

Preparation of pure gum raw materials-low brown algae application*

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Abstract Octenylsuccinate starch ester, also called pure gum, is non-toxic and odourless modified starch which is widely used in many food fields. This study synthesized pure gum in a reaction kettle using the low molecular weight trehalose and octenyl succinic acid. An orthogonal test was carried out to find how the reaction factors affect the synthetization of octenylsuccinic acid polysaccharide ester and to optimize the reaction at single factor level. The optimal products were obtained using 1:2 of octenylsuccinic acid: alginic acid, catalysed by 0.1% p-toluenesulfonic acid catalyst for 1.5 h at 200°C under vacuum conditions. The gained product contains up to 46% of seaweed gel monoesters. The degree of esterification of the polysaccharide is controlled by the use of the small-molecule trehalose. Compared with the traditional methods, our process can reduce raw material cost and improve emulsification stability of pure gum. These all can significantly improve the market competitiveness of pure gum products.

Keyword: trehalose; pure gum; synthesis

1 INTRODUCTION

Octenylsuccinic acid starch ester is usually obtained under the alkaline conditions by esterification between the active hydroxyl groups on the starch molecules and octenyl succinic anhydride, a highly safe emulsifier and thickening agent, first synthesized by Caldwell and Wurzburg in 1953, and patented (Caldwell and Wurzburg, 1953). Octenylsuccinate starch ester with amphiphilic properties, which improve the texture of the material and enhance structural stability. Compared with other macromolecular surfactants, it has a good market prospect and has great superiority. For example, it can be used as an emulsion stabilizer (Charoen et al., 2011; Sweedman et al., 2013), microcapsule wall material (Juansang et al., 2012), cosmetic components (Sarkar and Singhal, 2011), embedding flavor (Liang et al., 2013; Sarkar et al., 2013). At present, although there are many reports on the study of octenylsuccinate starch ester, more research is needed to break through

the reaction control. Most of them focus on the traditional optimization of the synthesis process, while less researches on the reorganization of raw materials (Qiu et al., 2012; Zheng et al., 2016; Li et al., 2018). On the one hand, starch does not contain small molecular substances, and its terrestrial plant origin produces a large difference in the nature, resulting in unstable quality of pure gum products (Królikowska et al., 2017); on the other hand, starch is hardly soluble in water, so the process of preparing octenylsuccinic acid starch ester by aqueous phase is slow (Wang et al., 2015; Fortuna et al., 2017); More importantly, it's often difficult for the enterprise to control the high technological content and complex process of controlling the starch reaction in the product, especially

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when involving the polyhydroxyl-denaturation of starch and the technical detection and index control of the final product.

The emulsification stabilizing mechanism of octenyl succinate starch is mainly steric hindrance effect, that is, the oil/water emulsion system has good stability due to the steric hindrance caused by the starch macromolecules adhered to the interface or dispersed between the colloidal particles (Jansson and Järnström, 2005; Torres et al., 2016). However, since the starch molecules are relatively large and need to be adsorbed on the newly formed interface and form a strong interface membrane at the oil-water interface, so the stability speed of the oil-water interface is relatively slow and takes a long time to see the effect of emulsification after homogenization (Jeon et al., 1999; Shogren et al., 2000; Tesch et al., 2002). Therefore, in order to accelerate the stabilization rate and enhance the stability of the prepared octenyl succinate-trehalose ester, it was first attempted to add trehalose in the experiment.

Trehalose, a non-reducing disaccharide composed of two glucose units linked by α,α -1,1-glycosidic bonds, is present in many organisms, including bacteria, fungi, plants and invertebrates, which has many excellent properties (Higashiyama, 2002; De Virgilio et al., 2010; Wang et al., 2017): 1. the molecular structure of trehalose is symmetrical, with minimal free energy and chemical bond energy, and has temperature stability and PH adaptability far beyond that of maltose, sucrose, glucose and other sugars (Iturriaga et al., 2009; Ohtake and Wang, 2011; Yang et al., 2017); 2. trehalose is easily soluble in water, and itself contains many hydroxyl groups, which can form more ester bonds with the water molecules and the carboxyl group of octenyl succinic anhydride. This enhances the spatial three-dimensional structure of esterified starch and speeds up the reaction. Its good water-holding capacity ensures that the bound water molecules are close to the starch molecules, which not only dilutes the starch sugar molecular chain, but also increases the microviscosity around the molecular chain, thus delaying the migration rate of the molecular chain and reducing the aging rate; 3. trehalose has a good freeze-thaw stability, so that the octenyl succinate-trehalose ester as emulsifier and thickener in the specific application, makes the product in a dry and frozen can be effectively protected (Brockbank et al., 2011; Shirakashi and Takano, 2018). Because of its high biocompatibility (Mandal et al., 2017), low

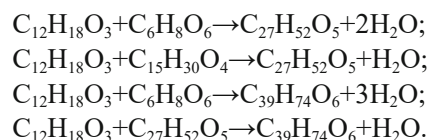
hygroscopicity (Apaliya et al., 2017), high stability (Sizovs et al., 2013; Domian et al., 2015), high gelation (Liyaghatdar et al., 2017), high oxygen permeability (Li et al., 2017), antibacterial (O'Neill et al., 2017) and antiradiation (Liu et al., 2017) in the biological (Lau et al., 2017; Lee et al., 2018; Hernández-Meza and Sampedro, 2018), food (Baker et al., 2018; Cai et al., 2018), health (Tanaka et al., 2004; Apaliya et al., 2018; Drić and De, 2018; Wu et al., 2018) and other fields (Beattie et al., 1997; Tang et al., 2018). Trehalose has attracted great interest of researchers in the research on the utilization of marine plants in recent years (Lin et al., 2017; Wen et al., 2017; Wu et al., 2017).

In this study, the pre-experiment were conducted before large-scale experiments to determine the optimal conditions for the preparation of algae gel monoester, low molecular weight trehalose was used to compound some high value octenyl succinate. The pre-experiment analysis results showed that the content of monoglyceride in algae prepared at the reaction temperature of 180°C, reaction temperature of 200°C, reaction time of 1.5 h and 2.5 h was relatively high under the conditions of three conditions of catalyst, pressure and molar ratio change. Therefore, we combined the results of the preliminary experiment to optimize the process steps, and finally adopt a new synthesis method to prepare seaweed gel monoesters, so that the degree of the esterification reaction could be controlled, the by-products could be recycled and the cost was greatly reduced. It will provide a theoretical reference for the future research and development of new octenylsuccinate products.

2 MATERIAL, INSTRUMENT AND METHOD

2.1 The chemical equation of the reaction

The key trehalose reaction equation is: $C_6H_8O_6 + C_{12}H_{18}O_3 \rightarrow C_{15}H_{30}O_4 + 3H_2O$. During the reaction, trehalose diester and triester are produced, and the reaction formula is as follows:



2.2 Preparation of Octenyl Succinate Starch Alginate

In the 1 000-L reactor kettle, 147.2 kg of octenylsuccinic acid, 160 kg of trehalose and starch,

Table 1 Factor and the level table

Level	Experimental factor				
	Catalyst (%)	Pressure	Molar ratio	Temperature (°C)	Time (h)
1	0.1% p-toluenesulfonic acid	Atmospheric pressure	1:1.5	180	1.5
2	0.2% p-toluenesulfonic acid	Low vacuum	1:2	200	2.5
3	No catalyst				

and 0.32 kg of p-toluenesulfonic acid catalyst were sequentially loaded under the ambient temperature conditions. The temperature was increased from room temperature to 180°C in 25 min with oil bath. When reached 180°C, started counting. The next reaction time lasted 1.5 h. At the first 30 min, the reaction speed was very fast and the exotherm was larger. Care must be taken to control the heating rate. From the room temperature to the end of the reaction, the total time was 2.5 h. Water is generated during the reaction and the water is removed by distillation. At the end of the reaction, the crude ester is condensed and contains about 46% seaweed gum monoester and about 3% trehalose. For polysaccharides can be removed by molecular distillation and then recycled.

2.3 Factor level Selection

The content of seaweed gel monoesters influenced quality of the product. So this study used monoester content as the reaction control index. The main influencing factors of monoester content were catalyst content, pressure, molar ratio, reaction temperature and reaction time. The investigation factor and the level table were shown in Table 1, according to Table 1 choose $L_{12}(3 \times 2^4)$ orthogonal table, as shown in Table 2.

3 RESULT

3.1 Experimental results

Experimental results are shown in Tables 2 and 3.

3.2 Result and discussion

Orthogonal test factors, the level shown in Table 1; orthogonal test results and analysis of variance were shown in Table 2 and Table 3. K1, K2 and K3 are the sum of the index values corresponding to the same level of each factor respectively; and k1, k2 and k3 are the average of the sum of the index values corresponding to the same level of each factor. The extreme difference value R is the difference between

Table 2 Orthogonal experimental results of monoester preparation

Order	Catalyst (A)	Pressure (B)	Molar ratio (C)	Temperature (D)	Time (E)	Monoester (%)
1	1	1	1	1	1	26.47
2	1	1	1	2	2	28.11
3	1	2	2	1	2	28.81
4	1	2	2	2	1	46.13
5	2	1	2	1	1	19.17
6	2	1	2	2	2	34.32
7	2	2	1	1	1	21.45
8	2	2	1	2	2	21.79
9	3	1	2	1	2	25.23
10	3	1	1	2	1	38.25
11	3	2	1	1	2	23.31
12	3	2	2	2	1	42.00
K1	129.52	171.55	159.38	144.44	193.47	
K2	96.73	183.49	195.66	210.60	161.57	
K3	128.79					
k1	43.17	85.78	79.69	72.22	96.74	
k2	32.24	91.75	97.83	105.30	80.79	
k3	42.93					
R	10.93	5.97	18.14	33.08	15.95	
Primary order	D>C>E>A>B					
Excellent combination	$A_1B_2C_2D_3E_1$					

Table 3 Results of analysis of variance of orthogonal experiment prepared with monoester

Dependent variables: monoester content						
Sources	Type III square sum	df	Mean square	F	Sig.	
Correction model	746.427 ^a	6	124.404	7.303	0.023	
Intercept	10 504.450	1	10 504.450	616.612	0.000	
A	175.297	2	87.648	5.145	0.061	
B	11.880	1	11.880	0.697	0.442	
C	109.687	1	109.687	6.439	0.052	
D	364.762	1	364.762	21.412	0.006	
E	84.801	1	84.801	4.978	0.076	
Error	85.179	5	17.036			
Total	11 336.055	12				
Total correction	831.605	11				

^a: R side=0.898 (adjust R side=0.775).

the maximum value and the minimum value of the average of indicators with different levels of the same factor. The greater the extreme difference value R is,

the greater the influence factor of the influence factor on the experimental result is. The significant $\text{sig} < 0.01$ of variance analysis indicated that the experimental factors had a significant effect on the content of seaweed gel monoesters.

From the results of Table 2 and Table 3, we could find that among the five factors, the reaction temperature had the most significant effect with the extreme difference of 33.08. The significant factor was $\text{sig.} = 0.006$. The reaction temperature had very significant influence on the content of seaweed gel monoesters prepared, followed by the molar ratio of octenylsuccinic acid to alginic acid, and the reaction time, the pressure was the least influential. The order was: $D > C > E > A > B$.

By comparing the K value in Table 2, we got the favorable reaction factors showing as below: catalyst (K1) 129.52; the pressure (K2) 183.49; the molar ratio (K2) 195.66; the temperature (K2) 210.60; the time (K1) 193.47. Therefore, the best synthesis process of monoester was $A_1B_2C_2D_2E_1$. That was to add catalyst, 0.1% p-toluenesulfonic acid under the condition of vacuum; octenylsuccinic acid: alginic acid=1:2; 200°C; and reaction 1.5 h. Under this conditions, the monoester content was the highest, could up to 46.13%.

In Table 3, the source is the source of the deviation. The sum of the squares of the type III is the sum of the squares of the deviations. The sum of the squares of the deviations of the correction model is equal to the sum of the deviation squares of the five main effects, and $F=7.303$, the level of significance $\text{Sig.} = 0.023 < P (=0.05)$, indicating that the model is statistically significant for further analysis. The size of the R -side means the fitness of the model. The R -side ranges from 0 to 1, and the closer the value is to 1, the better the fitting degree of the model. In this experiment, $R\text{-side} = 0.898$, indicating that the model has a good fit. D is the temperature principle effect, and the sum of deviation squares represents the difference in the influence of different temperatures on the content of monoester. The significant level $\text{Sig.} = 0.006 < P (=0.01)$ indicates that the temperature factor has a very significant influence on the monoester content. The significant levels of catalyst, pressure, molar ratio and time main effect were 0.061, 0.442, 0.052 and 0.076, respectively, which were greater than 0.05, on the one hand, shows that the experimental factors pressure, molar ratio and time had no significant effect on the monoester content, on the other hand, it also shows that our experimental factors

need to be further optimized.

The main reaction equation of trehalose and octenyl succinic acid is: The main reaction equation of trehalose and octenyl succinic acid is: $C_{12}H_{22}O_{11} + C_{12}H_{18}O_3 \rightarrow C_{15}H_{30}O_4 + 3H_2O$. In this experiment, the esterification reaction and the hydrolysis reaction were carried out simultaneously. At the beginning of the reaction stage, the esterification reaction dominated and the reaction proceeds to the esterification reaction. When the reaction to a certain period, due to the reduction of substrate concentration, the hydrolysis reaction would be in the dominant position. That was to say, when the reaction time of trehalose and octenyl succinic acid were prolonged, the reaction proceeds not only in the reverse direction, but also the monoester would react with trehalose to form diesters and triesters. So the reaction time is not as long as possible, we must control the reaction time.

In this study, a pre-experimental method was used to reduce the experiment factor to a lesser extent, reducing test times, reducing the costs of experiment, and adding a catalyst to a certain extent, increasing the reaction rate and shortening the reaction time. However, the crude ester synthesized by the scheme was color deepened and there were two ways to reduce the color deepening: firstly, nitrogen was introduced into the reaction process to keep the reaction vacuum and prevented or reduced the carbonization; secondly, to control the heating rate, kept the temperature rising slowly during heating. Under vacuum conditions, controlling the heating rate, could significantly improve the monoester content of the product.

4 CONCLUSION

Compared with other macromolecular surfactants, octenylsuccinate starch has several advantages. There has been many reports on starch octenylsuccinate, but more research is need to break through the reaction control. First of all, most of the researches focus on the traditional optimization of the synthesis process, while less researches on the reorganization of raw materials. Further, the preparation requires high molecular weight starch and octenylsuccinic anhydride as expensive raw materials. The most important thing is the stability of the product cannot be guaranteed. The method used in this experiment was to pre-test the factors that may affect the result before preparing the macromolecule gel, and to select the most influential factors by analyzing the pre-experimental results to prepare the pure gum, followed

by orthogonal test and analysis of variance. The advantages of this method were as follows: first, the experimental level was split in two steps to make the experimental results more accurate; second, the addition of the pre-experiment, which reduced the factors of the formal experiment, and accurately designs the orthogonal table and analyzes the contents; third, octenylsuccinic acid complex small molecule trehalose can shortening reaction time, lower production costs, and increase the yield of the major products. Through the use of the pre-experimental mode, it is easier to obtain the optimal preparation conditions, simplify the synthesis route, and improve the stability of the reaction products in the study of the preparation of other pure gums. This will provide a theoretical reference for the future research and preparation of starch octenylsuccinate.

5 DATA AVAILABILITY STATEMENT

Data sets generated and/or analyzed in the current research process have not been published because these data belong to Hangzhou Ruilin Chemical Co. Ltd., more data information can be obtained from Mr. YUAN Changgui on reasonable request and the number is 13806510398.

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