Research Article

Enzymatic Assisted Treatments of Lycopene Extraction from Tomato (*Lycopersicon Esculentum*) Peels using Rice Bran Oil

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Abstract
Although having the potential to prevent oxidation and cardiovascular diseases due to the substantial amount of lycopene it contains, the massive quantity of tomato peels is now solely discarded as a by-product of the processing industry. In the present study, the effects of enzymatic treatments on lycopene content extracted from tomato peels using rice bran oil were evaluated. A two-step protocol was followed: the tomato peels were treated with enzyme and then extracted with rice bran oil for 1 h at 25 °C and a solid percentage of 3.5%. Treatment factors investigated were Viscozyme L. concentrations (0.5–2.5 %), incubation time (30–150 min) and incubation temperatures (30–70 °C). Antioxidant capacity, peroxide value, acid value and color changes of the rich-pigmented oil product were analyzed. Under the best extraction conditions (Enzyme concentration = 2%; Incubation time = 90 min; Incubation temperature = 50 °C), the lycopene content was extracted up to 320 mg/100 g of dry weight. Results showed that using Viscozyme L. significantly ($p \leq 0.05$) increased the lycopene content in the pigmented oil product. These results suggested the idea of using a cell-wall degrading enzyme in the extraction to promote the use of tomato by-products as a rich source of lycopene and a good approach for waste utilization.

Keywords: Enzyme, Incubation temperature, Incubation time, Rich-pigmented oil product, Viscozyme L.

1 Introduction

Lycopene, a well-known carotenoid, is also one of the most dominant carotenoids in a person's diet. This substance naturally occurs in many fruits and vegetables such as watermelon, grapefruit, and guava and it is responsible for their deep red color [1]. The lycopene concentration varies significantly among these sources.

This phytochemical is an acyclic isomer of beta-carotene. It is a common bright red carotenoid that especially has no vitamin A activity but has protective effects against chronic and non-communicable diseases including neurodegenerative disorders, obesity, type-2 diabetes, some cancers, and cardiovascular diseases [2]. Lycopene is made of long unsaturated straight chain hydrocarbons with 13 double bonds, including 11 conjugated and 2 unconjugated double bonds and a molecular formula of $C_{40}H_{56}$ with a molecular weight of 536.89 g/mol. Several conjugated bonds make the compounds highly sensitive to degradation and isomerization. More clearly, lycopene is susceptible to light, oxygen, high temperatures, acids, catalysts and metal ions [3]. Lycopene has a distinct lipophilic character, which makes it insoluble in ethanol, methanol and water but soluble in nonpolar solvents such as carbon disulfide, ethyl ether, petroleum ether, chloroform, benzene and fats [4]. The beneficial effects of lycopene are thought to be due to its strong antioxidant activity, which can deactivate free radicals and reduce damage to the body's cells [5]. There are many studies showing strong correlations between lycopene consumption to a minor risk of growing chronic diseases comprising cancers (e.g., lung, prostate,

and stomach cancers) and cardiovascular diseases [6]. Furthermore, lycopene is used as a natural and functional ingredient in foods and beverages because of its strong color and non-toxicity [5], [7]. Lycopene can also be found in a concentrated and isolated form in several dietary supplements with different concentrations. Due to its remarkable health benefits and wide range of applications, high-purity lycopene is in high demand by many industries such as the food, pharmaceutical, nutraceutical, cosmetic and animal feeding industries.

The tomato (*Lycopersicon esculentum*) is considered as a rich source of dietary antioxidants including carotenoids, especially α-carotene, β-carotene, lycopene and lutein [8]. Among these, lycopene is predominant and exhibits the highest antioxidant activity. Especially, the lycopene concentration increases significantly during the ripening of tomatoes [9]. Among the red ripened tomatoes, watermelons, guavas, papayas, and grapefruits, which are the main dietary sources of lycopene. The tomato is an important crop, which is widely processed and consumed throughout the world, either as a raw fruit or as a processed product. It is predicted to be about 39 million metric tons of tomatoes in 2020. In Vietnam, tomatoes are grown and consumed throughout the country, which accounts for 7–10% of the country's vegetable area and 3–4% of the total amount of vegetables. It is considered the most economically important vegetable crop in Vietnam. The main vegetable production area is the Red River Delta which is a subtropical plain region in northern Vietnam [10].

Nowadays, a vast amount of tomato is processed into products including ketchup and sauce, resulting in large amounts of by-products, such as lycopene-rich peel and seeds that are discarded [11]. During post-harvest processing, the disposal of large amounts of tomato pomace generated manually has become a major problem for the tomato industry for years as the tomato pomace is highly perishable because it contains up to 95% moisture content [12]. It is discarded as solid waste or used for animal feeding. Meanwhile, it was documented that the lycopene content accumulated in the skin layer is five times higher than that in the pulp. It revealed that the tomato peel contains the highest amount of lycopene (417.97 μg/g) followed by industry waste (195.74 μg/g), whole tomatoes (83.85 μg/g) and pulp (47.6 μg/g) [13]. Similarly, Cuccolini *et al.* [14] stated that lycopene is found predominantly in the chromoplasts of the tomato peel tissues, which are explained by the transformation of chloroplast into chromoplast during the ripening stage [14]. Thus, a large quantity of lycopene is lost as waste in tomato processing. It is said that a significant amount of lycopene present in the skin and the water-insoluble fraction of tomato peels is nearly 72–98%, which could be used as a substantial cheap source of lycopene [13]. Therefore, tomato peel is particularly considered a desirable source of lycopene [15]. Utilization of the tomato waste could be a viable solution to the disposal waste problem and the environmental issues around the world. It can be an economic opportunity in the tomato industry and meet the lycopene requirement for various sectors including food, cosmetic, pharmaceutical and other industries [16].

The development of suitable techniques or ideal processing condition to stabilize lycopene in tomato products are an important issue for process optimization. It is worthy of note that the use of assisting technologies such as enzyme treatment, ultrasound waves, microwaves, high pressure extraction treatments can improve the efficiency of solvent extraction from biological materials. However, it is also important to be concerned about the degradation of lycopene. Applying enzymes in the extraction of lycopene can be a simple and effective way for enhancing the lycopene recovery from plant cell tissues and allowing the reaction under the mild operational conditions of pH, temperature and pressure in order to decrease the degradation and isomerization of lycopene [13], [14], [17]–[20]. It helps to degrade the cell wall as well as the polysaccharide network surrounding the cell, leading to the release of intracellular contents by extraction. Pretreatment of plant cells with enzymes also reduces the extraction time by producing the disruption of the cell wall and allowing better penetration of solvent, enhancing the solubility of oil solvent and lycopene in the tomato matrix in the extraction step. It also improves the release of other compounds in the plant cell such as phenolic compounds and aroma compounds [13], [21]. Enzymes like cellulase and pectinase have been employed commonly in several extractions of carotenoids from plant tissues [8], [13], [20]. Using an enzyme system such as the combination

of flavorzyme and alcalase assists in the digestion and extraction of amino acids and peptides in Lemna minor [22]. In addition, the utilization of pectinase in the extraction of essential oil from gac aril improved not only the yield of oil recovered but also the quality of the oil by enhancing lycopene and β-carotene content [23].

To the best of our knowledge, no study has evaluated the effect of Viscozyme L. in lycopene extraction using rice bran oil has been published. Viscozyme L. belongs to the cellulase enzyme class, it includes arabanase, cellulase, β–glucanase, hemicellulase and xylanase with optimal reaction conditions between pH 3.5–5.5 and 25–55 °C. Applying Viscozyme L. in lycopene extraction could be an efficient way in an enzyme treatment as the complex tomato cell wall consists of a variety of polysaccharides (cellulose, pectin, and hemicellulose) [14]. It is said that Viscozyme L. can effectively degrade cellulose/hemicelluloses and pectin components in tomato waste [24]. Furthermore, Viscozyme L. also is used in enzyme-assisted extraction from several plants such as apple pomace, gac, red capsicum, etc. [25]–[27]. The hydrolysis of plant tissues by enzymes varies significantly by differences in enzyme concentration, temperature reaction and incubation time [8], [28].

There has been a growing interest in seeking a suitable solvent for the extraction of lycopene from industrial tomato waste due to the difficult diffusion of the solvent to the highly structured tomato peel tissue containing polysaccharides such as cellulose, hemicelluloses, and pectin [29]. Thus, the type of extraction solvent and the polarity of which play a vital role in the extraction of lycopene [8]. Several organic solvents have been applied efficiently in the extraction of lycopene such as acetone, hexane, ethanol, ethyl acetate or a mixture of them. However, organic solvents can pose a threat to human health and the environment because of the large quantity of waste generated by poisonous and toxic solvents [30]. For this reason, it needs to search for a greener solvent for lycopene extraction. As lycopene is a hydrophobic pigment, which dissolves better in non-polar solvents and fats, there are several studies linking the extraction of lycopene by using vegetable oils [11], [31], [32]. The positive effects of oil containing lycopene are concluded in lycopene recovery, preservation against lipid oxidation and increased nutritional and health benefits [31]–[33]. Oil and hydrophobic fats are described as having a lycopene coating which prevents oxygen from reacting with them, leading to their possible oxidation and degradation reactions [5]. According to the research of Kehili *et al.* [31], it was stated that the use of refined olive oil in lycopene extraction from tomato peels has a positive effect on the stabilization of lycopene during long storage periods. The use of pigmented oil was found to be a colorant in fish sausage and the addition of carotenoids to the oil enhanced the sensory color, flavor, and overall quality score of the sausage [34]. In aquaculture feed provisions, pigmented oil could be typically employed as an energy source [35]. As a consequence, vegetable oils can be alternatives to organic solvents, meaning that there is no requirement for subsequent oil separation and lycopene.

Rice bran oil (RBO) is one of the most nutritious edible oils due to its balanced fatty acid profile [36]. Rice bran contains 15–20% lipids, 12–16% protein, 7–11% crude fiber, 34–52% carbohydrate and 7–10% ash [37]. It is also unique among edible oils because it is abundant in phytoceuticals such as oryzanol, lecithin, tocopherols, and tocotrienols [38]. India, China, Thailand and Vietnam are among the major producers of RBO. In 2008, the International Association of Rice Bran Oil (IARBO) was hold by Vietnam to share technologies and research as well as rice bran's benefits to users. Furthermore, Vietnam is predicted to have the potential to develop this healthy oil as Vietnam is the world's leading rice producer and exporter. However, the distribution of rice bran oil is still limited among local consumers in Vietnam and the large majority of rice bran oil is extracted specifically for export. For these reasons, it is necessary to leverage rice bran production to push for the manufacturing and consumption of rice bran oil. The use of rice bran oil in the extraction of lycopene is not only an opportunity to solve the tomato waste problem but also to make a lipophilic natural colorant and bioactive ingredient. Nonetheless, studies on tomato peels were scarce in this aspect. According to an investigation of the thermal stability of lycopene in vegetable oils, lycopene tended to be degraded more slowly in rice bran oil than in grape seed oil and sunflower seed oil [39]. The antioxidant capacity of the oil sample is determined using the DPPH method due to its cost-effectiveness,

convenience of conducting tests, consistency, and usefulness at room temperature. This assay has been successfully applied in several studies of the antioxidant activity of antioxidant substances from plant sources such as Vitamin D3 in canola oil [40], hydrolyzed proteins [22], and natural antioxidants in germinated Sangyod rice [41].

Because of those reasons, this study was conducted to determine the suitable enzymatic process for the extraction of lycopene from tomato peels using rice bran oil as a solvent. A fat-soluble substance containing lycopene can be used as a natural colorant or a functional ingredient in foods. The objectives of this research were to examine the impact of three extraction parameters including enzyme concentrations, incubation time and incubation temperature, thereby obtaining the highest carotenoid content in tomato peels.

2 Materials and Methods

2.1 *Research location*

The research experimental work was conducted at the laboratories of the Food technology department International University–Vietnam National University in Ho Chi Minh City.

2.2 *Experimental design*

2.2.1 Sample preparation

Forty kg of fully-ripe tomatoes used in this study were collected from Da Lat, Lam Dong province. Tomatoes were washed with water, removed the damaged parts and then measured the color based on the six stages from the tomato classifications of USDA [42]. Selected tomatoes were steamed for three minutes and hand peeled to separate the skin and the tomato pulp [43]. The peels were coarsely ground by a house grinder and were stored in plastic bags at –20 °C for further use. The moisture content was recorded for further discussion. Refined rice bran oil (Simply) was bought at local supermarkets.

2.2.2 Effects of enzyme concentration

Enzyme treatment was conducted based on the procedure suggested by Ranveer *et al.* [13] with some modifications. This study uses Viscozyme L. with enzyme activity 100 (FBG/g), density 1.21 (g/mL), optimum temperature (40–50 °C), and pH (3.3–4.5) from Novozymes Co., Denmark. In detail, 5 g of fresh tomato peels were added to Viscozyme L. at different concentrations: 0.5; 1; 1.5; 2; 2.5 (%, v/w). The reaction mixture was shaken using a shaking incubator (KS 4000 ICC, IKA, Germany) at 40 °C, 300 rpm, for 150 min. After that, the mixture was mixed with 143 mL of rice bran oil (v/w) and shaken at room temperature, for 1 h on a magnetic stirrer for lycopene extraction. The volume of oil was determined by screening studies with tomato percentage was 3.5% (w/v) according to the lycopene extraction study using refined olive oil [31]. The mixture was then centrifuged at 7000 rpm for 15 min at 4 °C using a refrigerated centrifuge (Z326 K, Hermle Labortechnik GmbH, Wehingen, Germany). The supernatant was collected, which was separated from the water content and prepared for further analysis. The enzyme concentration giving the highest lycopene content was applied for further experiments. The experiment was carried out in triplicate.

2.2.3 Effects of enzymatic incubation time

The suitable enzyme concentration chosen from experiment 1 was added to 5 g of fresh tomato peels. The mixture was shaken at 40 °C, 300 rpm for 5 intervals (30, 60, 90, 120, and 150 min) to access the effect of incubation time. Then the oleoresin was mixed with rice bran oil (143 mL) to obtain the fat-soluble pigment. The mixture was shaken on a magnetic stirrer at room temperature, for 1 h, under dim light conditions. The supernatant was collected after centrifuging at 7000 rpm for 15 min at 4 °C and used for measurements of the total lycopene content, antioxidant capacity, peroxide value, acid value and color values. The low water content also was mostly removed after centrifuging. The results were compared with the untreated one. The experiment was done in triplicate.

2.2.4 Effects of enzymatic incubation temperature

The enzyme concentration and incubation time chosen from the previous experiments were introduced into

this experiment. Briefly, add 2% (v/w) concentration of Viscozyme L. to 5 g of fresh tomato peels before incubating for 90 minutes using a shaking incubator (KS 4000 ICC, IKA, Germany) at 300 rpm. The different incubation temperatures employed in this experiment were 30, 40, 50, 60 and 70 °C. After incubating, the tomato mixture was mixed evenly with 143 mL of rice bran oil and centrifuged at 7000 rpm for 15 min at 4 °C. The supernatant was collected for further analysis. The experiment was designed in triplicate.

2.3 *Data analysis*

2.3.1 Determination of total lycopene content

Total lycopene content in the oil extracts was quantified using spectrophotometric analysis at 503 nm in a 1 cm path length cuvette. More clearly, 0.5 mL of the pigment-rich oil was dissolved with 9.5 mL of ethyl acetate before measuring the absorbance. The absorbance of the pigmented oil was calculated by subtracting the absorbance of the oil only. Lycopene standard was used to construct a standard curve. The results were expressed as mg of lycopene/100 g dry weight (DW) and µg of lycopene/mL oil.

2.3.2 Determination of antioxidant capacity

DPPH assay was modified from a method of Brand-Williams *et al.* [44]. 100 µL of the pigmented oil diluted in ethyl acetate was mixed with 3.9 mL of a 6×10^{-5} mol/L of DPPH-ethyl acetate solution. The mixture was vortexed well and kept in darkness for 30 min. The absorbance of the mixture was spectrophotometrically detected at 515 nm. The analysis was performed in triplicate. The standard curve was conducted by Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). The results achieved were displayed as µmol Trolox equivalent (TE)/mL oil.

2.3.3 Determination of peroxide value

The peroxide value of the pigmented oil was determined according to the procedure of Nielsen, [45]. The procedure was performed with 5 g of oil sample dissolved in 30 mL acid-chloroform solution in a 250 mL glass Erlenmeyer flask. Then, 0.5 mL of saturated KI solution was added and stood for 1 min before adding 30 mL of distilled water. Next, the mixture was titrated slowly with 0.005N sodium thiosulfate solution with vigorous shaking until the yellow color was gone. 0.5 mL of 1% starch solution was mixed and continued to titrate until the blue color disappeared. Record the volume of titrant used. A blank sample (omitting the oil) was titrated and recorded the volume of titrant used. The peroxide value was calculated by the following formula. The results obtained were displayed as mEq peroxide per kg of pigmented oil.

2.3.4 Determination of acid value

The acid value of pigment-containing rice bran oil was calculated by weighing the appropriate amount of the melted oil sample and adding 50 mL of neutralized hot ethyl alcohol and 1 mL of phenolphthalein indicator. Then the mixture was heated for around 15 min in a waterbath (75 °C). After that, the mixture was added 1 mL of phenolphthalein indicator and titrated with standard base (0.1 N NaOH) until the endpoint was reached when the light pink appears. Consequently, the acid value is calculated by the following formula [Equation (1)]:

$$Acid\ value = 56.1 \times V \times 100/W \quad (1)$$

Where W is the weight of oil that equals 5 g, V is the titer value of 0.1 N NaOH.

2.3.5 Color measurement

The color of the pigmented oil was measured using a portable colorimeter (Lutron RGB-1002). The R, G, B index was converted to L*, a*, b* for analysis.

2.3.6 Statistical analysis

Data were subjected to analysis of variance using the Minitab software (version 16, Minitab Inc., State College, PA, USA). One-way ANOVA was applied to determine the statistically significant differences in the lycopene content, antioxidant capacity, peroxide value, acid value and color of the pigmented oil between different extraction conditions. The significant

difference comparisons were executed by Fisher's test ($p \leq 0.05$). Results were presented as means ± standard deviations of triplicate experiments with a level of confidence of 95%.

3 Results and Discussions

3.1 *Effects of enzyme concentration*

From many previous studies, the enzyme pretreatment of tomato peels before extraction has been shown to be an efficient way to improve lycopene recovery [8], [17], [46]. Furthermore, from the environmental perspective, the enzyme pretreatment is opposed to be an eco-friendly manner to obtain a high lycopene yield under the mild extraction conditions of the tomato waste. Viscozyme L. is one of the common enzymes used in the food industry and is a multi-active enzyme with the capacity of hydrolyzing the polysaccharides in plant cells and leaving the linkages within the polysaccharide matrix to liberate the inner component of tomato cells. It can be responsible to conduct under a wide range of pH between 3 to 7 and the optimal pH is 3.3 to 5.5. Besides, Viscozyme L. has been applied in many studies to increase the efficiency of the extraction of carotenoids in general and of lycopene in particular [27], [28]. Nath *et al.* 2016 [28] stated that the application of Viscozyme L. was presented to be very effective in the extraction of total carotenoids and other bioactive compounds. To ensure the ideal conditions for Viscozyme L. to assist at its optimum in this investigation, the tomato peel sample had a moisture content of 86.78 0.9 wt% and a pH maintained in the range of 3.45–3.55 (Table 1).

Table 1: Moisture content and pH of tomato peels

Parameter	Tomato Peels
Moisture (%)	86.78 ± 0.9
pH	3.45–3.55

Table 2 showed that all of the enzymatic pretreatments with different enzyme concentrations increased the lycopene yield significantly in comparison to the untreated one ($p \leq 0.05$), from 83.07 ± 5.68 mg/100 g DW to 253.75 ± 5.56 mg/100 g DW. The control test showed the lowest lycopene yield of 83.07 ± 5.68 mg/100 g DW. When the enzyme concentration was increased, more lycopene was released. An increase in the lycopene extraction yield by 3 times after enzyme treatments was reported [17], [47]. This phenomenon could be explained by the presence of enzymes in the extraction mixture that enabled the easy extraction of bioactive compounds trapped in plant cells owing to the enzymatic hydrolysis. Tomato cell walls contain some major components such as pectin, cellulose, and hemicelluloses, which can be degraded by the Viscozyme L. that was possible to decompose these structures due to its pectinase, cellulase and hemicellulose activities. Thus, it could be easy for the solvent to penetrate the tomato tissue and achieve an improved extraction of lycopene in the tomato cell. In other studies, it was found that there was an increase of 1.5 to 20 times the initial lycopene yields from tomato waste as a result of enzyme pretreatment at different concentrations.

Table 2: The effects of enzyme concentrations on the lycopene content in the pigmented oil

Sample	Lycopene Content (µg /mL oil)	Lycopene Content (mg /100 g DW)
Control	3.84 ± 0.26^{e}	83.07 ± 5.68^{f}
0.5 %	6.21 ± 0.22^{d}	134.27 ± 4.70^{e}
1 %	8.42 ± 0.25^{c}	182.06 ± 5.49^{d}
1.5 %	9.95 ± 0.22^{b}	215.34 ± 4.70^{c}
2 %	11.73 ± 0.26^{a}	253.75 ± 5.56^{a}
2.5 %	10.61 ± 0.45^{ab}	229.57 ± 9.84^{b}

* The values are means ± standard deviations ($n = 3$). Mean values sharing different letters are significantly different ($p \leq 0.05$).

In this study, the highest lycopene content was obtained by the addition of enzyme at 2%, reaching 253.75 ± 5.56 mg/100 g DW followed by the addition of 2.5% and 1.5%, which reached 229.57 ± 9.84 and 215.34 ± 4.70 (mg/100g DW), respectively. The obtained results were higher than those of previous findings. According to previous views of enzymatic treatment of lycopene extraction using Viscozyme L., Nam [48] said that the highest lycopene was obtained with an enzyme concentration of 1%. These differences should be taken into account with respect to raw materials and the activity of enzymes and the successive solvent extraction after the enzymatic treatment. Many studies had demonstrated that lycopene content varied within the variety of tomatoes, agricultural practices, soil, climate factors, fruit growth, harvesting date, genotype, degree of maturity and post-harvest handling

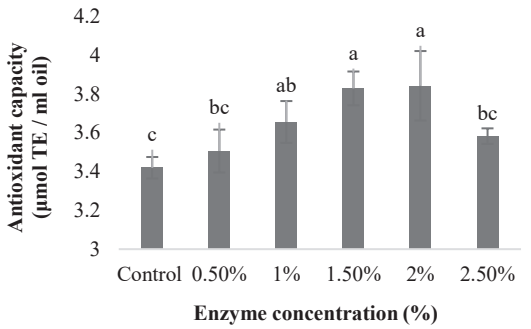

Figure 1: The effects of enzyme concentration on the antioxidant capacity in the pigmented oil. The values are means ± standard deviations ($n = 3$). Mean values sharing different letters are significantly different ($p \leq 0.05$).

[49], [50]. The increase in extraction yield was observed with increases in enzyme concentrations up to 2% but was not observed with the further increase in enzyme concentration to 2.5%. This could be due to the enzyme concentration reaching the point that the enzyme could interact with and degrade the lycopene released. Secondly, the enzyme might reach the saturation point at which no longer enzyme concentration could have an impact on lycopene content. The obtained results proved the evidence that the lycopene extraction should be conducted with 2% Viscozyme L. to obtain the highest lycopene content.

Figure 1 shows the antioxidant capacity increased significantly ($p \leq 0.05$) when the enzyme concentration increased from 0.5% to 2%. The highest value 3.84 ± 0.1795 (μmol TE/mL oil) obtained at an enzyme concentration of 2% and this value was not significantly different from the one collected at 1.5% (3.83 ± 0.09 μmol TE/mL oil). The phenomenon could be clarified by saying that the high content of many components in rice bran oil, such as vitamin E and the presence of mainly tocotrienols or the synergistic effect of tocopherols and tocotrienols [51], [52] also considered to affect the antioxidant activity of the pigmented oil. Moreover, a considerable decrease was witnessed in the antioxidant capacity of the following sample with an enzyme concentration of 2.5%. This could be a result of the decreasing lycopene content of the sample treated at 2.5% enzyme concentration, leading to a slight decrease in antioxidant capacity. Because of a high physical quenching rate of singlet oxygen, which is directly related to the antioxidant capacity, lycopene could stop lipid oxidation at an initial stage. This ability is twice as high as β-carotene and 100 times higher than α-tocopherol [53], hence the presence of these two substances in the oleoresin was insufficient to compensate for lycopene shortage. The control one had an antioxidant activity of 3.42 ± 0.01 (μmol TE/mL oil). Thus, it could be concluded that lycopene was capable of generating the antioxidant effect in the pigmented oil. This observation supported similar findings in previous studies. Lycopene–enriched walnut oil was also investigated an antioxidant capacity in a study by Xie *et al.* [54], which demonstrated that the 0.005% lycopene added to walnut oil exhibited the greatest antioxidant effect, extending the shelf life of the oil.

Table 1: Color changes of the pigmented oil with different enzyme concentrations

Sample	a*	b*	a*/b*
Rice bran	-1.87 ± 0.01^{d}	22.73 ± 0.02^{c}	-0.08 ± 0.01^{d}
Control	2.44 ± 0.41^{c}	24.98 ± 0.31^{c}	-0.11 ± 0.01^{c}
0.5%	16.68 ± 0.80^{b}	42.45 ± 0.55^{a}	0.39 ± 0.02^{b}
1%	16.57 ± 1.30^{ab}	43.19 ± 0.50^{a}	0.38 ± 0.03^{b}
1.5%	16.41 ± 0.65^{b}	43.92 ± 0.25^{a}	0.37 ± 0.01^{b}
2%	17.95 ± 0.52^{ab}	40.31 ± 0.71^{b}	0.44 ± 0.01^{a}
2.5%	18.63 ± 0.13^{a}	43.63 ± 0.63^{a}	0.43 ± 0.01^{a}

*The values are means ± standard deviations ($n = 3$). Mean values sharing different letters are significantly different ($p \leq 0.05$).

The grading of color in the oil sample was also vital in the oil sample and color was also one of the primary attributes used to sort the colorant and towards the customers about food products. Color change was quantified using the CIE (L*, a*, b*) system. In this coordinate system, the a* value represents the red/green axis and varies from −100 (greenness) to +100 (redness) and the b* value represents the yellow/blue axis and ranges from −100 (blueness) to +100 (yellowness). The values of a* and b* were used to determine the color index because the color index a*/b* was used to illustrate the redness of samples [8]. Table 3 showed the a*/b* values of both the initial rice bran oil, the pigmented oil with enzymatic treatment and the oil containing tomato peels treated without enzyme (the control one). The ratio of a* to b* significantly differed in the rice bran oil with treated samples and the non–treated ones as the ratio a*/b* increased dramatically, nearly 6 times indicating

the color of the oil was shifting to red. It was well reported that the application of lycopene content could change the color of the sample due to the red color of lycopene. Therefore, enriching lycopene could be an efficient way to improve the functionality, as well as color quality of food products. Furthermore, the highest color index was observed in the sample with 2% Viscozyme concentration, which possibly related to the highest lycopene content in this sample and that also was insignificant different with the pigmented oil with 2.5% enzyme. It might be because of the interfere of initial yellow color of rice bran oil. Except for the only rice bran oil sample, the control was obtained as the lowest color change compared to the treated ones. This could be explained by the higher lycopene content of these samples.

Peroxide value (PV) is widely used as a measure of the initial oxidation level causing rancidity in foodstuffs. It is also necessary to determine the acid value (AV) of the oil sample, which expresses the amount of free fatty acids in oil as the amount of KOH (in mg) to neutralize 1g of oil. These parameters are the main factors, which indicate the quality and stability of carotenoid-enriched oil.

In this research, the impact of enzyme concentration on peroxide value and acid value was significant ($p \leq 0.05$) [Figures 2(a) and (b)]. It was clear that the peroxide value of the treated sample was higher than the untreated one. It could be a result of enzymatic treatment during a long period of exposure to oxygen, elevated temperatures and peroxide compounds [55]. However, it showed the highest peroxide value in samples at 2.5% (1.21 ± 0.07 meq O_2/kg oil), followed by that of 2% (1.04 ± 0.07 meq O_2/kg oil) and no significant difference among samples treated with 0.5, 1, and 1.5% enzyme. The impossible cause of this effect was that the pro-oxidant effects of lycopene released from the sample with a high concentration of lycopene could promote the oxidation of vegetable oils by liberating radicals from fatty acids or hydroperoxides in certain conditions. Therefore, the pro-oxidation effect of lycopene in the extraction reaction should be considered. During the period of enzymatic treatment, the oxidation could occur by the interaction of the unsaturated fatty acids in rice bran oil with oxygen [56]–[58]. The enzyme concentration led to small differences between the other treated samples.

According to the data shown in Figure 2(b), the

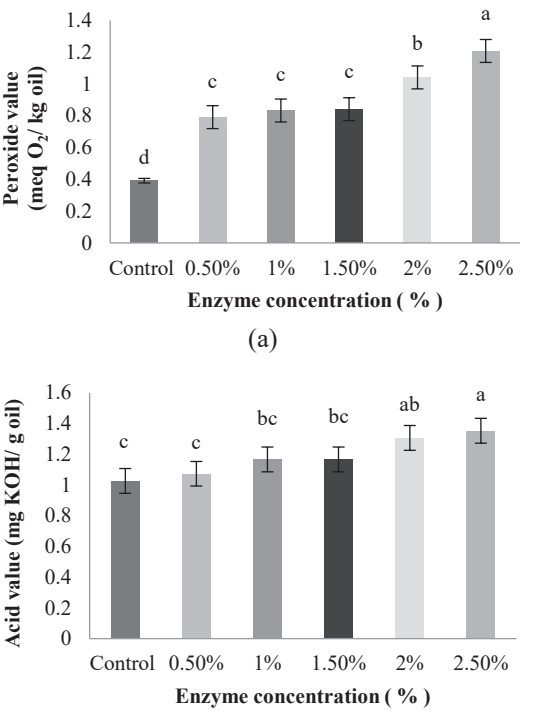

Figure 2: The effects of enzyme concentration on the peroxide value (a) and acid value (b) of the enriched-lycopene oil sample. The values are means ± standard deviations (n = 3). Mean values sharing different letters are significantly different ($p \leq 0.05$).

acid values of the oil samples at different enzyme concentrations were statistically different ($p \leq 0.05$) and the sample treated at 2.5% had the highest acid value (1.35 ± 0.08 mg KOH/g oil), followed by the sample with 2% enzyme concentration (1.31 ± 0.08 mg KOH/g oil). The control one and the sample treated with 0.5% both obtained low values of acid. This observation supported the reported studies. When studying the effects of enzyme concentrations on the acid value of oil extracted from wild apricot kernels, Bisht *et al.* [60] showed that the acid value of the wild apricot oil increased with increasing in enzyme concentrations. This phenomenon was due to the presence of enzymes and moisture content in the pre-treatment which triggered the rancidity in the pigmented oil. The acid values of samples with 1% and 1.5% Viscozyme L. concentrations were similar and slightly lower than those with 2%. However, the acid values also fluctuated

although the enzyme concentration increased. The PV and AV of samples with lycopene increased slower than those of samples without lycopene, therefore lycopene could be considered a positive barrier against the rancidity of the sample.

However, the peroxide and acidity values of all samples in the present study were lower than the TCVN specification according to which the edible oil qualifying as good quality oil should have a maximum AV of 4 mg KOH/g oil and a maximum PV of 15 meq O2/kg oil with the PV (1.21 ± 0.07 meq O_2/kg oil) and the AV (1.35 ± 0.08 mg KOH/g oil).

3.2 *Effects of enzymatic incubation time*

In the enzymatic treatment, incubation time also plays a crucial role in contributing to the carotenoid yields of the extraction. As mentioned above, the optimum temperature for Viscozyme L. activity is from 25–55 °C; However, to avoid the lycopene degradation caused by exposure to high temperatures, the experiments were conducted at 40 °C. After that, many intervals of incubation time were applied to estimate the suitable enzyme incubation duration.

Table 2: Lycopene content of the pigmented oil at different incubation times

Sample	Lycopene Content (µg/ mL oil)	Lycopene Content (mg/ 100 g DW)
Control	3.84 ± 0.26^{e}	83.07 ± 5.68^{e}
30 min	8.43 ± 0.46^{d}	182.06 ± 9.89^{d}
60 min	11.73 ± 0.57^{b}	253.75 ± 12.20^{b}
90 min	14.49 ± 0.44^{a}	313.48 ± 9.51^{a}
120 min	12.21 ± 0.89^{ab}	264.27 ± 19.20^{b}
150 min	9.74 ± 0.37^{c}	202.82 ± 8.07^{c}

*The values are means ± standard deviations ($n = 3$). Mean values sharing different letters are significantly different ($p \leq 0.05$).

From Table 4, the lycopene contents of the pigmented oil were observed to increase significantly ($p \leq 0.05$) when the incubation time increased from 30 min to 90 min. When increasing the incubation time, the enzyme was allowed to act more extensively on the plant matrix, thus enhancing the disruption of plant cells, consequently making the solvent penetration of the extraction step easier [39]. Similar results had also been obtained by other studies [8], [13], [23]. Catalkaya and Kahveci [8] also highlighted that the lycopene yield followed an increasing trend

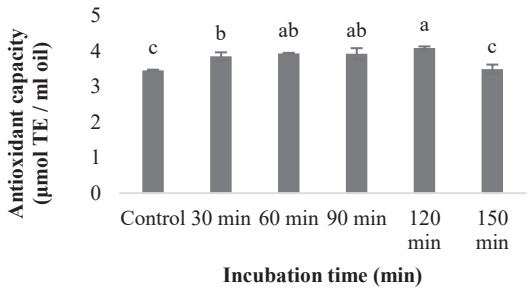

Figure 3: The effects of enzyme incubation time on antioxidant capacity. The values are means ± standard deviations ($n = 3$). Mean values sharing different letters are significantly different ($p \leq 0.05$).

up when prolonging incubation time; however, it was reduced thereafter. The greatest value of lycopene in the pigmented oil was achieved at the enzyme incubation time of 90 min, the lycopene content was 313.48 ± 9.51 (mg/100 g DW), which was 4-folds higher than the untreated one (83.07 ± 6.84 mg/100 g DW). After that, there was a progressive reduction in the lycopene content (at 120 min and 150 min). These values were 264.27 ± 19.20 (mg/100 g DW) and 202.82 ± 8.07 (mg/100 g DW), respectively.

Firstly, it was well known that lycopene was easily degraded by physical and chemical factors including heat, light, oxygen and extreme pH exposure [4]. Therefore, when the enzymatic reaction was prolonged, oxidation might occur as lycopene content was exposed to oxygen from the surroundings, which decreased the lycopene amount in the pigmented oil. Secondly, it might be due to the decrease in enzyme activity over time or the exhaustion of substrate. According to Lavecchia and Zuorro [17], they suggested that the enzyme activity would occur very fast, especially within the first hour during which lycopene trapped in the protective chromoplast structures of the tomato could release rapidly during the enzymatic incubation. Therefore, the results supported the view that enzymatic pretreatment should be conducted at the appropriate time to minimize the loss of lycopene extracted by several internal and external factors. As a result, the enzyme incubation time of 90 min was chosen from the obtained results.

Figure 3 indicated the incubation time had a significant ($p \leq 0.05$) influence on the antioxidant capacity of the pigmented oil since the incubation time

increased from 30 min to 120 min, the antioxidant capacity increased from 3.85 ± 0.11 (μmol TE/mL oil) to 4.09 ± 0.04 (μmol TE/mL oil). The increase in antioxidant capacity could be explained partly by the increase in the lycopene content of the pigmented oil. However, the sample for the incubation time of 90 min had the highest lycopene content but not the highest antioxidant capacity (3.92 ± 0.16 μmol TE/mL oil). This phenomenon could be explained by the fact that the antioxidant capacity of the samples was not only due to lycopene but also dependent on other antioxidants including phenolic compounds in the tomato peels [51], [60]. Thus, the presence of different types of antioxidant substances would also affect the antioxidant capacity of the pigmented oil. In addition, a combination of lycopene and other antioxidants was an important factor affecting the antioxidant capacity according to Shi *et al.* [61]. When examining the antioxidant properties of lycopene and other carotenoids in tomatoes, the authors indicated that a mixture of lycopene and vitamin E already presented in tomatoes and rice bran oil could obtain the highest synergistic antioxidant activity. Therefore, antioxidant capacity was also affected by the profile of many compounds in rice bran oil [62]. The antioxidant activity of the control, 30, 60, and 150 min were 3.85 ± 0.11 (μmol TE/mL oil), 3.93 ± 0.17 (μmol TE/mL oil) and 3.49 ± 0.13 (μmol TE/mL oil). It was all related to the lycopene concentration.

Table 3: Color values of a*, b* and a*/b* of the pigmented oil at different enzymatic incubation time

Sample	a*	b*	a*/b*
Control	–1.87 ± 0.01^{d}	22.73 ± 0.02^{c}	–0.11 ± 0.01^{d}
30 min	15.82 ± 0.82^{c}	44.62 ± 1.28^{ab}	0.35 ± 0.02^{c}
60 min	18.46 ± 0.50^{b}	44.64 ± 1.50^{ab}	0.41 ± 0.02^{b}
90 min	23.27 ± 0.99^{a}	46.31 ± 0.56^{a}	0.50 ± 0.02^{a}
120 min	15.96 ± 0.87^{c}	43.73 ± 1.24^{ab}	0.36 ± 0.01^{c}
150 min	18.16 ± 0.50^{b}	42.23 ± 0.26^{b}	0.43 ± 0.01^{b}

*The values are means ± standard deviations ($n = 3$). Means sharing different letters are significantly different ($p \leq 0.05$).

The color changes of rice bran oil and the pigmented oil of samples at different incubation times with Viscozyme L. were presented in Table 5. It showed that the enzyme incubation times significantly affected the color changes of pigmented oil. The increased ratio index of a*/b* illustrated the increasing redness of the lycopene-enriched oil. Compared to the non-enzyme treated sample, the color of the treated one changed to an intense orange-red color owing to the increased presence of lycopene. The redness color was obtained when the pigmented oil has treated for 90 min (0.5 ± 0.02), which the highest lycopene content was achieved. The control sample expressed the least amount of redness (–0.11 ± 0.01). Thus, it could be stated from the current work that the higher concentration of lycopene content imparted the higher red color to the oil sample. However, the redness of the sample incubated for 150 min (0.43 ± 0.01) was higher than that of the 120 min sample (–0.36 ± 0.01) although the lycopene content of the former was significantly lower than that of the latter. It might be a result of the higher peroxide value as an increase in peroxide value could bring a darker color to the oil sample.

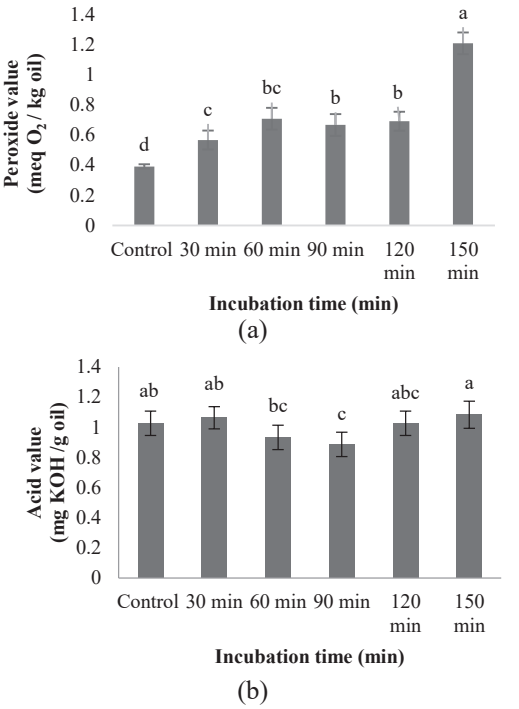

Figure 4: The effects of enzyme incubation time on the peroxide value (a) and acid value (b) of the pigmented oil. The values are means ± standard deviations ($n = 3$). Mean values sharing different letters are significantly different ($p \leq 0.05$).

Effects of the enzymatic incubation time on AV and PV values are presented in Figure 4(a) and (b), respectively. Both PV and AV of the pigmented oil increased significantly when the incubation time was

prolonged. These values of the pigmented oil incubated for 150 min were highest at 1.21 meq O_2/kg oil and 1.08 ± 0.09 mg KOH/g oil. It may be due to the impurity content of the tomato oleoresin added to the rice bran oil, leading to an increase in PV and AV values. Prolonging incubation time increased the number of contaminants in the tomato oleoresin, following a higher level of acid-forming oil oxidation. However, the PV values were unstable because the hydroperoxides of unsaturated fatty acids formed by lipid oxidation were very unstable [63]. In terms of AV, the two values of the control sample had insignificant differences as compared to those of the pigmented oil incubated for 30 min. They were 1.03 ± 0.08 (mg KOH/g oil) and 1.06 ± 0.07 (mg KOH/g oil), respectively. It was likely that the high lipases and lipoxygenases contents in the rice bran oil would make the oil more prone to oxidation during the experimental duration [64], making the non-treated sample have a higher acid value.

3.3 *Effects of incubation temperature*

Table 6 below showed the effects of incubation temperature on the yields of lycopene. The samples were treated with a 2% Viscozyme L. concentration and incubated for 90 minutes at different temperatures ranging from 30 °C to 70 °C. It can be seen that when the temperatures were increased from 30 °C to 50 °C, there was an increase in the lycopene content of the pigmented oil, from 246.35 ± 4.03 (mg/100 g DW) to 320.88 ± 8.54 (mg/100 g DW). The lowest value was obtained from the non-enzyme treated sample (83.07 ± 5.68 mg/100 g DW). At 50 °C, the experiment obtained the highest lycopene content (320.88 ± 8.54 mg/100 g DW) which supported the report studied.

Table 4: Lycopene content of the pigmented oil at varying incubation temperature

Sample	Lycopene Content (µg/mL oil)	Lycopene Content (mg/100 g DW)
Control	3.84 ± 0.26^{d}	83.07 ± 5.68e
30 °C	11.39 ± 0.19^{c}	246.35 ± 4.03^{d}
40 °C	11.59 ± 0.23^{c}	250.62 ± 4.92^{d}
50 °C	14.83 ± 0.40^{a}	320.88 ± 8.54^{a}
60 °C	13.36 ± 0.12^{b}	289.02 ± 2.61^{b}
70 °C	12.62 ± 0.28^{bc}	273.09 ± 6.16^{c}

*The values are means ± standard deviations (n = 3). Mean values sharing different letters are significantly different ($p \leq 0.05$).

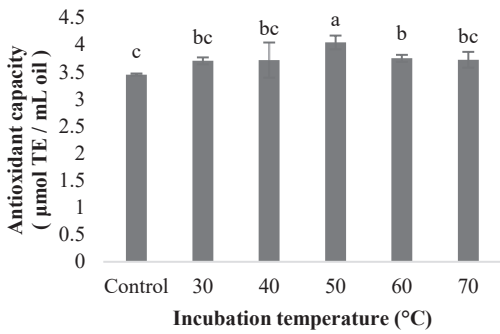

Figure 5: The effects of enzyme incubation temperature on antioxidant capacity. The values are means ± standard deviations (n = 3). Mean values sharing different letters are significantly different ($p \leq 0.05$).

In the study of enzyme-assisted treatment of lycopene extraction from tomatoes, it was found that the highest lycopene yield was obtained at 50°C after incubation for 60 min [48]. However, when the temperature increased to 60 °C and 70 °C, a decreasing trend was observed. The values were 289.02 ± 2.61 (mg/100 g DW) to 273.09 ± 6.16 (mg/100 g DW), respectively. It was well expected as lycopene could not be stable and was easy to degrade at higher temperature due to oxidation and isomerization. In addition, under a mild heat treatment, from 25 °C to 50 °C, temperature could be degraded mainly due to oxidation without isomerization. However, when temperatures were higher than 70 °C, lycopene could be susceptible to both oxidation and isomerization. Besides, it had been pointed out that lycopene was found in plant cells in a more stable all-trans form, which could transfer to the cis-form during a temperature and time-induced reaction. Thus, this change in lycopene form led to a decrease in the visible band absorption in spectrophotometric analysis because cis–form lycopene had different physical and chemical properties than trans–form lycopene [8]. For this reason, the lycopene content could be decreased. Therefore, the efficient incubation temperature of enzyme pretreatment for achieving a higher content of lycopene should be about 50 °C.

According to Figure 5, the antioxidant capacity increased significantly when the incubation temperature increased from 30 °C to 50 °C but changed insignificantly when the incubation temperature was elevated further to 60 °C and 70 °C. From 30–50 °C,

the lycopene concentration in the pigmented oil increased from 246.35 ± 4.03 (mg/100 g DW) to 320.88 ± 8.54 (mg/100 g DW). When increasing the incubation temperature to 60 °C and 70 °C, the lycopene content was 3.75 ± 0.06 (μmol TE/mL oil) and 3.73 ± 0.15 (μmol TE/mL oil), respectively. The changes in antioxidant capacity could be explained by the changes in lycopene content due to the antioxidant properties of the carotenoid compounds. Lycopene has quenching ability towards singlet oxygen (O_2), based on the excited energy state, and is greatly related to the length of the conjugated double bond system [3]. The highest antioxidant was observed in the sample incubated with 2% Viscozyme L. at 50 °C (4.04 ± 0.13 μmol TE/mL oil), which was followed by that of the one at 60 °C (3.75 ± 0.06 μmol TE/mL oil). There was a strong relation between lycopene and antioxidant activity (R = 0.9).

Table 5: Color values of a*, b* and a*/b* of the pigmented oil samples at different incubation temperatures

Sample	a*	b*	a*/b*
Control	–1.87 ± 0.00^{c}	22.73 ± 0.02^{b}	–0.11 ± 0.01^{e}
30 °C	13.87 ± 0.63^{b}	42.20 ± 0.50^{a}	0.33 ± 0.01^{bc}
40 °C	15.27 ± 0.50^{b}	45.80 ± 2.30^{a}	0.33 ± 0.02^{b}
50 °C	17.79 ± 0.32^{a}	43.63 ± 0.22^{a}	0.41 ± 0.01^{a}
60 °C	12.20 ± 0.72^{b}	40.02 ± 0.33^{a}	0.31 ± 0.02^{cd}
70 °C	12.58 ± 0.91^{b}	42.04 ± 1.15^{a}	0.30 ± 0.02^{d}

*The values are means ± standard deviations (n = 3). Mean values sharing different letters are significantly different ($p \leq 0.05$).

The ratio index of a*/b* in Table 7 indicated the color of the pigmented oil varied significantly according to different incubation temperatures. Similar to the trend of lycopene content, the redness of the 50 °C sample, which had the greatest lycopene content obtained the highest color index of 0.41 ± 0.01. The least reddish-yellow color was also obtained in the control sample. It was stated that the extended conjugated double bond system of lycopene was responsible for its visible color because the higher the number of conjugated double bonds, the higher the wavelength absorption [65]. Therefore, the more concentration of lycopene increased the more redness in color of samples. Besides, the yellow color of the rice bran oil also affected the ratio of a* and b*.

It can be seen from Figure 6(a), the incubation

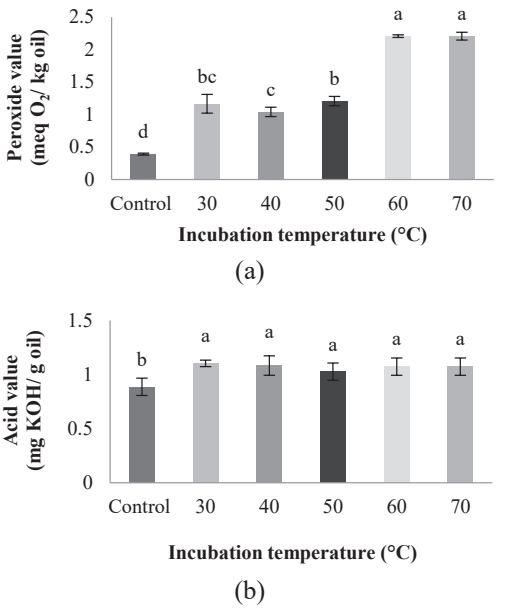

Figure 6: The effects of enzyme incubation temperature on the peroxide value (a) and acid value (b) of the pigmented oil. The values are means ± standard deviations (n = 3). Mean values sharing different letters are significantly different ($p \leq 0.05$).

time had a significant impact on the PV of the carotenoid-enriched oil ($p \leq 0.05$). Indeed, when the incubation temperature increased from 30 °C to 70 °C, the peroxide value of the pigmented oil increased. The values were ranging 0.39 ± 0.02 (meq O_2/kg oil) to 2.21 ± 0.06 (meq O_2/kg oil). The elevated temperature during enzyme pretreatment was supposed to favor the oxidation of the lipid fraction in the tomato oleoresin, leading to the formation of hydroperoxides, which increased the peroxide values.

In terms of AV, due to the elevated incubation temperature, there was a significant statistical increase of AV observed in enzyme-assisted samples compared to the control one (0.89 ± 0.08 mg KOH/g oil). Indeed, the extraction process promoted the acid value of the pigmented oil. Among enzyme-treated samples, AV values were similar, which indicated varying different temperatures did not significantly affect the AV value of the pigmented oil ($p > 0.05$). It may be due to the whole extraction process using rice bran oil was conducted under dim light and for the same period of time. Thus, the changes in the acid value are only significantly different between the non-treated sample

and the treated ones. According to TCVN (TCVN 7597:2018), it could be noticed that the peroxide and acid values of all samples studied in the current work were in the acceptable ranges of the standard for good quality oil.

4 Conclusions

From the work, it was found that the lycopene content reached the peak when tomato peels were incubated with 2% Viscozyme L. for 90 min at 50 °C. Although the extraction process increased the peroxide and acid values, they were still in the acceptable range according to TCVN 7597:2018 for the edible oil. In this way, the study provided a practical approach for producing a lycopene-enriched oil product which can also be an integrated way to utilize tomato waste. To enhance the lycopene content, besides enzymatic pre-treatments, other methods such as ultrasound–assisted extraction should also be studied. Response Surface Methodology should be conducted to optimize operating conditions to obtain the desired lycopene content as well as to minimize the lycopene degradation.

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Author Contributions

T.M.Tnguyen: investigation, writing an original draft; data analysis; H.V.H.Nguyen: conceptualization, data curation, writing—reviewing, editing, funding acquisition, project administration. All authors have read and agreed to the published version of the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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