

PROFILE OF LYCOPENE UV-SPECTRA OF LACTIC ACID BACTERIA FERMENTED MILK-TOMATO PASTE

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ABSTRACT

Lycopene is one of the famous ingredients in tomatoes, because of its important role as a potent anti-oxidant. Besides being consumed as fresh fruit, tomato paste can be combined with probiotic fermented milk to increase its health benefits. Probiotics are lactic acid bacteria (LAB), which when consumed in sufficient quantities will benefit health. Probiotic fermented milk is reported to have various properties including anti-microbial, antioxidant, immunomodulatory and anti-cancer, so the combination with tomato paste is expected to increase its benefits for health. This research aimed to study the effect of probiotic fermented milk on the content of tomato paste lycopene. *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, and *Lactobacillus casei* were used as mixed probiotic cultures. It was found that at the ratio of 2:8 for the LAB fermented milk and tomato paste, the highest lycopene drive was obtained by spectrophotometric measurement at 663, 645, 505, and 453 nm. Determination of the lycopene content was performed according to the Nagata and Yamashita simple methods. In addition, the lycopene content in the LAB fermented milk-tomato paste was greater than the content in the tomato paste without fermented milk addition and was tested stable after storage for 14 days.

Keywords: antioxidant; lycopene; probiotic fermented milk; tomato paste

INTRODUCTION

Yogurt is a product that is obtained from pasteurized milk and then fermented with bacteria to obtain a characteristic acidity, odor and taste with or without the addition of other permissible ingredients (Anonim, 2009). *Lactobacillus spp.* are the most predominate probiotic bacteria, and some of them are commonly used in making yogurt such as *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Streptococcus thermophilus*. Lactic acid bacteria (LAB) are the most important probiotics because of its beneficial effects on the human digestive tract or other activities, such as immunomodulatory (Isnaeni, 2016). Rajarajan *et al.* (2018) evaluated antimicrobial activity and probiotic potential of *Lactobacillus* strains against some human pathogens. Isnaeni and Mertaniasih (2015) reported the growth inhibitory activities of

mixed culture LAB against MRSA and ESBL. Inhibitory activity of LAB fermented milk and tomato juice combination against *Escherichia coli* and *Staphylococcus aureus* has been reported (Hidayah, 2014). Wang *et al.* (2017) reviewed antioxidant activity of the probiotic, although its mechanisms of action have not been completely understood.

Various food matrices have been developed with probiotics and were reported. With different technologies such as microencapsulation, nanoparticle (Sugiyartono, 2014; Sholeha *et al.*, 2014) and continuous fermentation, the probiotic will become an important ingredient in the functional foods (Soccol *et al.*, 2010). Yogurt product is one of the popular probiotic fermentation foods, found on the market in various forms including food, drinks and ice cream. The yogurt products are very popular in countries with four seasons, but in

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Indonesia the fermented milk containing LAB has also been popular, especially in cold climate cities with surplus milk dairy products. Yogurt preparations are often combined with fruits such as soursop, mangoes, avocados, durians, oranges, and others. Various tomato-yogurt combinations include yogurt curry tomatoes, tomato yogurt sauce, yogurt smoothie tomatoes, and salad tomatoes with chili yogurt and even for cosmetic preparations as a tomato face mask. Kaur *et al.* (2016) studied fermentation of tomato juice by probiotic LAB. They demonstrated that *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Lactobacillus casei* probiotics showing the ability to use the tomato juice as raw material to increase viability count.

Tomatoes (*Lycopersicon esculentum*) and its products contain lycopene, predominant carotenoid (5.7-26.3mg/kg) and an antioxidant which plays an important role in health and disease reduction (Erge and Karadeniz, 2011). The bioactive compounds in tomato fruit are carotenoid, phenolic and L-ascorbic acid; which act as antioxidants, which has a protective effect against various forms of cancer and cardiovascular diseases (Kopsel *et al.*, 2006). There are various manners of tomato processing to gain several kinds of tomato products, such as tomato juice, tomato paste, tomato boiled sauce, tomato ketchup with different specifications and lycopene content (Alda *et al.*, 2009). The lycopene content in tomato paste is higher than fresh tomatoes. Lycopene contents of fresh, sun-dried and canned tomatoes were found to be 1.74, 5.51 and 3.55 mg/100g, showing different antioxidant activities (Karakaya and Yilmaz, 2007).

This research was a breakthrough to make Nutraceutical food from tomatoes products combined with LAB fermented milk derived from LAB probiotics mixed culture (*Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, and *Lactobacillus casei*). A nutraceutical is a food or part of food that provides health benefits including prevention and treatment of diseases. From this definition, nutraceuticals can be in the form of isolated nutrients, food supplements, herbal

products or genetic engineering, or processed foods such as cereals, soups and drinks (Kaira, 2003).

Tomato-yogurt nutraceutical products were made by optimization of LAB probiotic fermented milk and tomato paste ratio to find the optimal composition with the highest lycopene content. The optimization was done by trying several compositions of fermented milk and tomato paste, which were then analyzed for their lycopene content using spectrophotometry method according to Nagata and Yamashita (1992).

METHODS

Material and chemicals

The tomatoes used were *Lycopersicon esculentum* or tomato fruit, obtained from the Karang Menjangan Market in Surabaya in one-time purchase to obtain a uniformity of the tomatoes condition. Based on the results of determination morphologically by Herbarium Malangensis, tomatoes fruit used in this study (Figure 1) included the family Solanaceae, genus *Lycopersicon*, species of *Lycopersicon esculentum* Mill (Pratiwi, 2014). Dairy milk was obtained from the Jemursari dairy cow, Surabaya, Indonesia. The probiotic fermented milk was obtained from plain yogurt containing LAB *Lactobacillus Acidophilus*, *Lactobacillus bulgaricus*, and *Lactobacillus casei* production of Microbiology Practical Room Faculty of Pharmacy, Universitas Airlangga. N-hexane and acetone p.a (Merck) were used as solvents.



Figure 1. Tomato fruits

Preparation of probiotic fermented milk-tomato paste

The tomatoes fruits used in this research were specifically selected to be red both in outside and inside color, round shape with diameter of 7-10 cm, juicy texture and sour-sweet taste. As much as 2 kg of tomato fruits

were washed, set aside to dry, and steamed for 10 minutes at $\pm 110^{\circ}\text{C}$. The tomato rind was removed and the fruit was crushed with a blender. Tomato juice was made by the same manner without steaming. Yogurt or probiotic fermented milk was prepared by mixing 1 liter of pasteurized fresh cow's milk (90°C , 15 minutes) with 25 mL plain yogurt containing a combination of LAB *Lactobacillus bulgaricus*, *Lactobacillus casei*, and *Lactobacillus acidophilus* 106-107 CFU/mL at 43°C . The mixture was left to stand for 24 hours at room temperature (30°C). Characteristics of the probiotic fermented milk were observed based on the acid pH and viscous texture. A mixture of the fermented milk-tomato pasta was made in 9 compositions (g/g), 9:1; 8:2; 7:3; 6:4; 5:5; 4:6; 3:7; 2:8; 1:9. Each combination was blended for homogenization.

Extraction of lycopene

Approximately 1 gram of each tomato juice, tomato paste, and fermented milk-tomato pasta at all composition were extracted with 8 mL acetone:n-hexane (4:6), then centrifuged $3000 \times g$ for 10 minutes at 4°C . The organic phase was separated after standing for 10 minutes and adding by 1 mL

water. The organic phase was added by n-hexane up to 10 mL and then dilution was done twice, before measurement by a spectrophotometer.

Qualitatively and quantitatively analysis of lycopene

The dilution results were measured quantitatively by spectrophotometer Agilent Technologies Cary 60 UV-Vis at wavelengths of 300-700 nm. Qualitative analysis of lycopene content was carried out by determination of the UV-Vis spectra profile of lycopene in a sample of tomato paste and probiotic fermented milk-tomato paste extract at 300-700 nm. Furthermore, quantitative analysis was done by measuring the absorbance at 663, 645, 505, and 453 nm using acetone-n-hexane (4:6) blank. The probiotic fermented milk extract in acetone-n-hexane (4:6) was used as a blank for probiotic fermented milk-tomato paste analysis, in order to evaluate the effect of the probiotic fermented milk addition to lycopene in pasta or tomato juice. The lycopene levels were calculated using the formula proposed by Nagata and Yamashita (1992) as follows:

$$[\text{lycopene}](\mu\text{g/mL}) = -0.0458 \times A_{663} + 0.2040 \times A_{645} + 0.3720 \times A_{505} - 0.0806 \times A_{453}$$

A_{663} , A_{645} , A_{505} , A_{453} , were absorbance at 663, 645, 505, and 453 nm, respectively.

RESULTS AND DISCUSSION

The studies by Bunghez *et al.* (2011) and Alda *et al.* (2009) on the determination of lycopene in tomato and tomato products reported spectra profile of the lycopene extract from tomatoes samples in hexane indicated that profile of the spectra consisted of three peaks at 340, 245, and 506 nm respectively. Based on the spectrophotometric data analysis, the profiles of tomato paste and probiotic fermented milk-tomato paste were obtained as shown in Figures 3a and 3b. Tomato paste and yogurt-tomato paste showed the same spectra profile measured at wavelengths between 300-700 nm. There were three peaks detected and the maximum wavelength was obtained at 472 nm. This phenomenon indicated that the spectra profile of the tomato pastes was similar to probiotic fermented milk-tomato

paste qualitatively. The spectra profile of lycopene extract of Bunghez's and Alda's research's showed the similar profile as spectra extract of tomato paste and probiotic fermented milk-tomato paste. Alpha-carotene and lutein had maximum wavelengths at 420 nm and 444 nm (Bunghez, 2011), while the lycopene has a maximum wavelength of 470 and 500 nm. Comparing with the profile of the probiotic fermented milk-tomato paste spectra, Bunghez's spectrum depicted peaks at 444 nm, 470 nm, and 502 nm with acetone:n-hexane (1:1, v/v) solvents. On the other hands, Suwanaruang (2016) used hexane:ethanol:acetone (2:1:1, v/v/v) as solvent and observed the lycopene extract at 503 nm. In this study, we found three peaks at 446 nm, 472 nm, and 502 nm (Figure 2). Alda *et al.* (2009) measured absorbance of the lycopene in n-

hexane at 472 and 502 nm. It can be seen that there was a shift in the wavelength of 2 nm might be due to differences in solvent composition. Djenni *et. al.* (2010) found that

dichloromethane lycopene extract from tomato fruit showed three peaks at 457, 483, and 516 nm in hexane solvent. Absorbance at 483 was selected for determination of the lycopene.

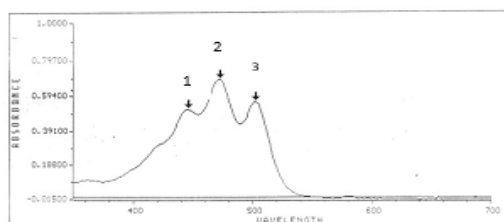


Figure 2. Spectra of yogurt-tomato paste scanned at λ 400-700 nm. (1) 446 nm, (2) 472 nm, (3) 502 nm

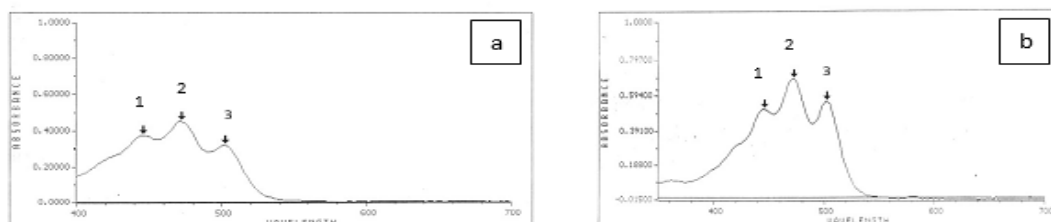


Figure 3. UV-Vis spectra of tomato paste (a) and tomato juice (b) scanned at λ 400-700 nm. (1) 446 nm, (2) 472 nm, (3) 502 nm

Calculations of lycopene levels in tomato juice, tomato paste, and probiotic fermented milk-tomato paste were based on the absorbance values at 663 nm, 645 nm, 505 nm, and 453 nm (Table I and Table II) following the method of Nagata and Yamashita (1992). All the composition of fermented milk and yogurt produced maximum absorption at 505 nm, both on day 1 and 2 weeks after storage. Optimum

composition was obtained on day 1 and 2 weeks after storage of 2:8 each then followed by 3:7. Profile of the lycopene levels for day 1 was different compared to spectra profile 2 weeks after storage. Decreased uptake on days 1 and 2 weeks after storage occurred in the ratio of 5:5 and 4:6, respectively, for a combination of fermented milk and tomato paste (Table III, Figure 4).

Table I. Absorbance of Sample Extract (first day)

No.	Sample	Fn1=663nm	Fn2=645nm	Fn3=505nm	Fn4=453nm
1	BLANK	-7.2E-04	-0.00122	-0.00110	-0.00108
2	Y:TP = 9:1	0.06888	0.06761	0.17310	0.15422
3	Y:TP = 8:2	0.08623	0.08516	0.27588	0.24934
4	Y:TP = 7:3	0.12714	0.12656	0.39714	0.35194
5	Y:TP = 6:4	0.16049	0.16457	0.53644	0.50517
6	Y:TP = 5:5	0.06808	0.06873	0.53687	0.46980
7	Y:TP = 4:6	0.09492	0.09697	0.56096	0.50552
8	Y:TP = 3:7	0.15346	0.15494	0.81306	0.71440
9	Y:TP = 2:8	0.15221	0.15500	1.1161	0.98357
10	Y:TP = 1:9	0.11249	0.11507	0.80241	0.76291
11	Tomato juice	-0.02861	-0.03575	0.54292	0.47690
12	Tomato paste	0.00862	0.00345	0.44748	0.48274

Y: TP was Yogurt: tomato paste

It was found that the highest lycopene content was found in probiotic fermented milk-tomato pasta with a composition of 2:8. The second highest content was found in the composition of 3:7. When converted, with the same number of samples, lycopene in

probiotic fermented milk-tomato pasta with a ratio of 3:7 and 2:8 is higher than both tomato paste and tomato juice. It was estimated that there was interaction between yogurt and tomato paste; which causes lycopene levels to increase. The LAB might be contributed for

the interaction, although the action mechanism of the interaction could not be understood. The effect of acid (CO₂) on the presence of lycopene has been reported by Helyes *et al.* (2011). The higher the CO₂ concentration the lower the level of lycopene. In this research, the longer storage time, the higher the number of LAB cells, the more acidic the pH, might

increase the influence on lycopene content. Combination of LAB fermented milk - tomato paste 3:7 and 2:8 was the optimal composition, resulting in higher lycopene. Further investigation needs to be done to examine the mechanism of the LAB to increase the lycopene levels.

Table II. Absorbance of Sample (after two weeks)

No.	Sample	Fn1=663nm	Fn2=645nm	Fn3=505nm	Fn4=453nm
1	BLANK	7.2E-04	4.3E-04	9.2E-04	8.5E-04
2	Y:TP = 9:1	-0.01102	-0.01251	0.02681	0.02177
3	Y:TP = 8:2	0.06377	0.06558	0.38768	0.36409
4	Y:TP = 7:3	0.11874	0.12413	0.41507	0.40907
5	Y:TP = 6:4	0.02751	0.02632	0.42239	0.36458
6	Y:TP = 5:5	-0.01244	-0.01270	0.33188	0.28586
7	Y:TP = 4:6	0.01268	0.00880	0.18983	0.16942
8	Y:TP = 3:7	0.05418	0.05327	0.45279	0.41934
9	Y:TP = 2:8	0.10034	0.09859	0.63196	0.56865
10	Y:TP = 1:9	-0.04057	-0.04414	0.31941	0.27258

Y:TP was Yogurt: tomato paste

The same results were shown in the observation of LAB fermented milk-tomato paste after storage for 14 days. However, there was a decrease of lycopene content in several compositions after storage. Acidity (pH 4-5.5) and concentration of the LAB fermented milk were critical factors that affected the lycopene concentration in the tomato-paste. At a ratio of LAB fermented milk and tomato pasta 9:1, the lycopene level decreased dramatically after 14 days storage. Alda *et al.* (2009) reported that lycopene content of tomatoes remained high during the multistep processing operations for the production of juice and paste. Kaur *et al.*

(2016) found that the fermented tomato juice could be used as a raw material for LAB fermentation. Stability of lycopene against degradation is affected by long heating time or at temperature above 50°C (Lambelet *et al.*, 2009). The lycopene will undergo isomerization under several conditions, such as heat, acids and light (Xianquan *et al.*, 2005). Degradation proceeds faster than isomerization at temperature above 50°C. This phenomenon is very interesting for future investigation to obtain an ideal formula with high lycopene content and antioxidant activities.

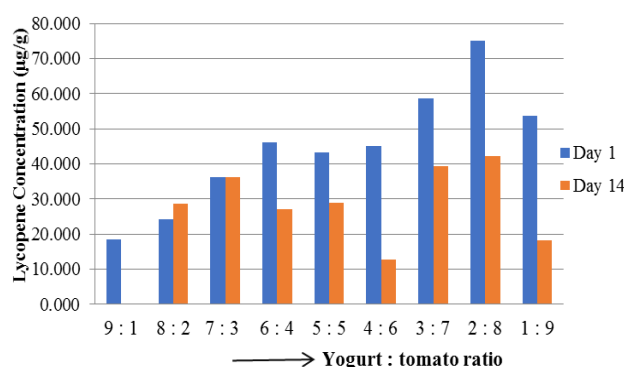


Figure 4. Lycopene content in yogurt-tomato paste

Lycopene content in tomato paste was higher than lycopene content in tomato fruit (Alda *et al.*, 2009), but in this study, lycopene content in tomato juice was higher than tomato

paste. Lycopene is known to exist in a variety of isomeric forms, including the all-trans, mono-cis, and poly-cis forms. The all-trans isomer is the predominant geometrical isomer

in fresh tomatoes. All-trans-isomer lycopene (C₄₀H₅₆) is an acyclic, open chain polyene hydrocarbon with 13 double bonds, of which 11 are conjugated in a linear array, but 7

bonds can isomerize from the trans-form to the mono or poly-cis form under the influence of heat, light, and certain chemical reactions (Su *et al.* 2002).

Table III. Lycopene Content in Yogurt-Tomato

Ratio of Yogurt-Tomato paste	Lycopene content	
	Day-1 (µg/g)	Day-14 (µg/g)
9:1	1.8491	0.1383
8:2	2.4232	2.8751
7:3	3.6174	3.6213
6:4	4.6011	2.7003
5:5	4.3300	2.8937
4:6	4.5090	1.2614
3:7	5.8652	3.9372
2:8	7.4993	4.2116
1:9	5.3730	1.8299

Fresh tomato = 3.7568 µg/g; Tomato paste = 2.8194 µg/g

Takeoka *et al.* (2001) studied processing effects of lycopene content in tomato and found that sample of raw tomatoes and tomato juice after adding hot scalding water and final paste indicated that the lycopene losses (9-28%) during processing into final paste. Isomerization and oxidation would degrade lycopene; by which its bioactivity is reduced. The lycopene oxidation and isomerization of all-trans form to cis form are induced by heat and light. Su *et al.*, (2002) have investigated the effects of thermal treatment and light irradiation on the stability of the lycopene. It has been found that lycopene stability depends on the extent of oxidation and isomerization. Cis-isomers are less stable than trans-isomers, conformed to studies results reported by Gupta *et al.* (2010). The major effect of thermal treatment and light irradiation was a significant decrease in the total lycopene content (Su *et al.* 2002). Honda *et al.* (2017) reported that Z-isomers of lycopene increased by thermal treatment at 120°C and 150°C for 1 hour to 10.0% and 56.2%, respectively.

This research was a preliminary study to obtain the tomato- yogurt paste composition with the most maximal lycopene content. Further investigations are needed to determine the probiotic fermented milk-tomato formula with the most optimal antioxidant activity with lycopene and other component content of the formula. Wang *et al.* (2017) reviewed

antioxidant activities showing some LAB, such as *Bifidobacterium animalis*, *Lactobacillus plantarum*, and *Lactobacillus rhamnosus*, although the mechanisms have not been completely understood. It was also found that there was a decrease in the amount of lycopene in the probiotic fermented milk-tomato paste after storage for two weeks, therefore in the next study it is necessary to investigate the effect of storage time on the lycopene levels and antioxidant activity of probiotic fermented milk-tomatoes products.

CONCLUSION

The highest content of lycopene was found in probiotic or LAB fermented milk-tomato paste 2:8. The lycopene content was higher than lycopene in tomato paste and tomato juice without addition of the probiotic fermented milk.

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