbance of the sample of the turmeric and tamarind leaves extract ratio (10:1, 10:1.5 and 10:2) is presented in Fig. 2. Based on the graph in Fig. 2, two equations were obtained and the induction time from each sample shown in Table 3.

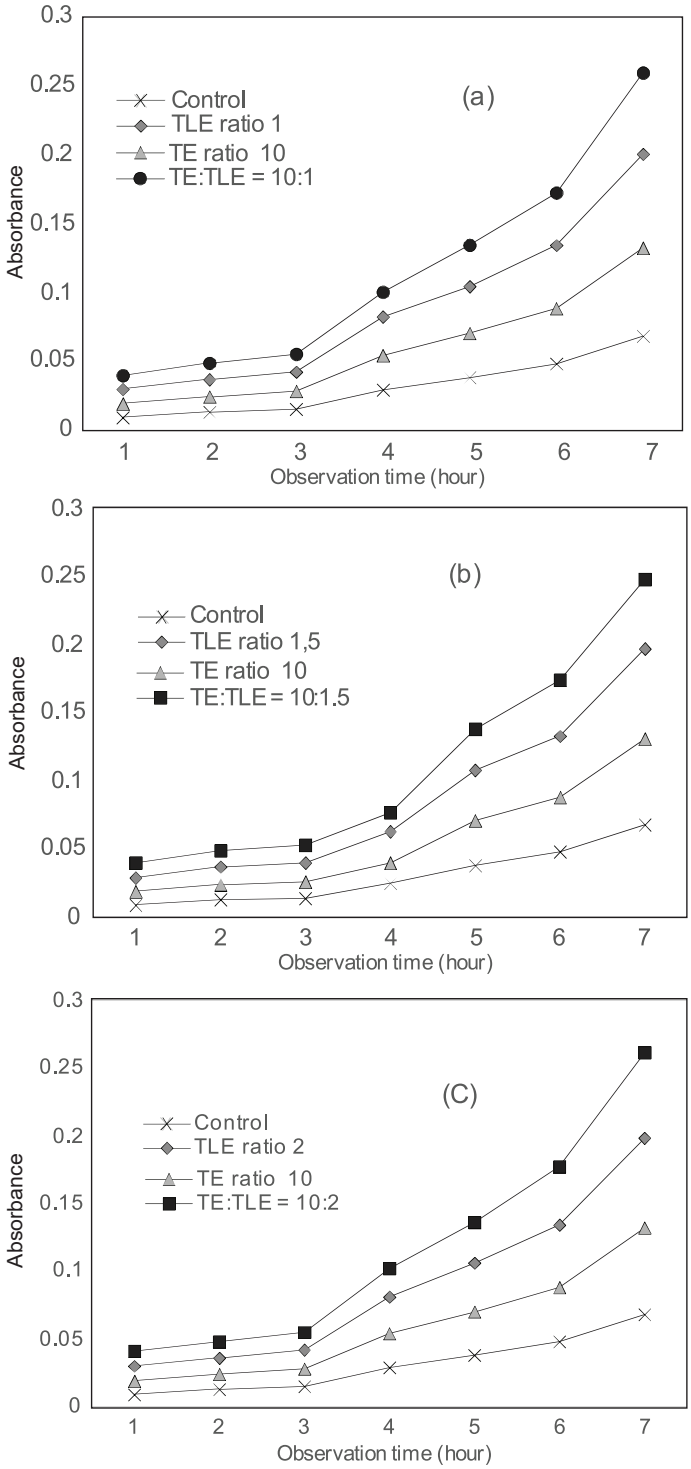

Fig. 2. Absorbance of turmeric and tamarind leaves extract in ratio10: 1 (a), 10: 1.5 (b) and 10: 2 (c) with TBA method at different time interval

Table 3 shows secondary lipid oxidation takes longer to produce malonaldehyde. The control produced malonaldehyde at 3.0935 hours. In the treatment given antioxidants, the induction time was between hours to 3.30172 (TLE ratio 1) to 3.8722 (TE:TLE = 10:2). Longer induction time is caused by the presence of phenolic compounds in turmeric and tamarind leaves.

Comparison of the synergism antioxidants with the FTC and TBA methods: There are various methods for determining the byproducts of lipid breakdown reactions, the most widely used methods are the FTC and TBA methods. Antioxidants are known to be able to inhibit lipid breakdown reactions. To determine

Table 3. Induction time and synergism of antioxidants TE: TLE (10: 1, 10: 1.5 and 10: 2) by the TBA method

	Treatment	Equation 1	Equation 2	Induction Time
TE:TLE = 10:1	C (Control)	y=0.002X+0.009	y=0.0127x-0.0241	3.0934
	A (TE ratio 10)	y=0.0009x+0.0098	y=0.0125x-0.0285	3.3017
	B1 (TLE ratio 1)	y=-0.0011x+0.0104	y=0.0132x-0.0276	3.1404
	AB1 (TE:TLE = 10:1)	y=-0.001x+0.01	y=0.0131x-0.0337	3.6116
		Synergism (%)		102.9393
TE:TLE = 10:1.5	C (Control)	y=0.002x+0.009	y=0.0127x-0.0241	3.0934
	A (TE ratio 10)	y=0.0009x+0.0098	y=0.0125x-0.0285	3.3017
	B1 (TLE ratio 1.5)	y=0.0014x+0.0101	y=0.0137x-0.0290	3.1789
	AB1 (TE:TLE = 10:1.5)	y=-0.0006x+0.0109	y=0.0122x-0.0321	3.7060
		Synergism (%)		109.5220
TE :TLE = 10:2	C (Control)	y=0.002X+0.009	y=0.0127x-0.0241	3.0934
	A (TE ratio 10)	y=0.0009x+0.0098	y=0.0125x-0.0285	3.3017
	B1 (TLE ratio 2)	y=0.0009x+0.0108	y=0.0127x-0.0275	3.2457
	AB1 (TE:TLE = 10:2)	y=-0.0006x+0.0109	y=0.0139x-0.0406	3.8722
		Synergism (%)		115.9693

Table 4. Comparison of the synergism of antioxidant extracts turmeric and tamarind leaves with FTC and TBA methods

Treatment		Induction time	
		FTC method	TBA method
TE : TLE = 10 : 1	C (Control)	2.0031	3.0934
	A (TE ratio 10)	2.3277	3.3017
	B1 (TLE ratio 1)	2.1475	3.1404
	AB1 (TE:TLE = 10:1)	2.9577	3.6116
	Synergism (%)	103.5339	102.9393
TE : TLE = 10 : 1.5	C (Control)	2.0031	3.0934
	A (TE ratio 10)	2.3277	3.3017
	B1 (TLE ratio 1.5)	2.1702	3.1789
	AB1 (TE:TLE = 10:1.5)	3.0206	3.7060
	Synergism (%)	106.9236	109.5220
TE : TLE = 10 : 2	C (Control)	2.0031	3.0934
	A (TE ratio 10)	2.3277	3.3017
	B1 (TLE ratio 2)	2.2409	3.2457
	AB1 (TE:TLE = 10:2)	3.1882	3.8722
	Synergism (%)	110.7045	115.9693

antioxidant activity, it is necessary to compare these two methods, considering that one method alone cannot recognize all possible mechanisms that characterize antioxidant activity (Dorman *et al.*, 2003; Erkan *et al.*, 2008). The results of the comparison of the two methods are listed in Table 4.

Discussion

Synergism of antioxidant turmeric and tamarind leaves by FTC method: The Ferric Thiocyanate (FTC) method is a testing method that aims to determine the antioxidant activity of a compound by measuring the inhibitory power of radical compounds that are reactive. The basic principle of this method is to measure: the antioxidant's ability to inhibit the formation of color characterized by its ability to maintain absorbance. The formation of peroxide is the beginning of the oxidation reaction, if antioxidants can inhibit peroxide formation, the rate of oxidation reaction initiation stage is inhibited so that the induction time is longer. The results of the measurement of induction time and synergism for each treatment are presented in Table 2. In linoleic oxidation, fat experiences reactions in two phases. The first phase of oxidation runs slow and tends to be stable or the changes that occur are not large, this stage occurs in the reaction time to 0 to the second hour (Fig. 1). The second phase of oxidation runs very fast and occurs between the second hour to the third hour, in this phase there is a rapid absorption of oxygen and fat oxidation and peroxide which is measured by a spectrophotometer**.**

The control treatment produced peroxide in 2.0031 h, while the treatment given antioxidants the induction time was between 2.1475 (TLE ratio 1) to 3.1882 h (TE: TLE = 10: 2). Curcumin in turmeric is a powerful antioxidant as a free radical scavenger (Anand *et al.*, 2008; Lee *et al.*, 2010; Mulyani *et al.*, 2014). The antioxidant properties of tamarind leaves are derived from the content of vitamin C (Mulyani *et al.*, 2014) and phenolic compounds (Bhadoriya *et al.*, 2011, De Caluwe, *et al.*, 2010).

The three treatments tested showed that the mixture of extract turmeric and tamarind leaves showed synergism, with a value that increased with increasing ratio of tamarind leaves. The treatment of turmeric extract and tamarind leaves 10: 2 produced the highest induction time of 3.1882 h. This is in accordance with report of Ock *et al.* (1991), which states that to produce an increased synergistic effect, 1 (one) to 2 (two) parts of acid with 10 parts d-tocopherol were needed to stabilize fish oil. This research has proved that with the increase in the ratio of tamarind leaves extract until 2 parts, synergistic effects increased up to 110.7045 %.

Synergism in antioxidants of turmeric and tamarind leaves with the TBA method: The graph of the relationship between the time of observation and absorbance of the sample is presented in Fig. 2. The TBA method shows the amount of peroxide in the secondary stage of lipid peroxidation (Rahmat *et al.,* 2003). This method measures the pink color produced by reagent TBA with malonaldehyde. Malonaldehyde is an advanced oxidation product derived from unsaturated aldehyde which is the result of the breakdown of hydroperoxide. In secondary stage oxidation of lipids will also experience reactions in two phases. The first phase of oxidation runs slowly and tends to be stable, occurs at the 0 to 3 hour reaction (Fig. 2),

followed by the second phase with a fast reaction (high amount of peroxide) occurring after the third hour. The first and second phase of the reaction phase can be seen in Table 3. The result shows in the absence of antioxidant, malonaldehyde has been formed at the 3.09346 hours. Turmeric, tamarind leaves and a mixture of both causes the time to produce malonaldehyde (induction time) to be longer as listed in Table 3.

In present study, secondary lipid oxidation took longer period to produce malonaldehyde. Longer induction time is caused by the presence of phenolic compounds in turmeric and tamarind leaves. Curcumin in turmeric has excellent properties as scavenger reactive oxygen species (ROS). Antioxidant are also derived from the vitamin C present in the leaves (Mulyani *et al.*, 2014). The content of phenolic compounds in turmeric and leaf acid has been shown to have antioxidant activity that can prevent free radical damage and lipid peroxidation (Bernardi *et al.*, 2008; Akhila *et al.*, 2009 and Mulyani *et al.*, 2014).

Comparison of the synergism in antioxidants with the FTC and TBA methods: The difference in induction time in both methods is because the FTC method measures the formation of peroxide lipid oxidation reaction at the initiation stage (stage 1), while the TBA method measures the reaction at the propagation stage (stage 2). Initiation is the reaction stage of the formation of free radicals, which is followed by the propagation stage, the reaction of the multiplication of free radicals (Gordon, 1990). The results showed that the initiation stage occurred from the second hour while the propagation stage occurred at the 3rd hour. The FTC and TBA methods showed that turmeric extract and tamarind leaf extract, as well as mixtures of both, had antioxidant activity. The two ingredients produced synergism. The value increased with increasing ratio of acid leaf extract and both methods showed the same results.

The synergism occurs because of the reaction of radical phenoxy regeneration. Peroxyl radical reactions with curcumin produce phenoxy radical curcumin, which is less reactive than peroxyl radicals, thus causing protection from ROS induced oxidative stress (Priyadarsini, 1997 and Jovanovic *et al.*, 2001). Synergism occurs because of the regeneration reaction of phenoxy radicals back to curcumin with antioxidants that are soluble in water such as ascorbic acid. This regeneration can also occur with vitamin E because of the ability of vitamin E to give molecules as chain-breaking antioxidants (Jovanovic *et al.*, 2001). The mechanism of phenoxy radical regeneration with ascorbic acid causes effective antioxidant activity. This increases the effectiveness of antioxidants due to combination of two types of antioxidants. Curcumin and ascorbic acid in turmeric and tamarind leaves are antioxidants that will inhibit the lipid oxidation. The mixture of these two ingredients will produce a synergistic effect in the linoleic system, which results in longer induction times. Antioxidants of turmeric and leaf acid can inhibit the oxidation reaction so that the impact of the induction time becomes longer. According to Mulyani (2017) the content of vitamin C in extract of turmeric and tamarind leaves in the ratio (8: 2) ranged between 4.63 to 6.72 %. This amount is greater than the requirement for the contribution of ascorbic acid in the mixture to produce a synergistic effect. Sufficient ascorbic acid content enables the antioxidant to survive during storage. The antioxidants in the mixture also showed stability at room temperature storage for up to eight weeks. This proves that during storage, antioxidants could show activity due to synergism.

Turmeric rhizome and tamarind leaf extracts have antioxidant activity that can inhibit lipid oxidation reactions at the initiation and propagation stages, at ratios (10:1, 10:1.5 and 10:2). Induction time determined by successive FTC methods (2.9577, 3.0206 and 3.1882 h) and TBA method (3.6116, 3.706 and 3.8722 h). The synergistic effect of antioxidants in three different ratios with the FTC method was 103.534, 106.924 and 110.705 %, and with the TBA method was 102.9393, 109.522 and 115.969 %. The highest antioxidant synergism in both methods was shown by the ratio of turmeric extract and tamarind leaves extract = 10:2

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