Optimization of Extraction Conditions and Design of Extraction the Technology of Lycopene from Strawberry

Lv Jing[1,2]
[1]*Nanchang Vocational University, Nanchang, Jiangxi, China*
[2]*Henan University of Technology, Zhengzhou, Henani, China*

Abstract: Based on the understanding of lycopene extraction technology at home and abroad, strawberry was taken as the research object. After the fruit was grouped and treated, acetone was used as the organic solvent for strawberry pretreatment. Lycopene has the maximum absorption peak at the wavelength of 472nm, so all the following tests are carried out under this wavelength. Firstly, the single-factor experiment was used to determine the data range of each of the four single factors that were beneficial to improve the extraction amount of lycopene in strawberries in different extraction temperatures, solvent amount, extraction time, and different extraction acid-base environment. After the completion of the single-factor experiment, the central group and the experiment were designed based on the experimental results. Then combined with the response surface analysis method, the above four conditions affecting the extraction rate of lycopene in strawberries were comprehensively analyzed. The results of the single-factor experiment showed that the extraction rate of lycopene reached the maximum when the solid-liquid ratio was 4:1, the extraction temperature was 40℃, the extraction time was 40min, and pH=5. The single factor of solvent removal was determined according to the effect of the single-factor experiment. The effects of extraction temperature, time, and pH value on the extraction efficiency of lycopene were investigated by response surface analysis with 3 factors and 3 levels, and the extraction process was optimized with absorbance as the corresponding value. Combined with response surface analysis, the extraction rate of lycopene was used as the experimental index, and the obtained quadratic multinomial mathematical model was analyzed by using Design-expert 7.0 software, and the best extraction process parameters of lycopene were determined as follows: The extraction temperature was 36℃, the extraction time was 49min, and the pH was 4. Under these conditions, the predicted value of lycopene content was 225.5mg/100g, and the actual measured value was 224.5mg/100g. In the empirical experiment, the relative error between the prediction results and the measured results is 0.433%, which has high reliability, indicating that the established mathematical regression model can accurately predict the extraction rate of lycopene within a certain range.

Keywords: Strawberry; Lycopene; Extraction and Separation; Response Surface Analysis; Optimal Extraction Process

1. Introduction

1.1 Research Significance of the Subject

Lycopene has been discovered and used in food for a long time, but it has only been used in the food field as a common plant pigment. With the gradual understanding of lycopene, researchers have found that tomato is not only a simple plant pigment but also has strong antioxidant properties. This pigment is not only present in tomatoes, but also in other plants, and even in human tissues and organs. Because lycopene does not have the biological activity of vitamin A, it has not attracted much attention for a long time. With the deepening of scientific research, researchers have found that lycopene has a strong antioxidant and is even one of the strongest antioxidants found in nature at present. Due to the discovery of lycopene antioxidants, it has gradually entered people's lives. At the same time, with the development of science and technology and scientific research, lycopene has great nutritional value, medical value, beauty, and other potential. Most modern people are in a sub-health constitution, lycopene just can well regulate this constitution. Therefore, it has a

broad development market. Researchers have also found that lycopene can improve people's immunity, strengthen bones, prevent adversely reactions caused by ultraviolet radiation, greatly reduce the incidence of hypertension, reduce the breathing disorders caused by exercise and other effects. In recent years, studies have confirmed that lycopene is not only distributed in tomatoes but also widely exists in other plants, such as strawberry, cabbage, mango, plum, wood onychia, guava, sweet potato, apricot, persimmon, papaya, grape, plum, and other fruits and carrot root[1].

It is because of the multiple effects of lycopene as mentioned above that lycopene has been highly recognized as A category nutrient by the Food and Agriculture Organization of the United Nations (FAO), the Committee on Food Additives (JECFA) and the World Health Organization (WHO). At present, the development of related products with lycopene as the main total ingredient has become a hot spot in the research of functional foods and new drugs in the world, and it is known as "the new darling of health products in the 21st century" [1]. So far, so strawberry has been used in many industries, especially in the fields of nutrition, health care, and beauty.

The strawberry is a berry fruit tree in horticulture. Originally from Europe, it was introduced into our country at the beginning of this century and was popular in China. It has been widely loved by people for thousands of years[2]. Because strawberries also contain lycopene and are widely loved by people, the content of lycopene in strawberries is still relatively vague in the field of scientific research, and there is no relevant literature to study it. Through this experiment, it can be determined that strawberries contain lycopene and determine the content of lycopene. So that people can eat their favorite food and supplement lycopene has achieved the purpose of strengthening the body, so this is also the significance of this experimental study.

1.2 Distribution of Lycopene

Lycopene is not only distributed in tomatoes but also the fruit of wood onyx, watermelon, strawberry, plum, pepper, peach, papaya, mango, guava, grape, grapefruit, molka , citrus, tea leaves, and roots of radish, carrot, turnip, and cabbage, especially in the mature red plant fruits. In nature, lycopene is not only found in plants but also in some microorganisms, fungi, and algae can produce carotenoids in their metabolic activities. Even if some algae do not contain lycopene themselves, they can also synthesize lycopene through changes in the external environment and microbial interactions. Studies have also found that lycopene can also be found in some tissues and organs of the human body. The human body can not synthesize lycopene, and lycopene plays a very important role in human health, so we should supplement lycopene properly[2].

1.3 Biochemical characteristics of lycopene

1.3.1 It has antioxidant properties

Lycopene has strong antioxidant properties because of its special molecular structure, which can inhibit or scavenge the generation of free radicals and quench the active singlet oxygen. Lycopene can also eliminate oxidative[3] free radicals by chemical reactions with reactive oxygen species such as hydrogen peroxide and nitrite. Lycopene can also promote the growth and regeneration of cells because of its 5antioxidant properties, which can achieve cosmetic wrinkles, maintain skin health, and delay aging.

1.3.2 It can Regulate Cell Growth and Metabolism

Usually, the cell membrane surface of active cells has a channel composed of membrane proteins. This channel has selective passability, allowing messenger and growth regulatory substances to pass through. Gap junction communication (GJIC) between cells transmits growth regulatory signals within the cell population, and regulates the normal proliferation and differentiation of cells. At the same time, experiments have shown that: Due to the weak or absence of GJIC function in most tumor cells, the GJIC function is reduced or inhibited after cell transformation. The inhibition or destruction of GJIC function is considered to be an important mechanism[1] [3] to promote the carcinogenesis stage. Lycopene inhibits the growth of tumors by inducing intercellular junctions, enhancing GJIC between normal cells, controlling cell growth, and inducing cell differentiation.

1.3.3 Can Regulate Cholesterol Metabolism

Enzymes can participate in some biochemical reactions in the human body and have inhibitory or promoting effects on some reactions. Lycopene can regulate cholesterol metabolism

because it is A rate-limiting enzyme, and it can inhibit the production of 3-hydroxy-3-methylglutaryl-coa in macrophages. It was found that the amount of cholesterol synthesis by macrophages in culture dishes with lycopene was decreased. It was also found that the activity of low-density lipoprotein (LDL)a receptor of macrophages in culture dishes was significantly enhanced[4-5].

1.3.4 Healthcare Role

The biological characteristics of lycopene and its unique molecular structure determine that it has a variety of health functions[5-6] such as anti-oxidation, delaying

aging, inhibiting mutation, inhibiting tumors, regulating blood lipids, preventing cardiovascular and cerebrovascular diseases, anti-radiation and beauty, breast and uterus health care, prostate health care, and improving male fertility.

1.3.5 The Role of Preventing and Inhibiting Tumors

Lycopene can prevent and inhibit tumors because of its special molecular structure, which makes it have strong antioxidant properties and can well prevent abnormal proliferation and gene mutation of human cells.

1.4 Pharmacological and physiological effects of Lycopene

Lycopene is a natural antioxidant, which has a strong ability to scavengers free radicals. In the gradual study of this ability, it is found that lycopene can well inhibit the spread and replication of cancer cells to prevent and treat cancer, and prevent cardiovascular and cerebrovascular diseases, improve immunity, etc [7].

1.4.1 Protect the Cardiovascular System

One data shows that patients with cardiovascular and cerebrovascular diseases have lower levels of lycopene than normal people. Some studies have shown that lycopene can effectively protect a type of lipoprotein particles that carry cholesterol into peripheral tissue cells from destruction, so lycopene plays an important role in the prevention and treatment of cardiovascular diseases[8].

1.4.2 Delay Aging and Enhance Immunity

Studies have found that this phenomenon is closely related to free radicals. If the number of free radicals is too large, it will cause cell damage and affect the normal function of cells. Otherwise, it will lead to the abnormal physiological function of people. Lycopene plays an important role in maintaining the health of the human body, and can effectively remove free radicals that are not conducive to human health. Therefore, preserving the normal amount of free radicals is an important way to maintain human health and vigorous energy. Lycopene can be used to regulate the balance of free radicals in the human body, and finally achieve the purpose of enhancing the body's immunity and strengthening the constitution[9].

1.4.3 Others

Lycopene can improve the dryness of the human body, allergic skin, and response to uncomfortable feelings. Lycopene also has a good anti-alcoholic effect. The practice has proved that usually, some drunk people will eat some raw tomatoes so that they will 7 feel a lot of headaches will be reduced, and many people who often drink alcohol have the habit of eating red wine. When people consume a large amount of alcohol, the alcohol is eliminated by chemical reactions in the body and a large number of harmful free radicals are generated in the process. Therefore, lycopene is very good to eliminate the adverse reactions caused by alcohol in the human body, especially the protective effect on organs due to its scavenging effect on oxygen-free radicals in the body. In addition, lycopene can also prevent osteoporosis, and reduce blood pressure, anti-ultraviolet radiation, and other physiological health functions[10].

1.5 Preparation of Lycopene

1.5.1 Extraction Method

Lycopene is a lipid-soluble carotenoid, so it is insoluble in water and soluble in organic solvents such as acetone, chloroform, benzene, and carbon disulfide. Using this chemical property of lycopene, lycopene will be extracted from substances containing lycopene and finally obtain lycopene products. Usually, the process of extracting lycopene with organic solvents is as follows: the lycopene-containing substances are washed and ground to facilitate the more effective extraction of the pigment by organic solvents; The crude lycopene product was obtained after the the filtrate was concentrated by the extraction with an organic solvent[11].

1.5.2 Supercritical Extraction Method

Supercritical extraction is an extraction method that combines traditional distillation with organic solvent extraction. It takes advantage of

the properties of a non-gas non-liquid state (supercritical fluid) between gas and liquid, and selects a the method that can dissolve the target material in the supercritical fluid under high pressure, and then separate the extract from the supercritical fluid through the process of stepping down and heating. Finally, the target material can be extracted. This the method can be treated at low temperatures and is suitable for the extraction of materials with high heat sensitivity such as lycopene[12].

1.5.3 Enzyme Reaction Method

In some experimental results abroad, a method of extracting and preparing lycopene by using the enzyme reaction of tomato has been introduced. Under micro alkaline conditions, pectinase and cellulase in tomato peel were allowed to decompress pectin and cellulose, releasing lycopene, and finally achieving the purpose of extracting lycopene[13].

1.5.4 Biological and Chemical Synthesis

Microbial fermentation studies have found that Gram-negative bacteria, fungi, algae and genetically engineered bacteria can synthesize lycopene by themselves. Among them, Boulardii transport is the most promising organism that can synthesize lycopene in a wide field of industrial applications[14]. With the development of science and technology, genetic engineering technology has become one of the latest sources of lycopene.Chemical synthesis There are many chemical reaction methods to synthesize lycopene. The common chemical synthesis methods include the Wittig reaction, Wittig Horner reaction, aldehyde-sulfone reaction, Heck arylation reaction, Ramberg acylation reaction, and McMurry acylation reaction[15].

1.6 Prospects of Lycopene

According to recent research results, lycopene plays a crucial role in maintaining human health. In addition, their products also have a certain effect on the prevention and treatment of chronic diseases through certain processing. Because tomato red is a natural and pollution-free functional pigment, it is used by most industries to develop new products. With the deepening of the research on the functional characteristics of lycopene, its application scope will be broader and broader. According to the current research results, lycopene has great potential in cancer prevention and treatment, anti oxidation and anti-aging, immunity enhancement, beauty, and so on. However, as a food additive or health medicine, there is no perfect conclusion to explain and solve these problems, such as the way for people to take it for a long time, the optimal dose, and whether there will be adverse reactions and drug combination after taking it for a long time. This makes lycopene can be widely promoted still have a long way to go. 9

At present, some developed countries such as the United States, France, Australia, and

Japan have successfully produced some drugs and health products with lycopene as the main ingredient and have been applied to clinical practice. The main functions of these drugs include reducing blood pressure, high blood lipids, reducing the activity of cancer cells, preventing prostate cancer, as well as preventing ultraviolet rays, protecting the skin, and nourishing the skin and other products[16].

With the improvement of the scientific level and the high attention of human beings, we have reason to believe that more beneficial functions of lycopene will be found, and we will make better use of it to serve us better. China is a big producer of tomatoes, but due to the lack of advanced equipment, resulting in a lot of resource waste, we currently do not have reasonable use of tomatoes and other ingredients such as skin and seeds. If these waste processing by-products are used to extract lycopene, it will bring huge economic benefits to the enterprise and ensure the maximum use of materials[17-20].

2. Materials and Methods

2.1 Test Materials and Reagents

Materials: Fresh commercial strawberry fruits purchased from off-campus supermarkets.

Acetone (analytically pure): Provided by Luoyang Haohua Chemical Reagent Co., LTD.

2.2 Main Instruments for the Experiment

This is shown in Table 1.

2.3 Experimental Design

Firstly, the main influencing factors were determined by a single-factor experiment, and then the influence of different single factors on the extraction rate of lycopene was evaluated by the Box-Behnken center combination experiment[21]. The main influencing factors in the extraction process of lycopene were screened.

Finally, the best extractor conditions were determined.

2.3.1 Treatment of Strawberries

Select fresh strawberry fruits with the same maturity, fruit size, and color, and no disease, insect, or mechanical damage. First, clean the strawberry fruits to remove the tail branches and leaves, and then put them in a mortar to mash and grind into a paste,
and put them in a clean conical bottle for use. The remaining strawberries should be stored in the refrigerator between 2℃ and 8℃ for fresh-keeping.

Table 1. Main Instruments for the Experiment

name of instrument	model	manufacturer
Instrument thermostatic water bath	Double row of four holes	Jintan Huafeng Co., LTD
ultraviolet and visible spectrophotometer	TU-1810	Beijing Pujie General Instrument Co., LTD
precision acidity meter	PHS-3C	Haidapu Instrument Co., LTD
Table top high speed refrigerated centrifuge	TGL-18MS	Shanghai Luxiang centrifuge Instrument Co., LTD
electronic scales	JA2003	Shanghai Shangping Instrument Co., LTD

2.3.2 Lycopene Extraction Process

Fresh strawberry → Pretreatment → Grinding → Acetone solution extraction → Centrifuge separation→Product → Absorbance value measured by spectrophotometer→ Extraction amount of lycopene calculated.

2.3.3 Determination of Test Single Factor

A large number of data have shown that lycopene's properties make it more sensitive to oxidation, and its solution is lost after 12 hours of sunlight irradiation. Lycopene is a lipid-soluble pigment, so it is insoluble in water and soluble in organic solvents such as acetone, chloroform, benzene, and carbon disulfide. The solubility of lycopene in various solvents will increase with the increase in temperature. It can be seen that the factors affecting the extraction effect of lycopene include light, solid-liquid ratio, time, temperature, pH, etc. Considering the operability of this experiment and the factors affecting the extraction efficiency of lycopene, the extraction time, extraction temperature, extraction pH value, and solvent amount of lycopene were finally selected as the four single factors of this experiment[22] .

2.3.4 Determination of Spectral Characteristics of Lycopene

Based on a large number of data, acetone solvent was selected to extract lycopene in strawberries and the absorption spectrum of the filtrate was measured by spectrophotometer[23]. The data showed that lycopene had an obvious absorption peak near 472nm. Therefore, the performance of lycopene was selected to measure the absorbance value at 472nm as the standard for all tests[24].

2.3.5 Determination of Lycopene Content

Processing and determination of tomato samples: 5.000g of strawberry ground samples were accurately weighed, then 40ml of acetone solvent was added for extraction, and centrifuged at 25 ° C for 20min. The absorbance of lycopene in strawberry was measured by the spectrophotometer which has been used to measure the absorbance value in the experiment, and the content of lycopene in strawberry (μg/100g) was converted to the standard curve.

2.4 Single-Factor Test of Lycopene Extraction Effect

In the process of single factor experiment, it is guaranteed that the experimental factors are verified change factors and other factors remain unchanged so that only the
factors that set up the gradient experiment can affect the experimental results are called single-factor experiments. The single-factor test is to select a numerical range for the later optimization experiment, to reduce the workload and determine the optimal the extraction process of lycopene faster and more accurately.

2.4.1 Effect of Solid-liquid Ratio on Lycopene Extraction Effect

Take fresh strawberries and mash them into a paste with mortar. Take 5 pieces of strawberry paste each of 5.000g and put them into 5 clean conical bottles (A, B, C, D, with marked numbers to add different amounts of acetone to them, so that the ratio of material to liquid is 4:1, 5:1, 6:1, 7:1, 8:1, under the condition of 35℃, Stir and soak for 30min, then take a small amount of extract, centrifuge for 20min, and measured its absorbance.)

2.4.2 Influence of Temperature on Lycopene Extraction Effect

Temperature Take fresh strawberries, and mash them into a paste with mortar, accurately 12 weigh 5 pieces of strawberry paste each 5.000g, and put the label A, B, C, D, and E in the clean cone bottle, add 40ml acetone, make the soaking temperature at 35℃, 40℃, 45℃, 50℃, 55℃, respectively, add 40ml acetone, make the soaking temperature at 35℃, 40℃, 45℃, 50℃, 55℃. Stir and soak for 30min, then take a small amount of extract, centrifuge for 20min and measure its absorbance.

2.4.3 Effect of pH Value on Lycopene Extraction

Take fresh strawberries for pH, mash them into a paste with mortar, and accurately weigh 5 pieces of strawberry paste each 5.000g, and put in the labels A, B, C, D, and E conical bottles, add 40ml acetone, and adjust the pH value to make the pH values 4, 5, 6, 7, 8. At 35℃, stir and soak for 30min, then take a small amount of extract, centrifuged for 20min, and measured its absorbance.

2.4.4 Effect of Extraction Time on Lycopene Extraction Effect

Time Take fresh strawberries, mash them into a paste with mortar, and accurately weigh 5 pieces of strawberry paste each 5.000g, placed in the label A, B, C, D, and E conical bottles, add 40ml acetone to them, let it at room temperature for 30min, 40min, 50min, 60min, 70min, respectively. The absorbance was measured after centrifugation for 20min.

2.5 Optimization of Lycopene Extraction by Response Surface Method

Response surface optimization experiment is a test method that comprehensively analyzes all the effects on the experimental results to obtain more scientific and reasonable experimental results. According to this experimental method, the experimental process conditions can be optimized. Its essence is actually to apply the theoretical knowledge in mathematics to the actual experimental operation. This method of combining theory with practice can achieve a more accurate expression of experimental results and is suitable for solving complex problems in nonlinear data processing. Through the regression fitting of the process and the drawing of the response surface, it is convenient to find out the response value of each factor level. It 13 is the most effective technical methodto obtain more and better production and research results in a short time with the least human and material consumption[25] . This is shown in Table 2.

2.6 Statistical Analysis

The experimental data were analyzed and graphed by response surface analysis software Design-Expert 7.0.

3. Results and Discussion

3.1 Construction of the Standard Curve

Take a solution of 1.000mg, select the organic solvent acetone and dissolve it into a standard sample of lycopene with different concentration gradient. After filtration and centrifugation, the spectrophotometer was used to measure the absorbance value at the maximum wavelength of 472nm. The standard curve was drawn by taking the concentration of lycopene as the horizontal coordinate and the absorbance value as the vertical coordinate (FIG. 1). The regression equation of the standard curve was y=0.08827x+0.00291, R2=0.99978.14

3.2 Results and Analysis of Single Factor Test of Lycopene Extraction Effect

3.2.1 Results and Analysis of the Leaching Effect of Lycopene by Solid Liquid Ratio

As can be seen from FIG. 2, with the increase of

Table 1 Three-Line Table of Corresponding Surface Analysis Factors and Levels

Factor	level		
	-1	0	1
Temperature/X1（℃）	30	40	50
Time/X2（min）	35	40	45
PH/X3	4	5	6

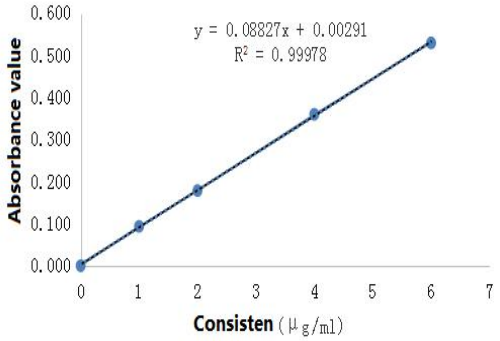

Figure 1. Standard Curve

the volume of extractant acetone, the amount of lycopene extracted decreases first and then increases. When the ratio of material to liquid reaches 1:4, the measured absorbance value reaches the maximum, that is, the amount of lycopene extracted is the largest. The decrease in extraction rate after 1:4 May be caused by the decrease in the concentration of lycopene caused by the increase in the amount of solvent and the decrease in the absorbance value. However, new growth began after the ratio of solid to liquid was 7:1. This may be due to the poor operation of the experiment or the extraction of other non-lycopene pigments under large solvent conditions. In conclusion, the ratio of material to liquid was finally selected as 1:4 as the most appropriate.

3.2.2 Results and Analysis of Lycopene Leaching Effect by Temperature

It can be seen from Figure 3 below that the absorbance value of strawberry jam increases with the increase of temperature before 40℃, and the increase the phenomenon is obvious, while it begins to decrease with the increase of temperature after 40℃. Because the boiling point of acetone is 56.5 ° C, in the process of the experiment, it was found that the solvent volatilization the phenomenon became more and more obvious after the immersion temperature reached 45 ° C, and the natural lycopene was unstable and easy to decomposition at higher temperatures. Therefore, it is more appropriate to choose 40 ℃ for extraction temperature considering comprehensively.

3.2.3 Results and Analysis of Lycopene Leaching Effect by pH

It can be seen from Figure 4 below that the absorbance value of lycopene increased first and then decreased during the whole process of pH increase. When the extraction Soak the ratio of material to liquid 16 pH=5, the extraction amount of lycopene reached the maximum. According to the data, lycopene is not stable for calculation but relatively stable for the base. Combined with Figure 4, it is found that the extraction amount of lycopene begins to decrease gradually after pH exceeds 5, which is exactly consistent with the previous data. Therefore, the extraction time of pH=5 is more appropriate.

3.2.4 Results and Analysis of Leaching Time on Lycopene Leaching Effect

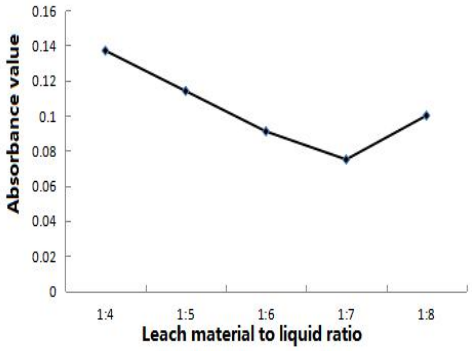

Figure 2. Ratio of Material to Liquid Lycopene Leaching Diagram

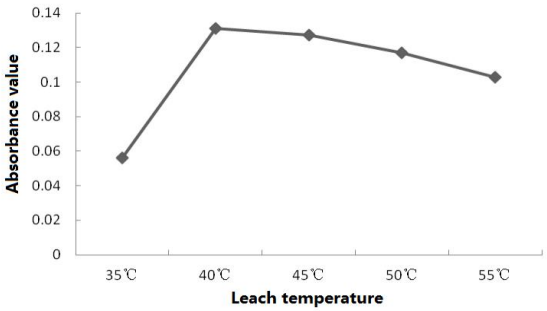

Figure 3. Lycopene Leaching Diagram by Temperature

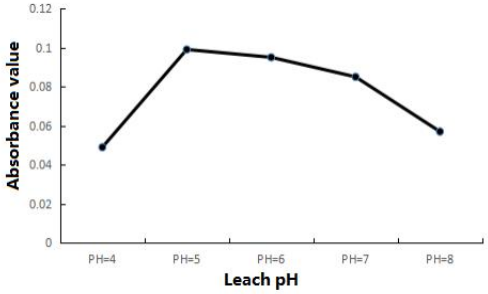

Figure 4. pH Leaching of Lycopene

As can be seen from Figure 5 below, the absorbance value of lycopene in the time range of 30-40 min, its absorbance value increases significantly with the increase of the leaching time, and the absorbance also reaches the

maximum value when the extraction time reaches 40min. It can be said that the amount of lycopene extracted in the experimental strawberry paste before 40min is positively correlated with the extraction time. However, after 40 minutes, the absorbance value of lycopene began to decrease gradually, the reason may be that lycopene itself is unstable, light, heating, and other factors can cause the decomposition of lycopene, resulting in the reduction of the amount of lycopene extracted. Therefore, the extraction time of 40 min is more appropriate.

3.2.5 Results of Single Factor Test

Based on the above single-factor test results, the optimal process of each single factor was experimental data in Table 3: Y=1.4433333+0.041X1 — 0.239125X2-0.351125X3 — 0.08225X1X3 +0.1445X2X3 -0.072667X1X1-0.242417X2X2 +0.3095833X3X3.In the experimental range, the effect of water bath leaching on lycopene absorbance, the absolute value of the partial regression equation coefficient X, X > X, indicating that extraction2 temperature and3 extraction pH1 had a greater impact on lycopene than extraction time. From the analysis of variance (Table 4) of the model, it can be seen that the quadratic multinomial model selected in this experiment is extremely significant (P < 0.0001), and the missing term is not significant obtained as follows: extraction temperature was 40 ℃, extraction time was 40 min, the pH value was 4, and the solid-liquid ratio was 4:1. based on the single factor test, it provides the test range for the corresponding surface experiments to be carried out in the following to improve the experimental efficiency.

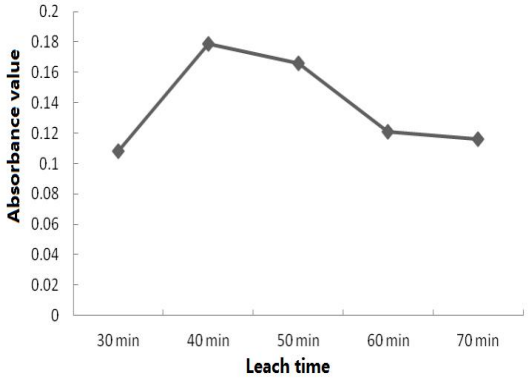

Figure 5. Leaching Time Versus Lycopene Leaching Diagram

3.3 The Extraction Conditions of Lycopene were Optimized by the Corresponding Surface Method

3.3.1 Box-Behnken Center Combined Experimental Design Method to screen Important Factors

According to the experimental results of the influence of single factor experiment on the extraction rate of lycopene, under the condition of the ratio of solid to liquid 4:1, according to the principle of Box-Behnken center combination experiment design, three factors that have an obvious influence on the extraction effect of lycopene are selected: Temperature, time, and pH values were coded as X1, X2, and X3, respectively. Each the variable was coded with -1, 0, and 1 to represent its low, medium, and high three levels, and the corresponding code number was -1, 0, and 1, respectively. The measured absorbance value was used as the evaluation index, and the results were shown in Table 3. The experimental arrangement and results are shown in Table 3 .

By using Design Expert software, the quadratic multinomial regression equation of lycopene absorbance on the encoding independent variables extraction time (X1), extraction temperature (X2) and pH (X3) was obtained by multiple regression fitting the absorbance (P=0.0876 > 0.05). The correlation coefficient of the regression model is 0.988992, indicating that the model can explain 98.8992% of the response value variation. This data shows that the model fits well and has a good reference value for the following experiments, that is, the model can be used to analyze and predict the absorbance value of lycopene. It can be concluded from the simulated data (Table 4) that the soaking temperature, pH, and time in the primary phase have quite significant effects on the response value. In quadratic terms, the extraction temperature and pH had significant effects on the response values. Among the interaction terms, soaking time and pH, soaking temperature, and pH had obvious effects on the response values, especially the effects of temperature and pH on the response values were the most significant. Extraction temperature and extraction time, extraction temperature and solid-liquid ratio, and extraction time and solid-liquid ratio had no significant effects on the response values.

The ANOVA of the experiments is shown in Table 5.

Table 3. Corresponding Surface Analysis Scheme and Experimental Results

Experiment number	X1（temperature/℃）	X2（time/min）	X3（pH）	absorbancy（A）
1	-1	-1	0	1.304
2	-1	0	-1	1.904
3	-1	0	1	1.315
4	-1	1	0	0.930
5	0	-1	-1	2.255
6	0	-1	1	1.315
7	0	0	0	1.451
8	0	0	0	1.417
9	0	0	0	1.462
10	0	1	-1	1.417
11	0	1	1	1.055
12	1	-1	0	1.360
13	1	0	-1	2.210
14	1	0	1	1.292
15	1	1	0	0.919

Table 4. Parameter Estimates

Item	Estimated value	Standard error	T-ratio	Probability >|t|
Intercept	1.4433333	0.036944	39.07	<.0001*
Time(30,50)X1	0.041	0.022624	1.81	0.1199
Temperature(35,45)X2	-0.239125	0.022624	-10.57	<.0001*
PH(4,6)X3	-0.351125	0.022624	-15.52	<.0001*
Time*pH	-0.08225	0.031995	-2.57	0.0423*
Temperature*pH	0.1445	0.031995	4.52	0.0040*
Time*Time	-0.072667	0.033301	-2.18	0.0719
Temperature*Temperature	-0.242417	0.033301	-7.28	0.0003*
pH*pH	0.3095833	0.033301	9.30	<.0001*

Table 5. Analysis of Variance

Source	Degree of freedom	Quadratic sum	Mean square	F-ratio	(p value) probability >F
Model (regression)	8	2.2073299	0.275916	67.3852	<.0001*
Error	6	0.0245677	0.004095		
Corrected sum	14	2.2318976			
Lack of fit	4	0.02346700	0.005867	10.6604	0.0876
Pure error	2	0.00110067	0.000550		
Overall error	6	0.02456767			
Among R2=0.988992 AdjR2=0.974316					

As can be seen from Table 5, the F value of the variance is 67.3852, P<0.0001, indicating that the model is extremely significant, that is, this experimental method is reliable. From the further test of the variance of the regression equation, it can also be seen that X2、X3、X1X3、X2X3、X22、X32 have a significant impact on the results (P<0.05), while X1 、 X12 no significant impact on the results. From the above analysis, it can be seen that the influence of each experimental factor on the response value is a nonlinear change relationship. According to the results of variance analysis, it can be seen that the loss of fitting term P=0.0876>0.05, indicating that the equation fits the experiment well (R2=0.988992), and the simulation is

suitable, so it can be used to analyze and predict the experimental results.

3.3.2 Prediction test

Prediction test: A prediction method that uses existing test methods and conclusions to infer the unknown from the known, the future from the present, and the whole from the local. The specific steps are as follows: first, the experimental method is assumed to be the most ideal, and then the most suitable environment for this method is designed according to the existing conditions and data. Finally, the comprehensive evaluation is carried out according to various situations, and the reasonability and feasibility of the expected results are compared. Finally, the desirability of the prediction experiment is tested through the verification test.

3.3.3 Response Surface Analysis

Response surface analysis is a method for comprehensive analysis of experimental influencing factors. To verify whether certain two factors affect each other will have different effects on the experiment, we keep the other factors unchanged as a quantitative method, and obtain a set of dynamic graphs of the influence of each two factors and their interaction with the experimental absorbance value (Figure 6~8). Furthermore, the optimal condition range of the factors affecting the experimental results was determined.

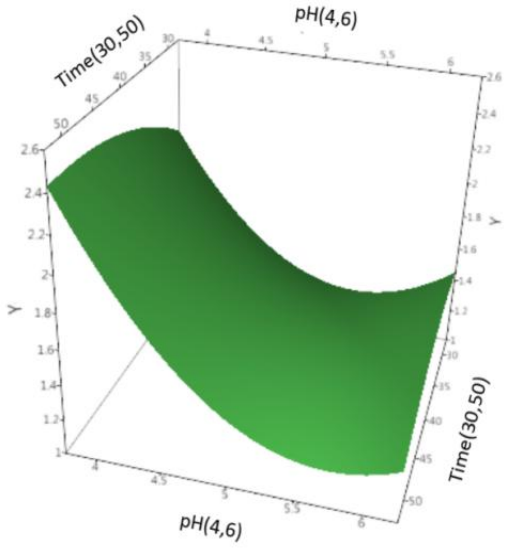

Figure 6. Surface Plot of the Interaction between Leaching pH and Leaching Time on Lycopene Extraction Yield

As can be seen from Figure 6, when the leaching temperature is regarded as a quantity, the influence of leaching pH value and leaching time on the extraction rate of lycopene is extremely significant. The interaction effect of the two on the extraction rate of lycopene was significant. The interaction was most significant, especially when the pH value was in the range of 4-5 and the time was in the range of 40-50 minutes. There was a significant interaction between extraction time and extraction temperature ($P < 0.5$) from the response surface extraction rate trend chart.

As can be seen from the response surface plot in Figure 7, when the extraction temperature is regarded as a fixed value, the interactive effect of the leaching pH value and leaching temperature on the extraction rate of lycopene is significant. In particular, when the leaching pH value was closer to 4 and the leaching temperature was closer to 38℃, the interaction was more significant.

According to the trend chart of the extraction rate of response surface and data analysis, there was a significant interaction between the leaching pH value and extraction time ($P < 0.05$).From the surface diagram of the interaction between leaching time and leaching the temperature on the extraction rate of lycopene (FIG. 8), it can be seen that when we consider the extraction pH value as a constant quantity, the changing trend of extraction

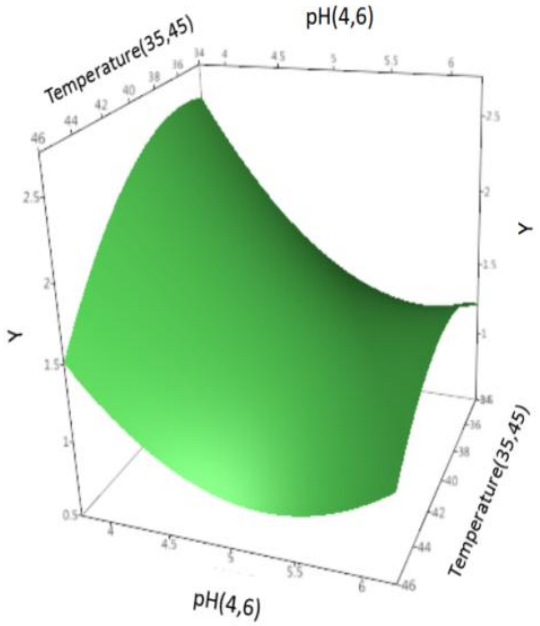

Figure 7. Surface Plot of the Interactive Effects of Leaching pH and Leaching Temperature on Lycopene Extraction yield

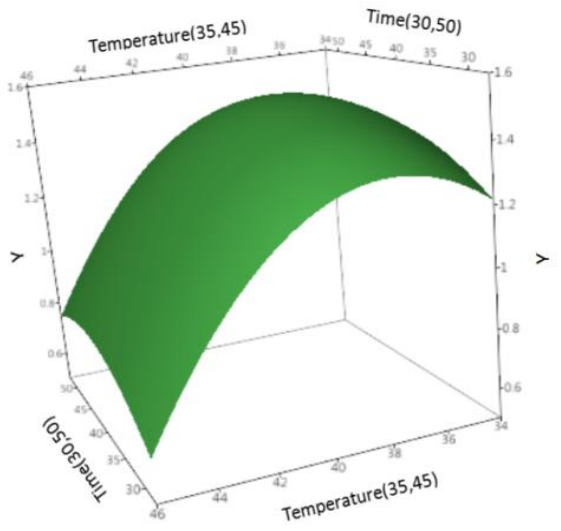

Figure 8. Surface Diagram of the Interaction between Leaching Time and Leaching Temperature on the Extraction Rate of Lycopene

temperature between 38 ℃ and 46℃: with the increase of extraction temperature, the extraction rate of lycopene increases; The variation trend of extraction time from 30 to 50min: with the extension of extraction time, lycopene extraction first increased and then decreased. According to the response surface diagram and the existing data analysis, there was no significant interaction between extraction time and extraction 24 time ($P > 0.05$).

Through the verification test of the best process conditions, through the solution of the equation, the best extraction process is predicted to be 36.04℃, with leaching time 48.51min, leaching pH=4, and the maximum extraction amount is predicted to be 2.31μg/ml under this condition. The optimal process conditions were modified as temperature 36℃, leaching time 49min, and leaching pH=4. After three verification tests, the average yield of lycopene was 2.30μg/ml, which was close to the predicted value of 2.31μg/ml, indicating that the model fitted well.

4. Conclusion

In this experiment, strawberry fruit was taken as the research object, the fruit was washed to remove the branches and leaves and ground into a paste in a mortar to be used. Other samples were stored at 2℃ to 8℃. In this experiment, the leaching temperature, leaching time, leaching pH, and solvent amount were selected as four factors affecting the extraction of lycopene. In this experiment, the leaching temperature, leaching time, leaching pH, and solvent amount were selected as four factors affecting the extraction of lycopene. In the single-factor experiment, the extraction effect of lycopene was the best at pH=5, solid-liquid ratio 4:1, temperature 40℃ and time 40min. Through this experimental study and data analysis, it can be concluded that the values of the single-factor experiment are within a reasonable range. Analysis of variance showed that the results could be used as a reference value ($p < 0.01$). The temperature, time, pH, and other technological conditions in the extraction process of lycopene was optimized by the optimization method of response surface analysis. Box-Behnken center combination experiment was used to comprehensively evaluate the influence of the three experimental factors on the extraction amount of lycopene. The best extraction process conditions for lycopene in strawberries were: pH=4, temperature 36℃, time 49min, which is conducive to the extraction of lycopene in strawberry pulp under the conditions of verification test measured that the absorbance value of lycopene extract in the sample was 0.202, the calculated content of lycopene 25 was 225.5mg/100g, and the verification test measured value was 224.5mg/100g. The verification test shows that the experiment is successful, and the verification the test shows that the prediction experiment meets the requirements. In conclusion, using the response surface method to optimize the extraction process of lycopene from strawberries can obtain the optimal process parameters, reduce the the blindness of the process operation, and lay a certain foundation for further experimental research.

References

[1] Zhou Ruili, Lu Feng. A kind of carotene-lycopene [J]. Journal of Military Economics University, 2007, 6: 24-26.

[2] LI Jing, HUI Bodi, PEI Lingpeng. [2] Lycopene: a functional factor of concern [J]. Food Science, 2005, 26 (8): 67-69. (in Chinese with English abstract)

[3] George B; Kaur C; Khurdiya D S, et a1. Antioxidants in tomato (Lycopersium esculent) as a function of genotype [J]. Food Chemistry, Food Chemistry, 2004, 84 (1): 89-94.

[4] Sun Qing-Jie, DING Xiao-lin. [4] SUN Q J, DING X L. Health effects of lycopene on development [J]. Food and Fermentation Industry, 1997, 23: 44-46.

[5] Bohm. F, et al. Carotenoid sprite against cell membrane damage by nitrogen dioxide radical. Nat. Med. 1995, 11: 101-105.

[6] Zhang Li, Wang Zhengjun. Review on the health function of lycopene [J]. Chinese Condiment, 2009, 9: 45-47.

[7] Marx J L. Oxygen free radicals linked to many diseases [J]. Science, 1987, 235: 4788-4794.

[8] Zhao Chunjing, Wei Lai. [8] WEI L, ZHAO C J. Research progress of lycopene in antioxidation and regulation of blood lipid [J]. China Pharmaceutical Industry, 2004, 13 (10): 56-58. (in Chinese with English abstract)

[9] Yang C W. Study on the purification conditions of lycopene extractor [J]. Northern Horticulture, 2010, 20: 33-35.

[10] YAO Yiliang, LI Rui, WU Qian. [10] Optimization of extraction of lycopene by 26 acetone [J]. Applied Chemical Industry, 2010, 39 (10): 56-59. (in Chinese)

[11] Rozzi N L; Singh R K; Vierling R A; Watkins B A. Supercritical Fluid Extraction of Lycopene from Tomato Processing Byproducts [J]. Journal of Agricultural and Food Chemistry, 2002, 50 (9): 237-242.

[12] Feng Xiaomei. Study on extraction technology of natural lycopene [C]. Ocean University of China, 2003. (in Chinese)

[13] Cheng Jian, Zeng Qingxiao. [13] Research progress on properties and physiological functions of lycopene [J]. Food and Fermentation Industry, 2000, 26 (2): 49-52. (in Chinese with Chinese abstract)

[14] Wang Lihua, Huang Mingfa. [14] WANG L H, HUANG M F. Several functional pigments with great development prospects [A]. Chinese Food Additives 1006-2513, 2008. (in Chinese with English abstract)

[15] Cheng Yang. Extraction technology and detection method of lycopene [J]. Food Research and Development, 2010, 3: 31-33.

[16] Wang C Y, Chen B H. Tomato pulp at the source for the production of lycopene powder contains a high proportion of customers [J]. Eur Food Res Technol, 2006, 22: 78-84.

[17] Zhang Zunying, He Yun, Liu Qianguang, et al. Determination OF ANTIOXIDANT ACTIVITY OF 20 Chinese HERBS in Taibai Mountain BY SPECTROPHOTometry [J]. Analytical Laboratory, 2002, 21 (2): 66-69.

[18] Wang Yong-fei, WANG Cheng-guo. Theory and Application of response surface Method [J]. Journal of Central Mingzu University, 2005, 8: 12-15

[19] LIU Dianfeng, WU Chunhao, WANG Jianjun, et al. [19] Optimization of lycopene extraction from tomato residue by response surface method [J]. Chinese Condiment, 2009, 12: 35-39. (in Chinese with English abstract)

[20] HU Wen-zhong, JIANG Ai-li, TIAN Mi-xia, et al. [20] HU W Z, JIANG A L, TIAN M X, et al. Extraction, separation, and purification of lycopene [J]. 2008, 7: 76-80.

[21] Chen L L, Zhang L. Study on optimization of extraction conditions and detection method of lycopene [J]. Science and Technology of Food Industry, 2012, 3: 33-36.

[22] Watzl B, Bub A, Briviba K, et al. Supplement of a low carotenoid diet with tomato or carrot juice modulates immune functions in healthy men [J]. Ann Nutr Metab, 2003, 3: 112-117.[23] Deng Yu. Preliminary study on the extraction process of lycopene [J]. Chemical Science and Technology, 2002, 10: 11-14. (in Chinese).

[24] LI Jing-dong, FU Shan-jiang. [24] LI J D, FU S J. Health effects and development prospects of lycopene [J]. Science and Technology of Food Industry, 2009, 4: 56-59. (in Chinese)27.

[25] Feng Yan-li, Jiang He-ti. Research on extraction technology of lycopene [J]. Southwest Horticulture, 2005, 33 (6): 44-47. (in Chinese).

