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Amylase Promotes Sustained Release Conditions of Extruded Starch-Proanthocyanidin-Gliadin Microcapsules

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Abstract: *In order to enhance the stability and utilization of proanthocyanidins, four kinds of microparticles with sustained-release ability of proanthocyanidins were prepared by using starch as the main raw material, embedding proanthocyanidins in low temperature extrusion process, adding or not adding zein and thermostable α-amylase. The optimal sustained release conditions of four kinds of microparticles were explored through the orthogonal experimental design of three factors and five levels. The release rates of proanthocyanidins reached 95.30%, 82.27%, 90.75% and 91.67%, respectively, with a pH of 9, a time of 8h and a different temperature. The results showed that the four kinds of microparticles could protect proanthocyanidins in alkaline condition, and had sustained release characteristics. The addition of zein enhanced the stability of proanthocyanidins in microparticles.The stability of proanthocyanidins can be enhanced by adding enzymes to starch. Adding enzymes to the mixture of starch and zein can promote the release of Proanthocyanidins from microparticles. At the same time, all four microparticles improved the instability of proanthocyanidins under alkaline conditions. The distribution of starch and zein in the microparticles before and after the release of proanthocyanidins was observed under fluorescence microscope after double staining, and the release characteristics of the microparticles were verified.*
Keywords: *Microparticles; Sustained-release; α-Amylase; Zein*

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I. Introduction

The extrusion technique is a method in which the material is conveyed, compressed, mixed, expanded, and polymerized by means of friction, extrusion and melting of the material by means of an extruder screw and a sleeve. Extrusion technology is widely used in the food industry, military industry, textile industry and chemical industry due to its advantages of simple operation, time saving and low energy consumption. Food extruders are initially used to process macaroni and ready-to-eat cereal pellets[1]. Along with the deepening of the research of extrusion technology, people have awareness of materials in the thermal extrusion process and the effect of shear force, nutrients in the food structure, chemical changes will happen, such as starch gelatinization and degradation, protein denaturation, lipid oxidation, the formation of flavor substances, minerals bioavailability and the increase of dietary fiber solubility, etc[2, 3] .Low temperature compound enzymatic extrusion technology is the research direction extending from extrusion technology. The enzyme preparation is added to the material before extrusion, and the material and the enzyme preparation are fully acted in the extruder at a suitable temperature for ensuring the activity of the enzyme preparation[4, 5].Therefore, on the basis of these complex biochemical reactions, we can get the target product according to the demand.

During the extrusion process, the carbon chain covalent bond in the starch molecule is cleaved by mechanical shearing, resulting in loss of crystallinity of the starch and fragmentation of amylopectin. The corn starch extrudate exhibits a loose structure and is more apt to absorb water, thereby increasing the digestibility of the starch[6, 7].

Compared with other cereal proteins, zein contains a high proportion of hydrophobic aliphatic amino acids such as leucine, phenylalanine and isoleucine[8]. Therefore, zein has unique functional properties such as anti-oxidation[9] , anti-hypertension and other functional properties[10].It is also quite common to use extrusion technology to process zein[11, 12]. Moderate extrusion will make zein plasticize[13], change its secondary structure[14], loose space structure, increase water holding capacity and improve thermal stability[15].

Polyphenols compounds are a group of compounds containing phenolic hydroxyl groups, which have attracted more and more attention because of their strong antioxidant activity. Proanthocyanidins are

polyphenolic compounds widely found in nature, with high antioxidant activity[16], anti-cancer and anti-tumor[17, 18], prevention and treatment of cardiovascular diseases[19], inhibition of bacterial activity and other physiological activities[20]. After a large number of acute toxicity and chronic long-term toxicity tests, no toxic and side effects of proanthocyanidins were found. However, proanthocyanidins are easily oxidized in natural environment, sensitive to light, heat, pH and other environmental conditions, and their poor permeability and low bioavailability determine that proanthocyanidins are very vulnerable to oxidative damage in production, storage, transportation and use, and their application scope and application conditions are greatly limited[21].

In order to improve the storage stability and bioavailability of proanthocyanidins, four kinds of microparticles with proanthocyanidins sustained release ability were prepared by using common corn starch, zein, thermostable α-amylase and proanthocyanidins through low-temperature extrusion and enzyme technology, and the optimal sustained release conditions of the microparticles were studied.

II. Materials And Methods

2.1 Materials

Thermostable α-amylase (CAS, 9001-19-8, 51 kDa, 40000 u/g, Solqrbio Life Science Co. Ltd., Beijing, China), corn starch (Food Degree, 13% water content, Shandong Hengren Food Industry co. LTD, Shandong, China), zein (CAS,9010-66-6/92%, Zhongcheng Jingnian Technology Co., Ltd., Beijing,China) , proanthocyanidins (CAS, 4852-22-6/95%, Heowns Technology Co. Ltd., Tianjin, China), Rhodamine B (CAS, 81-88-9, Merrill Chemical Technology Co., Ltd., Shanghai,China), FITC(CAS,3326-32-7/98%, Runzekang Biotechnology Co., Ltd.,Beijing,China) and distilled water (Made by Shandong University of Technology laboratory) were used.

2.2 Extrusion operation

Proanthocyanidin 100g, zein 200g and starch 2000g were accurately weighed and mixed evenly. Then, a suspension of heat-resistant α-amylase with distilled water was added to the mixed powder evenly, and the moisture content of the material was adjusted to 25%. The extruded material was extruded by a single screw extruder at 65°Cand 110rpm. After the extruded material was stabilized, the extruded samples were collected and the extruded samples were processed. The product is dried naturally at room temperature in the absence of light. After drying, it is crushed by a crusher through 40 meshes of screen and sealed for subsequent analysis.

The four particulate components are as follows:

SP: Starch + Proanthocyanidin

SZP: Starch + Zein + Proanthocyanidin

SAP: Starch+alpha-amylase+proanthocyanidins

SZAP: starch + zein + alpha-amylase + Proanthocyanidins

2.3 Determination of Proanthocyanidin Release

2.3.1 Standard curve of Proanthocyanidins

The content of proanthocyanidins was determined by catalytic colorimetry with iron salt. The standard product of proanthocyanidins was accurately weighed at 52mg (accurate to 0.0001g), dissolved in water and accurately volumed at 100mL. The standard solution with concentration of 0.52mg/mL was prepared. Then the solutions with concentrations of 0, 26.00, 52.00, 104.0, 208.0, 312.0 and 416.0 ug/mL were diluted with water.The standard solution with different concentrations of 1 mL was removed from the 10 mL calibrated test tube, and 9 mL mixed solution ($V_{butanol}$: $V_{concentrated\ hydrochloric\ acid}$: $V_{0.2\ kg/L\ ammonium\ ferric\ sulfate}$ = 85:5:0.4) was added to the test tube and heated in a boiling water bath. When the water bath temperature reaches 95±1°C, the time is started, and the stopper is tightened (not shaken evenly). After 40 minutes of heating, the ice water is quickly taken out and cooled to room temperature, the volume of n-butanol is fixed to the calibration line, the stopper is fully shaken, and the blank tube is set to zero at 550 nm. The absorbance value is determined and the proanthocyanidin concentration-absorbance curve is drawn.

2.3.2 Determination of Proanthocyanidin Release

1g of particulate matter was added into 40mL buffer solution, soaked at a certain temperature for a specific time, shaking and mixing. 30 minutes later, the supernatant was taken from a centrifuge of 5000rpm for 10 minutes. The release of proanthocyanidins was determined by 1 mL supernatant. The specific operation was the same as 2.3.1. The content calculation was based on the standard curve drawn by different concentrations of proanthocyanidins.

2.4 Orthogonal experimental design

The orthogonal experiment design of three factors and five levels was carried out by using L25 (5^6) table. The effects of temperature (A), pH (B), and time (C) on the sustained release ability of four kinds of

microparticles were studied to obtain the best release conditions of each particle. Each group was parallel twice. The orthogonal experiment factor level tables were as follows: Table 1 and Table 2.

2.5 Verification experiment

According to the optimal release conditions obtained by four groups of orthogonal experiments, the sustained release ability of proanthocyanidins under the optimal conditions of SP, SZP, SAP and SZAP were validated.

2.6 Fluorescence Microscope

In order to better explore the mechanism of sustained release and particle formation, and to provide a theoretical basis for judging the mechanism of particle formation and embedding effect, we used double staining method of fluorescein isothiocyanate and Rhodamine B to dye the microcapsules before and after sustained release. The appropriate amount of sample was dyed with Rhodamine B for 30 minutes, followed by isothiocyanate for 30 minutes, then the dye was poured out, and the sample was washed with distilled water until the cleaning solution was clear and colorless. The samples were dripped onto the slide and covered with the slide. The samples were observed under fluorescence microscope with a 20-fold objective lens.

III. Results and discussion

All experiments were performed in duplicate. The multivariate process in general linear model of IBM SPSS Statistics 20 was used to analyze the experimental data by variance analysis and multiple comparison.

3.1 Standard curve of Proanthocyanidins

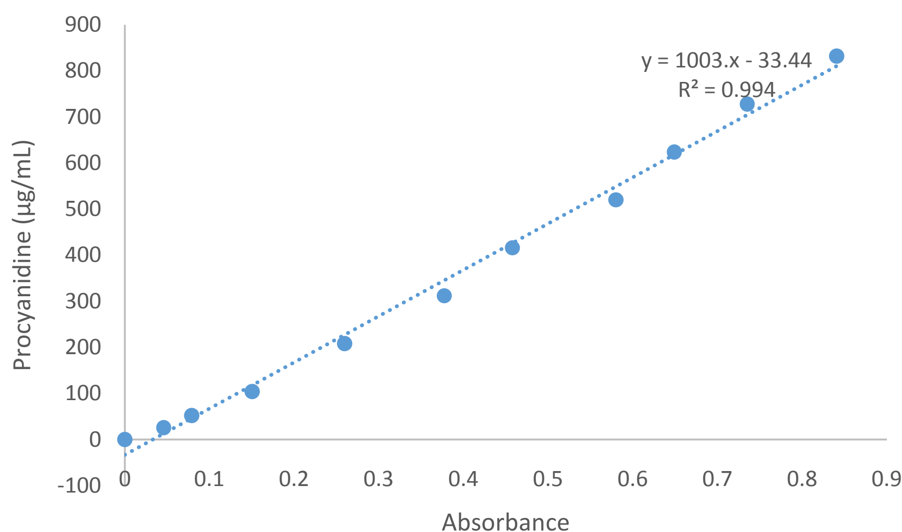

Fig. 1 Standard curve of Proanthocyanidins

3.2 Orthogonal Design and Result Analysis

Table 1 Factors and levels of SP and SAP orthogonal design and experimental results

Test	Factors			Experimental results	
	A Temperature (°C)	B pH	C Time（h）	SP	SAP
1	1(29)	1(8.5)	1(8)	916.36404	706.36074
2	1	2(9)	2(16)	949.98464	984.4583
3	1	3(9.5)	3(24)	556.52326	553.56264
4	1	4(10)	4(32)	520.74492	509.0028
5	1	5(10.5)	5(40)	456.86578	461.28162
6	2(32)	1	2	733.0565	587.0327
7	2	2	3	961.97766	982.35074
8	2	3	4	619.69988	563.29756
9	2	4	5	516.028	477.3894
10	2	5	1	539.46206	518.68754
11	3(35)	1	3	843.00088	747.70906
12	3	2	4	1036.49496	953.54742
13	3	3	5	477.08832	470.51474

14	3	4	1	726.784	619.34862
15	3	5	2	642.33106	576.34436
16	4(38)	1	4	716.748	751.82382
17	4	2	5	993.44052	878.42796
18	4	3	1	776.4622	738.8272
19	4	4	2	600.78202	544.73096
20	4	5	3	563.49828	520.14276
21	5(41)	1	5	759.1501	633.34884
22	5	2	1	1004.32958	859.15884
23	5	3	2	527.71994	555.06804
24	5	4	3	421.38852	443.96952
25	5	5	4	392.3343	378.23372

Table2 Factors and levels of SZP and SZAP orthogonal design and experimental results

Test	Factors			Experimental results	
	A Temperature (°C)	B pH	C Time（h）	SZP	SZAP
1	1(39)	1(8.5)	1(8)	174.65346	213.89422
2	1	2(9)	2(16)	745.25024	796.48402
3	1	3(9.5)	3(24)	328.80642	391.98304
4	1	4(10)	4(32)	293.4797	334.97856
5	1	5(10.5)	5(40)	246.0596	307.0283
6	2(42)	1	2	193.06952	233.56478
7	2	2	3	688.09522	806.8211
8	2	3	4	351.73868	425.40292
9	2	4	5	301.45832	371.91104
10	2	5	1	450.24202	444.2706
11	3(45)	1	3	207.62172	278.72678
12	3	2	4	629.08354	647.59996
13	3	3	5	268.03844	285.4509
14	3	4	1	524.35788	589.94314
15	3	5	2	326.3476	327.75264
16	4(48)	1	4	207.62172	259.45766
17	4	2	5	522.5514	539.76314
18	4	3	1	391.5816	469.96276
19	4	4	2	330.1111	393.99024
20	4	5	3	245.85888	333.42298
21	5(51)	1	5	243.14916	274.81274
22	5	2	1	705.2066	854.2412
23	5	3	2	383.60298	484.6655
24	5	4	3	270.6478	298.94932
25	5	5	4	208.02316	254.74074

Table 3 Variance analysis of orthogonal experiment results

Source of variation	Type Ⅲ Sum of Squares	df	Mean Square	F	P
SP					
Corrected Model	1811913.052[a]	12	150992.754	45.705	.000
Intercept	23811236.408	1	23811236.408	7207.54	.000
Temperature	97710.800	4	24427.700	7.394	.000
pH	1569226.322	4	392306.581	118.75	.000
Time	144975.930	4	36243.982	10.971	.000
Error	122235.314	37	3303.657		
Total	25745384.773	50			
Corrected Total	1934148.366	49			
SZP					
Corrected Model	1306380.043[a]	12	108865.004	35.371	.000
Intercept	6825266.248	1	6825266.248	2217.55	.000
Temperature	23101.206	4	5775.302	1.876	.135
pH	1170075.067	4	292518.767	95.041	.000
Time	113203.770	4	28300.943	9.195	.000
Error	113879.808	37	3077.833		
Total	8245526.098	50			
Corrected Total	1420259.850	49			
SAP					
Corrected Model	1416266.292[a]	12	118022.191	42.438	.000
Intercept	20517444.043	1	20517444.043	7377.519	.000

Temperature	78828.735	4	19707.184	7.086	.000
pH	1280213.313	4	320053.328	115.083	.000
Time	57224.244	4	14306.061	5.144	.002
Error	102899.826	37	2781.076		
Total	22036610.161	50			
Corrected Total	1519166.118	49			
SZAP					
Corrected Model	1485208.049[a]	12	123767.337	27.243	.000
Intercept	9022443.224	1	9022443.224	1985.966	.000
Temperature	19776.724	4	4944.181	1.088	.377
pH	1315987.860	4	328996.965	72.417	.000
Time	149443.465	4	37360.866	8.224	.000
Error	168094.754	37	4543.101		
Total	10675746.028	50			
Corrected Total	1653302.804	49			

Table 4 Range analysis on indicator parameters obtained from the orthogonal experiment

Indicators	(A) Temperature(°C)	(B) pH	(C) Time(h)
SP			
k1	680.096528	793.663904	792.680376
k2	674.04482	989.245472	690.774832
k3	745.139844	591.49872	669.27772
k4	730.186204	557.145492	657.204412
k5	620.984488	518.898296	640.514544
R	124.155356	470.347176	152.165832
Best level	A3	B2	C1
SZP			
k1	357.649884	205.223116	449.208312
k2	396.920752	658.0374	395.676288
k3	391.089836	344.753624	348.206008
k4	339.54494	344.01096	337.98936
k5	362.12594	295.306252	316.251384
R	57.375812	452.814284	132.956928
Best level	A2	B2	C1
SAP			
k1	642.93322	685.255032	688.476588
k2	625.751588	931.588652	649.526872
k3	673.49284	576.254036	649.546944
k4	686.79054	518.88826	631.181064
k5	573.955792	490.938	584.192512
R	112.834748	440.650652	104.284076
Best level	A4	B2	C1
SZAP			
k1	408.873628	252.091236	514.462384
k2	456.394088	728.981884	447.291436
k3	425.894684	411.493024	421.980644
k4	399.319356	397.95446	384.435968
k5	433.4819	333.443052	355.793224
R	57.074732	476.890648	158.66916
Best level	A2	B2	C1

From the range analysis in Table4, the order of influence of each factor on the release of proanthocyanidins is R(pH) >R (time) > R (temperature). The results of variance analysis in Table3 orthogonal test show that temperature, pH and time have significant effects on the release of proanthocyanidins. The optimum release condition of SP is A3B2C1, that is to say, the maximum release of SP can be obtained when the release time is 8 hours at 35°C and the buffer pH is 9.

From the variance analysis of Table3 variance analysis of orthogonal experiment results, it can be seen that both pH and time have significant effects on the release of proanthocyanidins, while temperature has no significant effect on the release of Proanthocyanidins from microparticles, which was consistent with the results of range analysis in table 4. Considering the K value of each factor, the optimum release condition of SZP is A2B2C1, that is to say, the maximum release of proanthocyanidins in SZP is achieved when the release time is 8 hours at 42°C and pH 9.

The results of variance analysis of Table3 showed that temperature, pH and time had significant effects on the release of proanthocyanidins. The best release condition of SAP is A4B2C1, which is the maximum release of SAP at 38°C and 9 pH for 8 hours.

The order of influence of three factors on the release of proanthocyanidins from SZAP microparticles is the same as that of SZP microparticles. The optimum release condition of SZAP was A2B2C1, the maximum proanthocyanidin release was achieved at 42°C and 9 pH for 8 hours.

3.3 The result of verification experiment

Table 5 The result of verification experiment

microparticles	Factors			Release (μg/mL)
	Temperature (°C)	pH	Time(h)	
SP	35	9	8	1134.59686
SZP	42	9	8	894.23466
SAP	38	9	8	1080.40246
SZAP	42	9	8	996.40114

The results of validation test under the optimum conditions in Table 5 show that the proanthocyanidin release of the four microparticles is increased compared with that of the orthogonal test. The release rates of SP, SZP, SAP and SZAP were 95.30%, 82.27%, 90.75% and 91.67% respectively, and the release rates were significantly improved.

Starch was the main material for the preparation of microparticles. Starch was denatured and stretched during extrusion, thus wrapping proanthocyanidins. Compared with the microparticles SP and SAP without adding zein , the release rate of proanthocyanidins in SZP and SZAP is relatively low, which indicated that the addition of zein makes the Proanthocyanidins in microparticles better encapsulated. This may be related to the properties of zein before and after extrusion. Zein itself is insoluble in water and is high in extruder. The spatial conformation of zein was changed under the synergistic action of high pressure, high shear force and high energy water molecule of extruder [15]. During the extrusion process, under the action of high pressure and high shear force, the hydrophobic amino acid inside the zein was exposed, so that the surface hydrophobicity was enhanced[22]. At the same time, it has been reported in the literature that under the conditions of drying or regeneration after high-pressure gelatinization of amylose and alcohol-soluble protein, amylose and alcohol-soluble protein bind by hydrogen bond, making the release of proanthocyanidins in the microparticles with zein added slower[23].

Compared with SP, the release of proanthocyanidins in SAP is relatively slow. In the extrusion process, starch granules mainly undergo two processes of gelatinization and fragmentation. Yuhong Zhao and other studies have shown that proanthocyanidins can combine with sugar molecules to form macromolecular substances, which can enhance the stability of proanthocyanidins[24]. The crushing mechanism of starch granules during extrusion is believed to be the decrease of amylopectin branching, which results in the decrease of amylopectin content[25]. The main components of the microparticles SP and SAP are starch granules and starch mixtures of different degrees of gelatinization, and these mixtures are wrapped around proanthocyanidins. Thermostable α-amylase acts on the 1,4-glycoside bond in starch. The addition of high-temperature-resistant alpha-amylase in SAP causes starch to hydrolyze into small molecule sugars. At the same time, the carbon chain covalent bond in starch molecule breaks down into small molecule sugars during extrusion due to mechanical shear stress. The proanthocyanidins in the particle SAP bind to smaller molecules of sugar, allowing the proanthocyanidins in the particle to be better embedded, thus increasing their stability. Therefore, the stability of SAP proanthocyanidins was stronger than that of SP with the addition of thermostable α-amylase..

However, the particle SZAP, which was also added with heat-resistant alpha-amylase, had the opposite effect as compared with the particle SZP due to the addition of zein. The verification experimental results show that the release amount of proanthocyanidins of microparticle SZP under optimal conditions is slightly lower than that of microparticles SZAP. In the actual test process, the microparticles with thermostable α-amylase added were soaked in the buffer solution, and the microparticles became extremely small, and became a paste with the buffer solution. Because of the low extrusion temperature, the activity of thermostable α-amylase was not affected. Under the action of water and high pressure, starch molecules were decomposed during extrusion, and proanthocyanidins were wrapped by zein. The thermostable α-amylase in the extrudate still maintains a certain vitality. Compared with the SZP without enzyme preparation, the starch, dextrin and sugar in the SZAP microparticles were more, and the contact with the enzyme preparation was increased. Therefore, adding enzyme preparation to the mixture of starch and zein gliadin was more conducive to the release of Proanthocyanidins from the microparticles.

3.3 Fluorescence Microscope

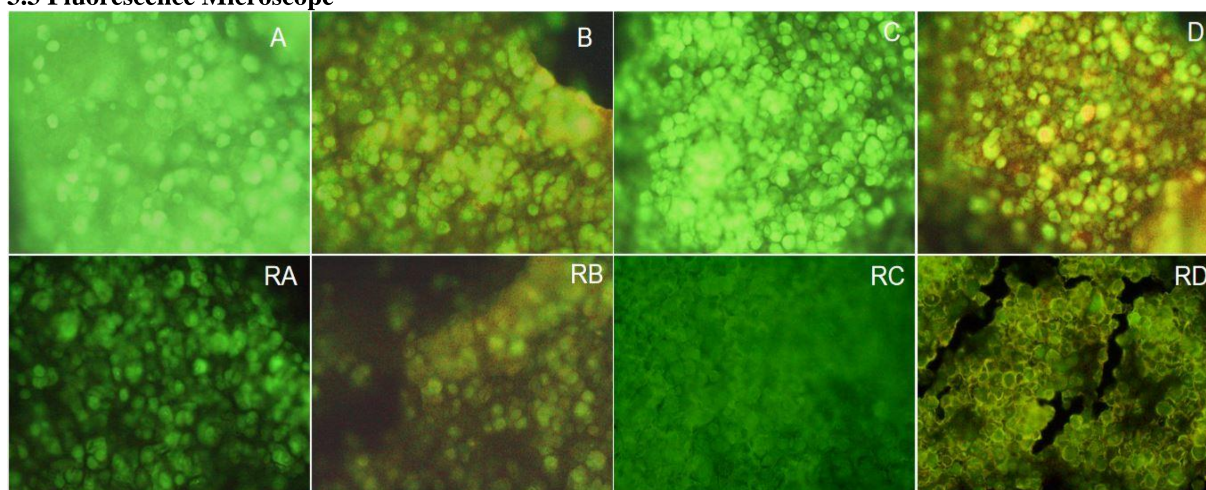

Figure 2 Fluorescence Microscope of starch (green) and protein (red);
(A,B,C,D represent microparticles SP,SZP,SAP,SZAP; RA,RB,RC,RD correspond to the complete release of proanthocyanidins in buffer solution SP,SZP,SAP,SZAP respectively)

Microparticles before and after the release of proanthocyanidins were stained with fluorescein isothiocyanate and Rhodamine B, and starch (green) and protein (red) components were identified respectively. In the samples without protein addition, no red (Fig. 2A and C) was observed. The Red areas of starch with zein microparticles (Fig. 2B and D) were dotted, indicating that zein was densely dispersed among starch molecules. Zein has the characteristics of hydrophobicity, barrier and film formation. The addition of zein makes the Proanthocyanidins in microparticles better embedded. Therefore, the slower the release of proanthocyanidins in SZP and SZAP microparticles with zein is, the stronger the sustained release ability is. The original sample (Fig. 2A - D) had bright green and red fluorescence images, and starch and gliadin molecules in the microparticles were more distinct than those of the sample (Fig. 2RA - RD) released from proanthocyanidins soaked in buffer solution. At the same time, the fluorescence image of the sample (Fig. 2RA - RD) released from the proanthocyanidins soaked in buffer solution was darker and weaker than that of the original sample (Fig. 2A - D). In the actual experimental operation, the changes of particle size after soaking were observed. After soaking, the particle size of SP and SZP decreased slightly, while the particle size of SAP and SZAP after soaking was unknown and almost turned into paste. Therefore, the starch in the granules released by the buffer solution was completely destroyed, and the red areas in SZP and SZAP (Fig. 2RB and RD) were significantly reduced, and the content of zein was decreased, indicating that the corn was soluble during the soaking process. The protein molecule is partially destroyed, allowing the proanthocyanidins to be released. The original four kinds of microparticles are more compact and complete, which can block oxygen, light and water molecules. The contact between starch molecules in the soaked microparticles becomes slightly looser, which also provides the possibility for the release of proanthocyanidins.

IV. Conclusion

In this study, starch, zein, thermostable α-amylase and proanthocyanidins were used as raw materials to prepare four kinds of sustained-release microparticles at low temperature by extrusion technology. Through orthogonal experiment, the influences of temperature, pH and time on the sustained release ability of microparticles were investigated, and the optimal release conditions of four kinds of microparticles were obtained, respectively. Under the optimal conditions, the release rates were 95.30%, 82.27%, 90.75% and 91.67%, respectively. The starch and protein in the microparticles were stained, and the distribution of starch and zein in the microparticles was observed by fluorescence microscopy, the structure comparison of the four microparticles before and after sustained release was carried out. The results showed that the granule prepared by the extruding technology could protect the proanthocyanidins to some extent and had the characteristics of sustained release. Adding enzyme preparation to starch can enhance the stability of proanthocyanidins, while adding enzyme preparation to the mixture of starch and zein can promote the release of proanthocyanidins. Meanwhile, the four kinds of microparticles improved the instability of proanthocyanidins under alkaline conditions. This study provides a theoretical basis for the encapsulation of functional substances with poor stability and the preparation of sustained-release microparticles.

Acknowledgements

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References

[1]. Faraj, A., T. Vasanthan, and R. Hoover, *The effect of extrusion cooking on resistant starch formation in waxy and regular barley flours.* Food Research International, 2004. **37**(5): p. 0-525.
[2]. Alam, M.S., et al., *Extrusion and Extruded Products: Changes in Quality Attributes as Affected by Extrusion Process Parameters: A Review.* Critical Reviews in Food Science & Nutrition, 2015. **56**(3): p. 445-473.
[3]. Alam, M.S., et al., *Extrusion and Extruded Products: Changes in Quality Attributes as Affected by Extrusion Process Parameters: A Review.* Critical Reviews in Food Science & Nutrition, 2016. **56**(3): p. 445-473.
[4]. Vasanthan, T., J. Yeung, and R. Hoover, *Dextrinization of Starch in Barley Flours with Thermostable alpha-Amylase by Extrusion Cooking.* Starch, 2015. **53**(12): p. 616-622.
[5]. Govindasamy, S., O.H. Campanella, and C.G. Oates, *Enzymatic hydrolysis and saccharification optimisation of sago starch in a twin-screw extruder.* Journal of Food Engineering, 1997. **32**(32): p. 427-446.
[6]. Chiang, B.Y. and J.A. Johnson, *Gelatinization of starch in extruded products.* Cereal Chemistry, 1977.
[7]. Sarawong, C., et al., *Effect of extrusion cooking on the physicochemical properties, resistant starch, phenolic content and antioxidant capacities of green banana flour.* Food Chemistry, 2014. **143**(1): p. 33-39.
[8]. Shukla, R. and M. Cheryan, *Zein: the industrial protein from corn.* Industrial Crops & Products, 2001. **13**(3): p. 171-192.
[9]. Wang, J.Y., et al., *Antioxidative mechanism of maize zein in powder model systems against methyl linoleate: effect of water activity and coexistence of antioxidants.* Journal of Agricultural & Food Chemistry, 1991. **39**(2): p. 351-355.
[10]. Ariyoshi and Yasuo, *Angiotensin-converting enzyme inhibitors derived from food proteins.* Trends in Food Science & Technology, 1993. **4**(5): p. 139-144.
[11]. Ying, W. and G.W. Padua, *Tensile Properties of Extruded Zein Sheets and Extrusion Blown Films (p 886-893).* Macromolecular Materials & Engineering, 2010. **288**(11): p. -.
[12]. Selling, G.W., et al., *Reactive extrusion of zein with glyoxal.* Journal of Applied Polymer Science, 2010. **113**(3): p. 1828-1835.
[13]. Selling, G., *The effect of extrusion processing on zein.* Polymer Degradation & Stability, 2010. **95**(12): p. 2241-2249.
[14]. Verbeek, C.J.R. and L.E.v.d. Berg, *Extrusion Processing and Properties of Protein-Based Thermoplastics.* Macromolecular Materials & Engineering, 2010. **295**(1): p. 10-21.
[15]. Batterman-Azcona, S.J., J.W. Lawton, and B.R. Hamaker, *Microstructural changes in zein proteins during extrusion.* Scanning, 2010. **21**(3): p. 212-216.
[16]. Negro, C., L. Tommasi, and A. Miceli, *Phenolic compounds and antioxidant activity from red grape marc extracts.* Bioresour Technol, 2003. **87**(1): p. 41-44.
[17]. Feng, L.L., et al., *Effect of Grape Procyanidins on Tumor Angiogenesis in Liver Cancer Xenograft Models.* Asian Pacific Journal of Cancer Prevention Apjcp, 2014. **15**(2): p. 737.
[18]. Miura, T., et al., *Apple procyanidins induce tumor cell apoptosis through mitochondrial pathway activation of caspase-3.* Carcinogenesis, 2008. **29**(3): p. 585.
[19]. Corder, R., et al., *Oenology: Red wine procyanidins and vascular health.* Nature, 2006. **444**(7119): p. 566.
[20]. Alejo-Armijo, A., et al., *Antimicrobial and antibiofilm activities of procyanidins extracted from laurel wood against a selection of foodborne microorganisms.* International Journal of Food Science & Technology, 2016. **52**(3): p. 679-686.
[21]. Khanal, R.C., L.R. Howard, and R.L. Prior, *Effect of heating on the stability of grape and blueberry pomace procyanidins and total anthocyanins.* Food Research International, 2010. **43**(5): p. 1464-1469.
[22]. Selling, G.W. and K.D. Utt, *Effect of multiple extrusion passes on zein.* Polymer Degradation & Stability, 2013. **98**(1): p. 184-189.
[23]. Guo, J., et al., *Property analysis of resistant wheat amylose and amylopectin with wheat gliadin.* Transactions of the Chinese Society of Agricultural Engineering, 2018.
[24]. Zhao, Y.H., et al., *Stability of proanthocyanidins from Picea koraiensis Nakai in solution.* Journal of Beijing Forestry University, 2016.
[25]. Davidson, V.J., et al., *A Model for Mechanical Degradation of Wheat Starch in a Single-Screw Extruder.* Journal of Food Science, 2010. **49**(4): p. 1154-1157.

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