

# Enzyme-Assisted Extraction of Lycopene from Watermelon Fruits: Effect of Hydrolysis Parameters on Lycopene Yield

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**Abstract**— This study aims to explore influence of process parameters on lycopene recovery from watermelon fruits. Investigations were carried out with respect to lycopene content as the main indicator following randomized single factor routine. Examined hydrolysis parameters included used enzyme, enzyme concentration and hydrolysis duration. In addition, physicochemical measurements of the watermelon fruit was also performed. Results showed that the highest lycopene content ( $14,125 \pm 0.271 \mu\text{g/ml}$ ) could be attained at enzyme concentration of 2.4% (v/v), hydrolysis time of 60 min under cellulose enzyme treatment. The enzymatic treatment was also shown to be capable of significantly lowering viscosity and improving lycopene yield.

**Keywords**— lycopene, enzyme, watermelon, extraction.

## I. INTRODUCTION

Carotenoid represents a pigment class consisting of valuable bioactive compounds with diverse applications in pharmaceutical and functional food industry. Among carotenoids, lycopene is a typical compound known for its strong antioxidant activity and red color due to its structure having eleven conjugated double bonds. Lycopene has been pointed out to exhibit preventive effects against several cancer diseases including stomach cancer and prostate cancer [1-3]. Other beneficial functions of lycopene may include inhibition of cholesterol synthesis, boosted production of low-density lipoprotein[4] and prevention of atherosclerosis and myocardial infarction. Lycopene exists predominantly in red-colored plants such as tomato, watermelon, papaya and red guava. Notably, watermelon has been shown to be a promising source of lycopene, amounting to  $4532 \mu\text{g}$  per 100gr of watermelon pulp [5].

Enzyme-assisted extraction has been one of the most well-studied approaches to obtain lycopene from plant materials[6]. The technique involves the use of enzymes to penetrate cells of plant material, allowing for better expulsion of carotenoids and phenolics[7]. While enzymatic extraction

has certain drawbacks including high cost and susceptibility to degradation, its merits justify the technique in industrial applications. Recent investigations have demonstrated superiorities of enzyme-assisted extraction over conventional methods including more efficient extraction, reduced solvent and power usage, renewability and specificity[8,9]. Kong et al. has reported that, in comparison with conventional techniques, significant improvement of up to 20 times in lycopene recovery could be attained via enzymatic extraction [10]. Against tomato tissue, it was found that enzymatic extraction of lycopene is particularly effective due to pectinolytic, cellulolytic and hemi cellulolytic activities of certain enzymes, possibly giving rise to a 6-fold improvement over lycopene yield of the unhydrolyzed sample [8].

Apart from process conditions, enzyme selection plays a crucial role in enzyme-assisted extraction. Depending on the substrate and the desired compound of extraction, different individual enzymes or a multi-enzyme system could be utilized. For example, hydrolytic enzymes such as pectinase and cellulase have been shown to be effective for extraction of carotenoids from chili pepper [11], lutein from marigold flowers [12], and flavonoids from Ginkgo biloba

[13]. Particularly for tomato substrates, pectinase has been suggested as a suitable enzyme in industrial scale production of lycopene [14-16], enabling further possibilities for combining multiple enzymes or multiple extraction methods simultaneously [6, 16-18].

To the best of our knowledge, enzyme-aided recovery of lycopene from watermelon substrate has not been fully explored. In this study, we attempted the extraction process and examined effect of several process parameters including used enzyme (cellulases, pectinases and viscozyme), enzyme concentration and hydrolysis duration on the extracted lycopene content.

## II. MATERIALS AND METHODS

### 2.1. Plant materials and reagents

Selected plant materials are watermelon (*Citrullus lanatus*) fruits of Hac My Nhan variety, originating from Long An province, Vietnam. Selected fruits were intact, dark green in color and had uniform hardness. Fruits were harvested after 25 – 30 days after pollination or after 65 – 70 days after cultivation. Harvested fruits were maintained at 2-8 °C for not more than 48 hours and then dehulled, deseeded and pressed to afford the pulp.

Enzymes used in this study including Viscozyme L, Pectinex Ultra SPL and Celluclast 1.5L (700EGU/g, 60 FPU/ml). All were obtained from Novozymes Company. Chemicals including lycopene, acetone, ethanol, n-Hexan and BHT (Butylated Hydroxy Toluene) were obtained from Sigma-Aldrich.

### 2.2. Enzymatic treatment of watermelon pulp

The enzymatic treatment commenced with homogenization of pulp materials for 5 min using a blender. Then, a pre-determined amount of enzyme with specific concentration was added into 0.2 M acetate buffer. Following that, 3 g of material was introduced into a container sealed with aluminum foil followed by addition to the mixture and blending for 2 min. The incubation was carried out under 55°C for samples treated with cellulase and under 60°C for samples treated with pectinase. After incubation, the filtrate obtained from the mixture was extracted with solvent extraction using 20 mL of hexane and acetone (1:1 v/v). The residue obtained from the mixture was extracted using 30 mL of hexane and acetone (1:1 v/v), followed by double extraction with 10 mL of hexane and acetone (1:1 v/v) each. Separation funnel was used to discard

the lower aqueous phase to obtain the upper phase, which consists of lycopene and other lipophilic carotenoids. Lycopene extracts from the filtrate and the residue were then mixed and passed through desiccant and 1 g of sodium sulphate. Final samples were stored at 2-8°C. Control samples were prepared identically but without enzyme treatment.

### 2.3. Determination of lycopene

To determine lycopene content, a method adapted from a previous study (Ranveer et al., 2013) was employed. First, extraction of sample was carried out using acetone (containing 0.05% w/v BHT) ethanol and hexane (1:1:2 (v:v:v) ratio). The mixture was then agitated at 100 rpm and added with deionized water under shaking. Phases of the mixture were separated by allowing the mixture to cool to room temperature and collecting the upper solvent phase. The collected solvent was diluted with hexane 1:50 ratio (v:v) before being spectrophotometrically measured for absorbance at 503 nm. The lycopene content was determined as in equation 1:

$$H = \frac{OD_{503} \times 31.2 \times N}{m} \quad (1)$$

H: lycopene content (mg/kg pulp);

OD (503): absorbance at the wave length 503nm

31.2: coefficient

N: dilution factor;

m: gram of sample

The lycopene extraction yield of each experiment was calculated as in equation 2:

$$G = \frac{H \times M}{1000} \quad (2)$$

G: lycopene extraction yield (mg/kg whole watermelon)

M: g of obtained pulp after centrifugation.

### 2.4. Determination of moisture content, ash content and viscosity

Moisture of the material was determined by drying at 75°C. Ash content was determined via heating (550-600°C). The remain was then weighed to calculate the ash content. Viscosity was determined using capillary tube viscometer and calculated based on the time the fluid takes to pass through the viscometer following Hagen-Poiseuille equation.

### III. RESULTS AND DISCUSSION

#### 3.1. Proximate analysis

Several indicators of the sample were presented as shown in Table 1 and Table 2

Table 1. Mean weights of watermelon fruits

Indicator	Mean
Moisture content (dry basis)	10.224±0.5
Ash content (%)	0.285±0.005
Viscosity (Pa.s)	0.001046±0.000001

Table 2. Proximate composition of the watermelon pulp

	Mean	% Total weight
Total weight of watermelon fruit (g)	2411,67 ± 10,40	100,00
Weight of shell (g)	565,89 ± 35.83	23,46±1,49
Weight of seeds (g)	36.92 ± 6,25	1,25±0.25
Weight of pulp prior to enzyme treatment (g)	1640.28±99.2	68.01±9.53

#### 3.2. Effect of used enzyme on the obtained lycopene content

Figure 1 demonstrated lycopene contents with regard to different used enzymes, showing significantly improved contents in enzyme-treated samples. The non-enzyme treated sample (control sample) showed the lowest lycopene content at 4.906 ±0.124µg/ml. The peak lycopene content was observed in sample treated with cellulase enzyme, at 8.502 ±0.301µg/ml. Those results suggest that enzyme could aid in breaking down of fruit tissues, accelerating the release of cells into the medium. ANOVA results also confirms the influence of cellulase and pectinase enzyme on lycopene content with confidence intervals of 95% (p<0.05). LSD multiple range test comparing influences of the three enzymes also indicates that cellulase gave higher and significant improvement in lycopene yields in comparison with other enzymatic treatments. This could be explained by the cellulolytic nature of the enzyme. To be specific, cellulase could act as a catalyst facilitating the breakdown of cellulose, which is present in primary walls, into glucose, cellobiose and higher glucose polymers. For comparison, obtained results are different from another study

[14] who found that lycopene, rather than cellulase, gave superior lycopene content with materials of tomatoes.

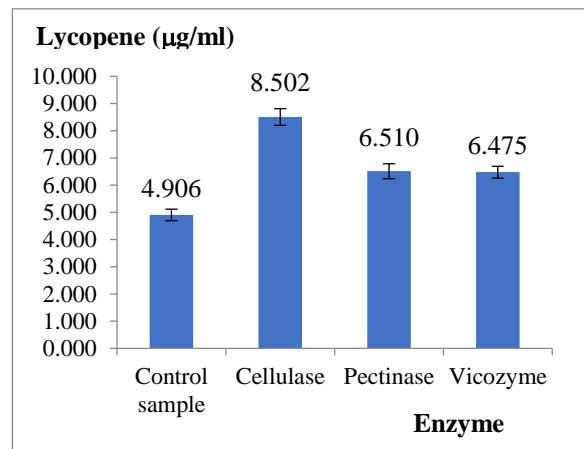


Fig.1. Lycopene content in relation to different enzymes

#### 3.3. Effect of enzyme concentration on the obtained lycopene content

Figure 2 demonstrated variations in lycopene contents with different concentrations of cellulase enzyme. Rising the concentration from 0.8 to 2.4% induced a significant improvement in lycopene content, from 7.05 to approximately 14.054 ±0.271µg/ml. However, rising concentration higher than 2.4% seemed to cause no clear improvement in the lycopene content. Pre-treatment of watermelon juice with cellulase assists in breaking of cellular structure, in turn facilitating the release of lycopene into the solvent media. These results are further confirmed by ANOVA test, indicating that enzyme concentration significantly affected lycopene in watermelon juice (p<0.05). LSD multiple range test also indicated that at 2.4% enzyme concentration, the lycopene content peaked and was significantly indifferent to the content achieved at 3.2%. The strong influence of enzyme content on the first stage of the reaction could be explained by the accelerated enzymification that improved the release of lycopene out of cells. However, at higher enzyme concentration, due to substrate saturation, increasing enzyme content is no longer impactful on reaction speed. Another explanation for the phenomenon was proposed by Cinar [19] and Ranveer [16] suggesting that, in a given duration of reaction, more release of lycopene may lead to increased oxidation of hydrolysate, in turn causing lycopene to remain stagnant at very high enzyme concentration [16, 19].

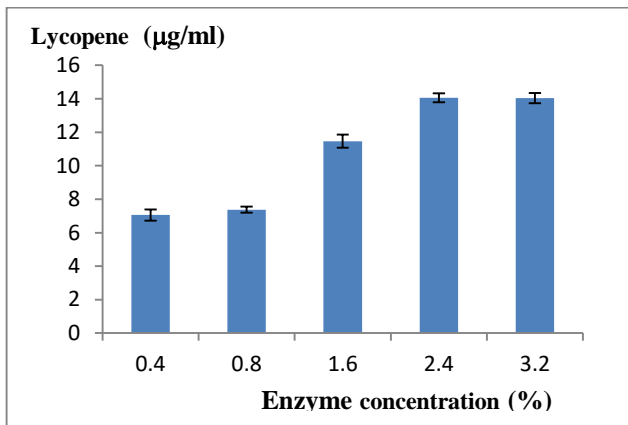


Fig. 2. Lycopene content in relation to different enzyme concentrations

In comparison with previous reports, our results showed many differences. A previous study showed that to hydrolyze the whole tomatoes, pectinase with the concentration of 0.5% was required[14]. Meanwhile, Ranveer et al. reported the pectinase concentration of 2% for efficient recovery of lycopene from tomato wastes[16]. The differences with current results (2.4% of cellulase enzyme) could be attributed for differences in raw materials and used enzyme. Therefore, enzyme concentration of 2.4% will be used for subsequent experiments

#### 3.4. Effect of hydrolysis duration on the obtained lycopene content

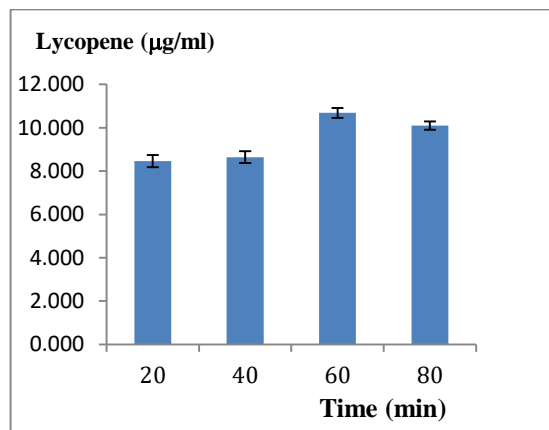


Fig 3. Lycopene content in relation to different enzyme treatment periods

Figure 3 illustrated effect of duration on obtained lycopene content. In general, the increasing trend of the content could be observed in the first 60 minutes. The highest lycopene content,  $10.687 \pm 0.231 \mu\text{g/ml}$ , was achieved

at the duration of 60 min and gradually decreased thereafter. ANOVA results indicated significant influence of duration on lycopene content ( $p < 0.05$ ) and LSD test also confirmed that differences between lycopene obtained at 60 min and those obtained at other durations were statistically significant.

These results suggest that cell-walls tend to degrade rapidly within the first 60 min of hydrolysis. Furthermore, extending the duration from 60 to 80 min did not seem to cause any noticeable improvement in obtained lycopene. This is explained by the increased quantity of hydrolysable substrate under longer durations, resulting in improvements in lycopene content. However, eventual depletion of substrate occurring when carrying out hydrolysis in a very long period may reduce the reaction speed, causing lycopene loss. In addition, long hydrolysis time may also cause spoilage, which also contributes to decreased recovery efficiency. Alternatively, other studies also suggest the decline in lycopene under long hydrolysis time may be attributable to generation of cleavage products having shorter wavelengths than that of lycopene[14, 20]. Current hydrolysis time results are similar to the findings of Lavecchia and Zuurro [15] showing lycopene yield from tomato peels could be improved by enzymatic treatment within 1 hour[18]. Therefore, one hour is selected as the optimal time.

#### IV. CONCLUSION

The present study has attempted the enzyme-aided recovery of lycopene from pulp of watermelon fruits and optimized the parameters of the said process. It was shown that the assistance of enzyme (Cellulase 1.5L and Viscozyme L) could greatly enhance the lycopene yield of the recovery process. Optimal hydrolysis conditions consisted of 2.4% (w/w) of enzyme concentration, incubation time of 60 min and treatment with cellulase enzyme. These conditions corresponded with the highest lycopene yield, at  $14.125 \mu\text{g/ml}$ . Further studies should explore the possibility of a larger scale extraction system and attempt on other enzymes and watermelon varieties for determining more efficient lycopene recovery.

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