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

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ABSTRACT

Pectic oligosaccharide (POS) obtained by partial hydrolysis of pectin is proposed as a new class of prebiotic which has many beneficial properties for the health of humans and animals. Currently only a small number of researches have 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 a recovery process of POS powder from the pectin hydrolysate have 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 °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 hydrolysate usually contains different components such as product, enzyme, substrate, buffer salt, water, etc which requires different recovery steps. The aim of the present work is to 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); Pectinex Ultra - 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 °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. Identification of the POS components by Thin Layer Chromatography (TLC) [7]: solvents system: butanol: acetic acid: water = 9 : 4 : 7 (v/v/v), colored by 10 % sulfuric acid, 120 °C dryer for 5 minutes.

2.2.3. Determination of the POS content was performed by using Kit D - Galacturonic [3]: treatment with H2SO4 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 conducted with 0.3kDa nanofiltration column (Model: DL2540F1072), pressure maintained on the column at 15 to 20 bar. Filtration rate 170-180 L/h. Hydrolysate was 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° 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 in powder to initial POS in first suspension.

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

\[ H = H_1 \times H_2 \times H_3 \times 100 \, (\%) \]

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 %.

**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 (%) - H₁ |
|----------|----------------|---------------------|---------------|-------------------------|
| Hydrolysate | 100            | 8.47                | 847           | 100                     |
| Concentrate | 20             | 37.35               | 747.05        | 88.2                    |

3.2. POS precipitation by ethanol

Analytical results above show that concentrate still contains considerable amounts of buffer salts (sodium citrate), which will 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 salt are shown in Table 3.2.

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

M. Standard oligosaccharide
1. Concentrated solution (uppermembran)
2. The filtrate removal (behindmembran)

**Table 3.2**

| Sample   | Volume (liter) | POS content (mg/ml) | POS total (g) | Recovery efficiency (%) - H₂ |
|----------|----------------|---------------------|---------------|------------------------------|
| Hydrolysate | 100            | 8.47                | 847           | 85.2                        |
| Concentrate | 20             | 37.35               | 747.05        | 85.2                        |

Results in Table 3.2 show that when the ethanol/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 (%) H₂ | 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) and adding 5% maltodextrin. The most suited drying condition at input air temperature 170 oC and input flow rate 2.5 L/h was determined for best quality and the highest efficiency (Table 3.3).

Table 3.3. Effects of drying parameters on the efficiency and product quality.

| Test | Drying parameter | POS total (kg) | Recovery yield (%) H₃ | Notes |
|------|------------------|----------------|-----------------------|-------|
|      | Inlet air temperature (°C) | 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 °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 be 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    | PE bag            | ND |     |     |     |     |     |     |
| (CFU/g)              | 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 bags could be used to preserve the POS powder for at least one year in 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 show that there is no 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.

| 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 x 10² |
| 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 hydrolysate obtained using first step of concentration (5 times) by column 0.3 kDa follow the step of remove salt by ethanol (ratio of ethanol/concentrates: 3/1) and final step of spray drying (7 % POS, 5 % maltodextrin, inlet air temperature 170 °C, liquid flow rate 2.5 L/h). POS preparation satisfies the demand of
quality, food safety and having prebiotic activity, this has a great potential for manufacture of functional foods.

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TÓM TÁT

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

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Pectic oligosaccharide (POS), sản phẩm thủy phân không hoàn toàn của pectin, là một prebiotic thể hiện có nhiều đặc tính quý có lợi cho sức khỏe của người và vật nuôi. Hiện tại mỗi chi 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 chao 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 quy trình thủy phân pectin: có đặc (5 lần bằng lọc tiếp tục với cột lọc nano 0,3 kDa), kết tủa (tỉ lệ ethanol/dịch cỏ đặc: 3/1), say phun (bổ sung 5% maltodextrin, nhiệt độ không khí đầu vào 170 °C, tốc độ tiếp liều 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 tới vi nấm, kim loại nặng và vi sinh vật gây bệnh cũng như không xác định được liệu gây chết LD₅₀ khi thử nghiệm trên chuột.

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