RECOVERY OF PECTIC OLIGOSACCHARIDE (POS) FROM PECTIN HYDOLYSATE FOR FUNTIONAL FOODS

Nguyen Thi Kim Dung[1], Vu Kim Dung[2] , Chu Thi ThuyTrang[1], Hoang Van Anh[1], Nguyen Tien Thanh[1], Nguyen Thi Xuan Sam[1, *]

[1]*Hanoi University of Science and Technology,No.1 Dai Co Viet road, Hanoi, Vietnam*

[2]*Vietnam Forestry University, Xuanmai town, Hanoi, Vietnam*

Email: sam.nguyenthixuan@hust.edu.vn*

Received: 15 August 2016; Accepted for publication: 5 October 2016

ABSTRACT

Pectic oligosaccharide (POS) obtained bypartial hydrolysis of pectin is proposed as a new class of prebioticwhich has many beneficial propertiesfor the health of humans and animals. Currently only a small number of researcheshave explored the production process of POS products in laboratory-scale and pilot, however the manufacturing process, as well as the product, has not yet been offered for sale on the market. In this study, several parameters of arecovery processofPOS powder from the pectin hydrolysatehave been established: condense(5 times, by tangential filtration with nanofiltration column 0.3 kDa), precipitate (ratio of ethanol / concentrates: 3/1), spray drying (5 % maltodextrin, inlet air temperature 170 ^{o}C, liquid flow rate 2.5 L/h). The total yield of the recovery processes is 67.7 %. The POS product is still stable after 12 months of storage in plastic bags and in bags of tin. Food safety analysis indicate that POS products do not contain mycotoxins, heavymetals, pathogenic microorganisms and the lethal dose LD 50 can not be detected.

Keywords: pectin, pecticoligosaccharide, hydrolysis, spray drying, food safety.

1. INTRODUCTION

Pectic oligosaccharide (POS) made of 2-10 D galacturonic acid units, bound to each other through α glycosidic bonds, is usually obtained by partial hydrolysis of pectin with pectinase preparations [1 - 4]. Recent studies reported healthy effects for POS, including regulation of lipid and glucose metabolism with decreased glycemic response and blood cholesterol levels, anticancer and immunological properties, anti-obesity effects, antibacterial and antioxidant properties [2, 5, 6].To avoid product inhibition in hydrolysis of pectin, membrane bioreactors have been often applied, where the small molecule inhibitors (e.g. product) can easily pass through the membrane and be removed continuously from the system, while the large molecules (substrate and enzyme) are retained by the membrane [4, 7]. The hydrolysateusualycontents diferent components such as product, enzyme, substrate, buffer salt, water, etcwhich requires different recovery steps.The aim of the present work isto develop a recovery process of POS

products from the pectin hydrolysate.The conditions for packaging, storage and assessment of food safety for POS products are also surveyed.

2. MATERIALS AND METHODS

2.1. Materials

Polygalacturonic acid; Mono, di, tri galacturonic acid (G1, G2, G3 respectively); DNS , A thin silica TLC, butanol, acetic acid (Merck);PectinexUtra - SPL (Novozymes);); D - galacturonic Kit (Megazyme, Ireland); Maltodextrin (China); Pectin (extracted from the peel of passion fruit, DE 38 %, 95 % pectin content - Hanoi University of Science and Technology).

2.2. Methods

2.2.1. Pectin hydrolysis: the reaction is carried out in system integrating two membranes (50 kDa and 1 kDa). Reaction conditions: 1 % pectin , 42 ^{o}C, pH 4 , the enzyme 24 U/g pectin , 225 rpm, retention time on the system 2.5 hours). Hydrolysate is taked for testing.

2.2.2. Identificationof the POS components by Thin Layer Chromatography (TLC) [7]: solventsystem: butanol: acetic acid: water = 9 : 4 : 7 (v / v / v), colored by 10 % sulfuric acid , 120 ^{o}C dryer for 5 minutes.

*2.2.3. Determination of the POS content*was performed by using Kit D - Galacturonic [3]: treatment with H_2SO_4 2M, POS will be completely hydrolyzed into galacturonic acid. The galacturonic acid is determined by D - galacturonic kit according to manufacturer's instructions.

2.2.4. Recovering POS from hydrolysate

Concentration: experiment was conductedwith 0.3kDananofiltration column (Model: DL2540F1072), pressure maintained on the column at 15 to 20 bar. Filtration rate 170-180 L/h.Hydrolysatewas concentrated 5 times. The recovery efficiency of the condense(H1) was determined by ratio of POS in concentrate to initial POS in hydrolysate.

Precipitation: different ratios of ethanol/concentrates 1/1; 2/1; 3/1 and 4/1 (v/v) were tested in conditions of 4 °C, 4 hours. The precipitate was washed with ethanol 96^{o} followed centrifuged 10000 rpm for 15 minutes. Similarly, the recovery efficiency of the precipitation step (H2) was determined by ratio of POS in precipitate to initial POS in concentrate. On the other hand, the desalination was also estimated.

Spray drying: This tests were performed on LPG5 spray drying system with 3 modes of input temperature (°C) - input flow rate (L/ h) as follows: (200 - 2.5); (170 - 2.5) and (150 - 2). Atomizer speed 23,000 rpm. The recovery efficiency of the spray drying step was noted as H3, calculated by ratio of POS inpowder to initial POS in firstsuspension

Determine the recovery efficiency of the whole process (H):

$$H = H1 \times H2 \times H3 \times 100 (\%)$$

H1, H2, H3 indicate the recovery efficiency corresponds to the step of concentration, precipitation and spray-drying, respectively. Number of 100 is calculated in %.

2.2.5. Determination of sodium citrate as following the standard of NTR 4-11 : 2010 / BYT.

2.2.6. Identifying microorganisms: microorganisms total aerobic (TCVN 9977 : 2013), *E. coli* (ISO 9974, 2013), *Salmonella* (ISO 4829 : 2005)

3. RESULTS AND DISCUSSION

3.1. Concentration of hydrolysate

Hydrolysate in this case is quite dilute (1 %) and hence needs to be concentrated to achieve higher levels of dry matter for the precipitation process or spray drying followed.The trial was conducted with 100 liters of hydrolysate, concentrated 5 times by 0.3 kDa filtration column. The analysis results of POS content in Table 3.1 shows the POS recovery yield of this step (H1) is at 88.2 %. TLC analysis results in Figure 3.1 showthat the concentrate still contains monogalac turonic (line 1) while the filtrate behind the membrane does not contain these monosacharides (line 2). The conductivity of concentrate increased while this parameter of the filtrate is close bythat of water (results not shown here). These results indicated that the nano membrane used in this experiment only has a condensing effect, but notdesalination and removal of simple sugars.

Table 3.1. The POS recovery efficiency of concentrate stage determined by nanofiltration.

Sample	Volume (liter)	POS content (mg/ml)	POS total (g)	Recovery yield (%) - H_1
Hydrolysate	100	8.47	847	100
Concentrate	20	37.35	747.05	88.2

*Figure 3.1.*Chromatography of test solutions with 0.3kDa membrane

M. Standard oligosaccharide
1.Concentrated solution (upermembran)
2. The filtrate removal (bihindmembran)

M 1 2

3.2. POS precipitation by ethanol

Analytical results above show that concentrate still contains considerable amounts of buffer salts (sodium citrate), whichwill affect the taste of the final product and hence should be removed. There are many solutions to remove salt from a product, such as using of membranes with suitable pore size, dialysis or precipitation, etc. In this case ethanol was selected to precipitate the POS.

The trial was conducted as described above and the results of the recovery efficiency of the precipitation step (H2) and the ability to remove the saltare shown in Table 3.2.

Results in Table 3.2 show that when theethanol/concentrate ratio increases, recovery efficiency also increases and reached the highest value is 85.2 % at the ratio of 5/1. However, at

the ratio of 3/1, POS recovery efficiency is also achieved at nearly 83 %. Therefore, based on product price, this ratio was selected for the precipitation POS. A. Lama - Munoz et al (2012) also obtained POS having size from 0.3 to 1 kDa at 80 % ethanol segment. Moreover, at this ratio, 96.6 % of salt was detected in liquid phase (after precipitation of POS), this indicates the efficiency of remove salt by ethanol precipitation.

*Table 3.*2. The influence of the ethanol/concentrates ratio to precipitate efficiency and to ability to remove salt.

Ethanol /concentrates ratio (v/v)	Total POS (g)	Recovery yield (%)-H_2	Total salt in liquid phase (g)	Ability to remove the salt (%)
0:1	3.70	-	0.766	-
1:1	1.8	48.60	0.754	98.43
2:1	2.65	71.64	0.750	97.91
3:1	**3.05**	**82.56**	**0.740**	**96.61**
4:1	3.09	83. 60	0.740	96.61
5:1	3.15	85.20	0.740	96.61

3.3. Spray drying

The trial was conducted on LPG5 spray drying system with 100 L of suspension which was prepared from precipitate above (70 mg POS /ml) andadding 5 % maltodextrin. The most suitabledrying conditionat input air temperature170 ^oC and input flow rate2.5 L/h was determined for best quality and the highest efficiency (Table 3.3).

Table 3.3. Effects of dryingparameters on the efficiency and product quality.

Test	Dryingparameter		POS total (kg)	Recovery yield (%) - H_3	Notes
	Inlet air temperature (^oC)	Feed flow rate (l/h)			
First suspension			7.00	-	-
1	200	2.5	5.88	84	High lost on the wall of spray chamber. Powder is hygroscopic quickly
2	170	2.5	6.51	93	Powder is dry, less lost, easy to manipulate
3	150	2	6.30	90	Powder is dry, not sticky, easy to manipulate

Dehydration by spray drying is used in the wide range of products in food industries to produce dry powders and agglomerates. In powders, it results in good quality, low water activity, easier transport and storage. The physicochemical properties of powders produced by spray drying depend on the variables of process and/or operating parameters, such as inlet temperature, feed flow rate, types of carrier agent and their concentration.Normally, the inlet temperature used for spray drying technique for food powder is 150 – 220 ^{o}C. The increase of inlet air temperature has reduced the yield which might be caused by the melting of the powder and cohesion wall [8].

Base on the results of the above stages, the total yield of the recovery process (H) from hydrolysate to POS powder was determined to beat 67.7 % (as shown in 2.2.4).

3.4. Storage of POS preparation

The packaging material is used to protect the product and prevent contamination from external sources. The packaging environment should be able to slow down or prevent the growth of undesirable microorganisms in or on the product by use of anaerobic conditions or inert gas atmosphere.

All of the POS samples were kept in a dry place sealed in PE bag or tin bag (light protection) and stored at ambient temperature. For every 2 months, samples were taken to determine the quality and microorganisms indicators.

Table 3.4. Effect of type of packaging on the parameters of POS powder.

Evaluation indicator	Type of packaging	Storage time (months)						
		0	2	4	6	8	10	12
POS content (g/g)	PE bag	0.55	0.55	0.54	0.54	0.53	0.53	0.53
	Tin bag	0.55	0.55	0.55	0.54	0.54	0.54	0.53
aerobic microorganism (CFU/g)	PE bag	ND	ND	ND	ND	ND	20	30
	Tin bag	ND						
E. coli (CFU/g)	PE bag	ND						
	Tin bag	ND						
Salmonella (CFU/g)	PE bag	ND						
	Tin bag	ND						
Total yeast, mold (CFU/g)	PE bag	ND						
	Tin bag	ND						
Moisture content (%)	PE bag	5.18	5.21	5.21	5.23	5.33	5.35	5.45
	Tin bag	5.18	5.20	5.21	5.21	5.30	5.30	5.34

As shown in Table 3.4, the composition and moisture content of product are almost consistent after 12 months of storage in both types of packaging. Such low moisture content (5

%) is in agreement to the absence of the growth of harmful bacteria such as *E. coli*, *Salmonella* or yeast and mould. Overall, these results suggest that both PE bags and tin bagscould be used to preserve the POS powder for at least one yearin under normal conditions.

3.4. Assessing the quality and safety of POS product

The results of quality and food safety tests of POS product, presented in Table 3.5, were obtained from the National Institute of Nutrition. The data shows that the POS product satisfies the microbiological and chemico-physical requirements of the National technical regulations for fungal toxins (NTR 8-1: 2011/BYT), for heavy metals (NTR 8-2: 2011/BYT) and for pathogenic microorganisms (NTR 8- 3: 2011/BYT).

Toxicological test results at the Central Drug Testing Institute also showthatthere is not unusual expression and nor toxicity expression when testing on rats with 20– 60 g POS sample/kg of rats. The lethal dose LD 50 can not be detected.Therefore it could be conclude that POS preparation is in the nontoxic material group.

Table 3.5. The results of quality analysis POSpreparation.

No.	Analysis Indicator (Methods)	Unit	Result
1	Protein *(AOAC991.20)*	g/100g	1.06
2	Total sugar *(AOAC920.183)*	g/100g	69.14
3	Total aflatoxin (B1, G1, B2, G2) *(AOAC990.33)*	µg/kg	ND
4	Asen *(AOAC999.10)*	µg/kg	ND
5	Lead *(AOAC999.10)*	µg/kg	0.12
6	Mercury *(AOAC999.10)*	µg/kg	ND
7	Cadimi *(AOAC999.10)*	µg/kg	0.012
8	Total number of aerobic bacteria (TCVN 4884:2005)	CFU/g	7.9×10^2
9	*Coliforms* (TCVN 6848:2007)	CFU/g	ND
10	*E. coli* (TCVN 7924-2:2008)	CFU/g	ND
11	*Cl. Perfringens* (TCVN 4991:2005)	CFU/g	ND
12	*Salmonella* (TCVN 4929:2005)	CFU/25g	ND
13	Total number of yeast, moldy spores (TCVN 8275-2:2010)	Spore/g	ND

ND: Not Detected

4. CONCLUSION

The highest recovery yield (67.7 %) of POS from pectin hydrolysateobtained using first step ofconcentration (5 times) by column 0.3 kDa follow the step of remove salt by ethanol (ratio of ethanol /concentrates: 3/1) and final step ofspray drying (7 % POS, 5 % maltodextrin, inlet air temperature 170 ^{o}C, liquid flow rate 2.5 L /h). POS preparation satisfies the demand of

quality, food safetyand having prebiotic activity, this has a great potential for manufacture of funtional foods.

Acknowledgements. The research work was supported by the project "Production of Pectic oligosaccharide (POS) using enzyme for the application in functional food". 04-14/CNSHCB , Ministry of Industry and Trade.

REFERENCES

1. Lama-Munoz A., Rodriguez-Gutierrez G., and Rubio-Senent F. - Production, characterization and isolation of neutral and pectic oligosaccharides with low molecular weights from olive by-products thermally treated, Food Hydrocolloids **28** (2012) 92-104.
2. Gullón B., Gómez B., Martínez Sabajanes M., Yáñez R., Parajó J. C., Alonso J. L. - Pectic oligosaccharides: manufacture and functional properties, Trends Food Sci. Technol. **30** (2013) 153−161.
3. Megazyme - D-glucuronic acid & D-galacturonic acid assay procedure,Megazyme International Ireland (2014) 1-5.
4. Olano Martin E., Mountzouris K. C., Gibson G. R., Rastall R. A. - Continuous production of pectic oligosaccharides in an enzyme membrane reactor, Food Engineering and Physical Properties **66** (7) (2001) 966-971.
5. Mandalari G., Nueno P. C., Tuohy K., Gibson G. R., Bennett R. N., Waldron K. W., Bisignano G., Narbad A., Faulds C. B. - In vitro evaluation of the prebiotic activity of a pectic oligosaccharide – rich extract enzymatically derived from bergamot peel, Appl. Microbiol. Biotechnol. **73** (2007) 1173−1179.
6. Martínez Sabajanes M., Yáñez R., Alonso J. L., Parajó J. C. - Pectic oligosaccharides production from orange peel waste by enzymatic hydrolysis, Int. J. Food Sci. Technol. **47** (2012) 747−754.
7. Kiss K., et al. - Vacuum assisted membrane bioreactor for enzymatic hydrolysis of pectin from various agro-wastes, Desalination **241** (2009) 29-33.
8. Phisut N. - MiniReview:Spray drying technique of fruit juice powder: some factors influencing the properties of product, International Food Research Journal **19** (4) (2012) 1297-1306.

TÓM TẮT

THU NHẬN PECTIC OLIGOSACCHARID (POS) TỪ DỊCH THỦY PHÂN PECTIN CHO SẢN XUẤT THỰC PHẨM CHỨC NĂNG

Nguyễn Thị Kim Dung[1], Vũ Kim Dung[2], Chu Thị ThùyTrang[1], Hoàng Vân Anh[1], Nguyễn Tiến Thành[1], Nguyễn Thị Xuân Sâm[1, *]

[1]Trường Đại học Bách khoa Hà Nội, số 1 Đại Cồ Việt, Hà Nội, Việt Nam

[2]Trường Đại học Lâm nghiệp, Thị trấn Xuân Mai, HàNội, Việt Nam

[*]Email: *sam.nguyenthixuan@hust.edu.vn*

Pectic oligosaccharide (POS), sản phẩm thủy phân không hoàn toàn của pectin, là một prebiotic thế hệ mới có nhiều đặc tính quý có lợi cho sức khỏe của người và vật nuôi. Hiện tại mới chỉ có một số công bố về quy trình sản xuất POS ở quy mô phòng thí nghiệm và pilot, tuy nhiên công nghệ sản xuất và sản phẩm vẫn chưa được chào bán trên thị trường. Nghiên cứu này xác định được các điều kiện thích hợp cho việc thu nhận chế phẩm POS dạng bột từ dịch thủy phân pectin: cô đặc (5 lần bằng lọc tiếp tuyến với cột lọc nano 0,3 kDa), kết tủa (tỉ lệ ethanol/dịch cô đặc: 3/1), sấy phun (bổ sung 5 % maltodextrin, nhiệt độ không khí đầu vào 170 ˚C, tốc độ tiếp liệu 2,5 lít/giờ). Hiệu suất thu hồi của toàn bộ quá trình từ dịch thủy phân tới chế phẩm dạng bột đạt 67,7 %. Sau 12 tháng bảo quản trong túi PE 2 lớp hoặc trong túi thiếc, chất lượng của chế phẩm vẫn giữ được ổn định. Các kết quả đánh giá về an toàn thực phẩm cho thấy chế phẩm POS không nhiễm độc tố vi nấm, kim loại nặng và vi sinh vật gây bệnh cũng như không xác định được liều gây chết LD_{50} khi thử nghiệm trên chuột.

Từ khóa: pectin, pectic oligosaccharide, thủy phân, sấy phun, an toàn thực phẩm.