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FULL PAPER

Characteristics of inulin from mangrove apple (*Soneratia caseolaris*) with different extraction temperatures

[1,3]Wibawanti, J.M.W., [2,*]Mulyani, S., [2]Legowo, A.M., Hartanto, R., [2]Al-Baarri, A. and [2]Pramono, Y.B.

[1]*Department of Animal Science, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang, Indonesia*
[2]*Department of Food Technology, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang*
[3]*Department of Animal Science, Faculty of Agricultural Science, (Universitas Muhammadiyah Purworejo*

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Abstract

Mangrove apple (*Soneratia caseolaris*) is a mangrove species from *Sonneratiaceae* family, rich in dietary fibre, from which they are composed of soluble dietary fibres. Inulins are carbohydrates and act like dietary fibre. The study on elucidating the characteristics of inulin from mangrove apples with different extraction temperatures has never been published. This present study aimed to determine the characteristics of inulin extracted from mangrove apples at different temperatures during the extraction process. The data were analyzed using Analysis of Variance (ANOVA) and further test Duncan's Multiple Range Test ($P<0.05$) and the experimental design in this study used a Completely Randomized Design (CRD) with four different extraction temperatures (60, 70, 80, and 90°C). They were relatively higher total yield ($p<0.05$), inulin content ($p<0.05$), solubility ($p<0.05$), soluble dietary fibre ($p<0.05$), FOS, but lower in the total sugar and reduction sugar in the extraction temperature at 90°C. The study indicated that the temperatures during the extraction process were able to improve the characteristics of inulin from mangrove apple.

1. Introduction

Prebiotics are a group of compounds that can serve as the substrate for microflora in the gastrointestinal tract (Sugiharto *et al*., 2014; Davani-Davari *et al*., 2019). Inulin, fructooligosaccharides (FOS), lactulose, and galactooligosaccharides (GOS) have been identified as the prebiotics in functional food (Mensink *et al*., 2015). Inulins are polysaccharides and part of soluble dietary fibre mainly containing numerous fructose units and a terminal glucose unit (Mudgil and Barak, 2013; Li *et al*., 2019). They are characterized by various degrees of polymerization (DP) ranging from 2-60 monosaccharide units, which are connected by β-(2-1) glycosidic bonds (Kim *et al*., 2001; Yudhistira *et al*., 2020). Previous research showed that prebiotics can be produced from a variety of plants such as gembili tubers (Setyaningrum *et al*., 2019), chicory root (El-Kholy *et al*., 2020), Jerusalem artichoke, asparagus, salsify, dandelion, and garlic (Kaur and Gupta, 2002). Inulin can also be found in several fruits such as banana (Mensink *et al.,* 2015) and red fruits (*Pandanus conoideus* L) (Murtiningrum *et al*., 2019).

Mangrove apples (*Soneratia caseolaris*) is a type of mangrove plant that grows in tropical areas. It is included as a mangrove species family *Sonneratiaceae* containing about 15.95% carbohydrate, 2.24% protein, 0.86% fat, 77.10% moisture, and 3.85% ash (Jariyah *et al*., 2014). The fruit also contains some functional compounds such as polyphenols, flavonoids, anthocyanin, vitamins, antioxidants, and antibiotics (Avenido and Serrano, 2012; Thuoc *et al*., 2018). Moreover, it is rich in dietary fibre (63.70% dry mass), from which it consists of 9.8% soluble dietary fibre (Jariyah *et al*., 2016). For this reason, the mangrove apple has the potential to be developed as a natural prebiotic source candidate.

It has been reported that there are a number of factors determining the quality of inulin, including the extraction process and inulin source (Gholami *et al*., 2018; Li *et al*., 2019). Temperature is one of the most important factors in the extraction process to produce inulin (Kim *et al*., 2001). To date, the study elucidating

*Corresponding author.
Email: *srimulyani@lecturer.undip.ac.id*

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the characteristics of inulin from mangrove apples with different extraction temperatures has never been published. Therefore, the objective of the present study aimed to determine the characteristics of inulin extracted from mangrove apple at different temperatures during the extraction process.

2. Materials and methods

2.1 Methods inulin extract

Inulin extract was prepared from mangrove apple (*Soneratia caseolaris*) obtained from the beach of Purworejo city, Central Java, Indonesia. Inulin was extracted by the method of Setyaningrum *et al*. (2019) with some modifications. Mature, yellowish and soft mangrove apples were selected and carefully washed with water to remove undesirable materials and the edible parts of the mangrove apple were separated from the seeds. Mangrove apple was cut into small pieces for the extraction and the Spectrophotometric method was performed at the temperature of 60, 70, 80, and 90°C. Each extraction with different temperatures was measured five times. Mangrove apple was extracted at a ratio of 1:4 (fruit: hot water at 90°C w/v) for 60 minutes. The filtrate was precipitated with 70% ethanol (1:4 w/v), and frozen at - 18°C for 6 hrs. The filtrate was thawed at room temperature. Following this, the filtrate inulin of mangrove apple was centrifuged at 5000 rpm for 5 mins, and the supernatant was removed. The inulin of mangrove apple was dried at 50°C in the cabinet dryer for 12 hrs. The dried inulin of mangrove apple was dry milled, and the resulting was sieved 60 mesh. They were then stored in an airtight container in a refrigerator until the analysis.

2.2 Determination of total yield

The inulin extraction yield was carried out using the method according to Lingyun *et al*. (2007). Final inulin sample of mangrove apple divided initial sample weight x 100.

2.3 *Determination of inulin content*

The determination of inulin content in the mangrove apple was performed based on the Spectrophotometric method according to Petkova *et al*. (2013); Petkova and Denev (2015) with some modifications. The sample of 1 g of mangrove apple was dissolved in distilled water (1:4 v/v) and put in the water bath at a temperature of 80°C for 5 mins. After cooled down, the samples were added with Seliwanoff reagent (1:5 v/v) and mixed by Vortex for 5 mins. The samples were heated for 5 mins and the mixture was centrifuged at 5000 rpm for 5 mins. The absorbance of the solution was then measured at 520 nm with a spectrophotometer (Shimadzu, Kyoto, Japan). A standard curve was plotted using the inulin standard.

2.4 Determination of soluble dietary fibre

The determination of soluble dietary fibre of mangrove apple was analyzed by a multienzyme (AOAC, 1995).

2.5 Determination of inulin solubility

Inulin solubility of mangrove apple was performed based on AOAC (1995). However, a few modifications were incorporated into this procedure. Before being used, the Whatman filter paper was heated at 105°C in an oven until constant weight is achieved (W1). The inulin of mangrove apple was scaled at 1 g (W2) and dissolved in 20 mL distilled water at a temperature of 90°C. They were then filtered by Whatman filter paper 41. The sample in the filter paper was heated in an oven at 105°C for about 3 hrs until reaching the constant weight. Furthermore, it was taken out and cooled in a desiccator (W3). The inulin solubility of mangrove apple was later calculated.

$$\text{Solubility (\%)} = 1 - \left(\frac{W3-W1}{W2}\right) \times 100\%$$

Where W1 = the filter paper, W2 = initial weight sample, and W3 = the filter paper + final weight sample

2.6 Determination of total Sugar

Total sugars of inulin of the mangrove apple extract were determined based on a D-Glucose curve the method according to the Yuliana *et al*. (2014) with a few modifications. The samples of about 1 g were added with 50 mL distilled water. Then, the samples were added with 3 mL HCL 25% and heated in a water bath at 100°C for 10 mins. The samples were diluted water distillate until 100 mL before being filtered using Whatman paper. The filtrate (1 mL) was added with 1 mL Nelson reagent (Nelson A 25:1 Nelson B). The mixture filtrates were heated in the water bath at the temperature of 100°C for 30 mins. After cooling, the samples were added with 1 mL Arseno Molibdat and shaken until homogeneous. Distilled water was added to the samples until the volume reached 10 mL before being vortexed. The samples were determined by *spectrophotometer* with an absorbance of 540 nm.

2.7 Determination of reducing sugar

Reducing sugar in the inulin of the mangrove apple extract was determined using the Somogyi- Nelsen method previously described by (Susilowati *et al*., 2015) with modification. The inulin of mangrove apple was prepared 1 g into Erlenmeyer. Thereafter, the samples were added with distilled water (100 mL). The sample was centrifuged and filtered by Whatman paper. The

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filtrate (1 mL) was dissolved in 1 mL Nelson reagent and the sample was heated over a water bath at the temperature of 100°C for 30 mins. The solution was cooled to 25°C, added with 1 mL of arsenomolybdate reagent, and was shaken. The distilled water was added into 10 mL and the solution was mixed by a vortex. The reducing sugar of inulin mangrove apple ware determined with a spectrophotometer at a wavelength of 540 nm. The concentration of reducing sugars was estimated using a D-Glucose standard curve.

2.8 Determination of fructo oligosacaride

Fructo oligosaccharide (FOS) of mangrove apple was determined by HPLC the method based upon Petkova *et al*. (2013) with some modifications. The calibration curve was built using fructose as a standard. The linearity of the standard curve was obtained at the concentration ranges of 25-500 µg/mL and characterized with the equation: $Y = 32209.4x - 318697$; $R^2 = 0.999$. Where y is the absorbance at 480 nm and x is the concentration of FOS, µg/mL.

2.9 Statistics Analysis

Data collected from the research were analysed by SPSS 16 method. The statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's test to assess the difference between mean values. The differences, at this point, were considered significant when P< 0.05.

3. Results and discussion

3.1 The total yield of inulin of mangrove apple

Table 1 shows the amounts of yield from mangrove apple. The extraction temperature had a significant effect (p<0.05) on the yield of mangrove apple. The yield of inulin of mangrove apple might be due to the process of the increasing extraction temperature in which it was found higher than that of Yudhistira *et al*. (2020) which showed the blanching white sweet potato yield of 22.53%. Winarti *et al*. (2011) reported that with precipitation temperature at -20°C the yam tubers with obtained inulin yield was 21.08%. According to Kosasih *et al*. (2015), the yield of inulin dahlia tuber was caused by the precipitation of ethanol solvent.

The results showed the increase of inulin yield from mangrove apple by the difference of extraction temperature. When compared with another treatment, the extraction temperature at 90°C had a higher total yield. However, no difference in total yield was observed between the extraction temperature at 80 and 90°C. The total yield of inulin mangrove apple in the extraction temperature at 60 and 70°C was not different (p>0.05). The difference of yield from mangrove apple extract was probably due to the influence of the extraction temperature during the hydrolysis process. It was related to the solubility by inulinase enzyme activity to form fructooligosaccharides that it could increase the total yield. In addition to temperature, the total yield of mangrove apple was also influenced by the type of solvent. High-temperature water solvent can assist the diffusion process. In literature, Kim *et al*. (2001) reported that the heating process can affect the increasing of gel formation of inulin. According to Petkova *et al*. (2020), the yield of inulin of Burdock (*Arctium lappa* L.) roots with ultrasound-assisted water extraction was revealed as the preferred method for extraction.

Table 1. Yield, total sugar, and reduction sugar of inulin from mangrove apple

The Extraction Temperature	Yield (%)	Total Sugar (%)	Reduction Sugar (%)
60°C	9.44 ± 0.36^{a}	2.23 ± 0.76^{a}	1.94 ± 0.81^{a}
70°C	9.37 ± 1.07^{a}	1.17 ± 0.39^{b}	0.90 ± 0.36^{b}
80°C	10.08 ± 0.93^{ab}	1.57 ± 0.13^{b}	1.23 ± 0.24^{b}
90°C	10.86 ± 0.54^{b}	1.13 ± 0.19^{b}	0.99 ± 0.18^{b}

Values are expressed as mean ±standard deviation. Values with different superscripts within the same column are significantly different (p<0.05).

3.2 Inulin content

Figure 1 shows the amounts of inulins from mangrove apple extract. The extraction temperature had a significant effect (p<0.05) on the inulin content of mangrove apple extract. In this research, the extraction temperature had an effect on the hydrolysis process where that process had a number of changes in the fructose and glucose structure. The extraction temperature increased inulin content in the mangrove apple. As the inulin from mangrove apple also contains inulase enzyme; thus, it can support the hydrolysis process. Enzymes act as catalysts in the hydrolysis process at 1→-4- glycosidic bonds with the increasing inulin content. In the literature, Susilowati *et al*. (2015) inulin fibre is obtained through enzyme inulin derived from plants and microbial isolation. As reported in a previous study, the inulin content (Glibowski and Bukowska, 2011) depended on the temperature, pH of treatment and heating time. Inulin extracted by hot water extraction would have inulin-type fructans that vary in the number of fructose units from a low to a maximum (Kelly, 2008). The long inulin chains were broken into several smaller chains during the hydrolysis process. Li *et al*. (2019) reported that the short-chain inulin is the replacers for sugar in low-sugar foods.

In this research, the inulin content of mangrove apple

FULL PAPER

was caused by the total soluble dietary fibre. The amount of inulin increased with the increase of the total soluble dietary fibre through the difference in extraction temperature. In the literature of Mensink *et al*. (2015) inulin is a fructan-type polysaccharide including soluble dietary fibre.

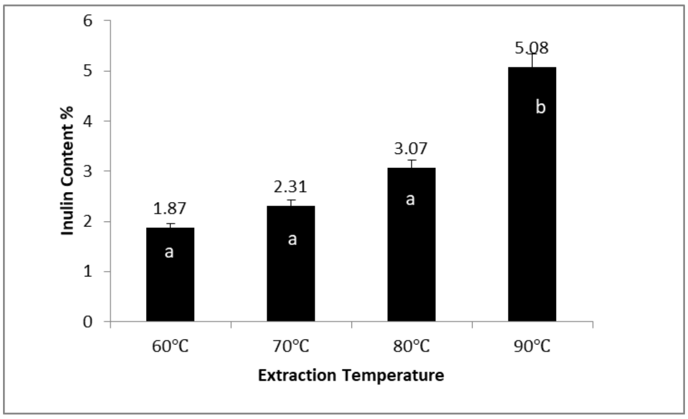

Figure 1. Inulin content of mangrove apple. Different alphabet notations in the bar indicate significant difference ($p<0.05$).

3.3 Soluble dietary fibre

Figure 2 illustrates the soluble dietary fibres of inulin from mangrove apple extract at different temperatures. The figure also shows that the differences in extraction temperature had a significant effect ($p<0.05$) on the soluble dietary fibre of inulin from mangrove apple extract. The amount of soluble dietary fibre is related to the inulin content of mangrove apple extract. The extraction temperature causes a hydrolysis process in which is fibre produces soluble dietary fibre. It might be due to the ability of the hydrolysis process of fructant polymers to form oligosaccharides, namely fructooligosaccharides. The soluble dietary fibre has been studied previously by Melanie *et al*. (2015) stated that enzymatically hydrolysis of Dahlia red is soluble dietary fibre. Inulin is an oligosaccharide group, as the soluble dietary fibre. Both inulin and inulin fructans are known as soluble dietary fibres (Roberfroid, 2005).

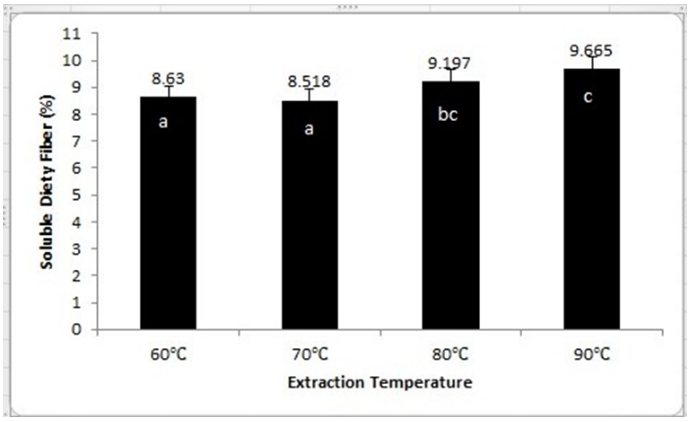

Figure 2. Soluble dietary fibre of inulin from mangrove apple. Different alphabet notations in the bar indicate significant difference ($p<0.05$).

In the research, as the extraction temperature increased, the soluble dietary fibre of the inulin from mangrove apple extract increased ($p<0.05$). The extraction temperature at 80°C had stronger soluble dietary fibre when compared with the extraction temperature at 70 and 60°C. The highest soluble dietary fibre was found in the extraction temperature at 90°C; it was probably due to the high temperature causing hydrolyze glucose at the last chain, which is the increase of the soluble dietary fibre of inulin of mangrove apple. The high soluble dietary fibre corresponded with the increase of inulin content of mangrove apple. According to Melanie *et al*. (2015), the soluble dietary fibre in the red Dahlia tubers was found at 9.73% at the temperature of 90°C.

3.4 Inulin solubility

As shown in Figure 3, the inulin solubility of all samples were correlated well with the increase of extraction temperature ($p<0.05$). Here, that solubility was partly attributed to the chains of the inulin. The high extraction temperature increased the solubility rate. In this research, the high solubility of inulin from mangrove apple extract corresponded to the temperature extraction. According to Toneli *et al*. (2008), inulin was soluble in water with solubility dependent upon the temperature. Inulin solubility of mangrove apple was found lower than the one by Kim *et al*. (2001) reporting that a temperature of 90°C increased solubility by as much as 34% (w/v). Inulin solubility of mangrove apple extract was also caused by process extraction. A similar result was obtained by Naskar *et al*. (2010) finding out that the solubility of inulin was dependent on the medium used in the process extraction.

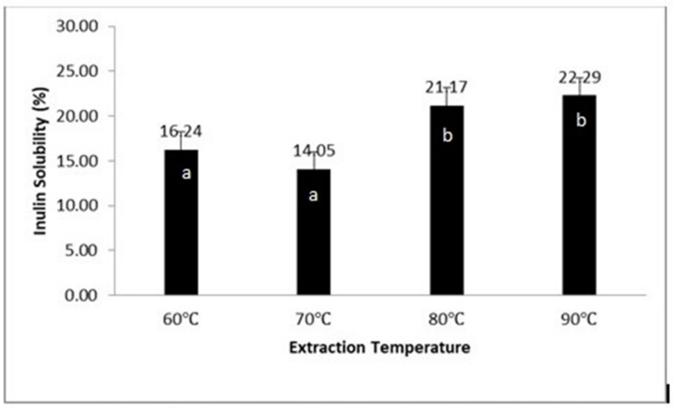

Figure 3. The solubility of inulin from mangrove apple. Different alphabet notations in the bar indicate significant difference ($p<0.05$).

The amount of inulin solubility increased with the increase of extraction temperature ($p<0.05$). The extraction temperature at 90°C had higher inulin solubility when compared with another extraction temperature. Inulin solubility on the extraction temperature at 80°C and 90°C were higher than that of at

60°C and 70°C ($p<0.05$). The inulin solubility on the extraction temperature at 80°C, however, were not different from the one at 90°C ($p>0.05$). Furthermore, no difference in inulin solubility was observed between extraction temperature at 60°C and 70°C ($p>0.05$). The differences between the inulin solubility of extract mangrove apple in this study might be due to the temperature of the extraction process. Inulin extract of mangrove apple has hydrophilic characteristics, which is easily soluble in water with high temperature. The enhancement of the intermolecular cavity is caused by the increasing kinetic energy of water molecules. Inulin extract mangrove apple is dissolved in the water making them have a hydroxyl component (OH). The inulin solubility of extract mangrove apple is also affected by the polymerization degree. Inulin with a low degree of polymerization is soluble in hot water. As stated by (Gupta *et al*., 2019), the solubility of natural inulin is dependent upon the degree of polymerization, temperature and molecular weight.

3.5 Total sugar of inulin mangrove apple

Based on Table 1, shows the total sugars of inulin. The extraction temperature had a significant effect ($p<0.05$) on the total sugar of mangrove apple extract. It might be due to that mangrove apple extract has an inulase enzyme that is an activity breaking down in the inulin polymer contained in the inulin substrate of mangrove apple extract into a component of free monomer. Therefore, the inulin polymer becomes shorter, as indicated by the decrease in the value of total sugar content. In the literature, (Yuliana *et al*. 2014) reported that the decrease of total sugar of inulin is caused by dahlia tuber containing inulase enzyme.

In this research, the total sugar gradually decreased with the increasing extraction temperature ($p<0.05$). The total sugar in the temperature extraction at 60°C was found higher ($p<0.05$) than that of 70, 80, and 90°C. However, the extraction temperatures at 70, 80, and 90°C were not different ($p>0.05$) in the total sugar of inulin of mangrove apple extract. The differences in total sugar, where it can cause the hydrolysis process into reducing sugar. The total sugar of inulin from mangrove apple extract was affected by the reduction of sugar and non-reduction sugar. The total sugar of inulin is lower than that of (Yudhistira *et al*., 2020), stating that the total value of a white sweet potato was at a value of 15.06%. Inulin degradation does not occur until at a temperature of 100°C with a pH equal to or greater than 5. The inulin was influenced by the number of fructans and total sugar through the difference in the extraction temperature. The decrease in the total sugar of inulin from mangrove apple was caused by the accumulation of fructose and glucose during the hydrolysis process.

3.6 Reduction Sugar

Table 1 shows the effects of the difference in extraction temperature on reducing sugar. The extraction temperature showed a significant effect on sugar reduction value ($p<0.05$). The value of reducing sugar was affected by the temperature of extraction in which the reducing sugar concentration showed the hydrolysis rate during the heating process. The presence of high temperatures caused the inulin to break down the molecule into shorter chains. Reduction sugar is a sugar (carbohydrate) group that can reduce electron-accepting compounds. Kim *et al*. (2001) reported that the reduction of sugar is used to measure the hydrolysis rate during the heating process. The result showed that the extraction temperature decreased inulin from mangrove apple extract. Yuliana *et al*. (2014) reported that the low reducing sugar value indicates that the treatment is getting better.

In this research, the reduction in sugar gradually decreased with the increase of extraction temperature ($p<0.05$). The sugar reduction values were extracted with different temperatures of 60°C until 90°C varied from 1.938 to 0.987%. Sugar reduction in the treatment of extraction temperature at 60°C was greater ($p<0.05$) than another extraction temperature; while the value of sugar reduction was relatively similar to the extraction 70, 80, and 90°C ($P>0.05$). The lowest value was found in the extraction temperature at 70°C. The variation of sugar reduction may be explained by the extraction temperature of inulin mangrove apple. The temperatures were caused by a change in the oligosaccharide chain. The reduction sugars were associated with the enzyme activity where the higher the enzyme activity, the higher the reducing sugar produced. The reaction rate of inulin also affected the temperature of extraction. Kim *et al*. (2001) reported the temperature at 80°C with 5% (w/v) inulin, after five mins heating, the reduction in sugar content was 0.0489% (w/v).

3.7 Fructo oligosacaride content

Figure 4 presents the results of the fructooligosaccharide content (FOS) in the inulin from mangrove apple extract. As seen in Figure 4, the FOS of inulin mangrove apple retention time was T 12.45 with a molecular weight of 28.28 µg/mL (60°C), 12.50 with a molecular weight of 38.54 µg/mL (70°C), 12.50 with a molecular weight of 33.59µg/mL (80°C) and 12.47 with a molecular weight of 65.32µg/mL (90°C). In the literature (Petkova *et al*., 2018) the fructooligosaccharides of Dahlia tubers by ultrasound-assisted extraction composed 1-Kestose (R_f = 0.37),

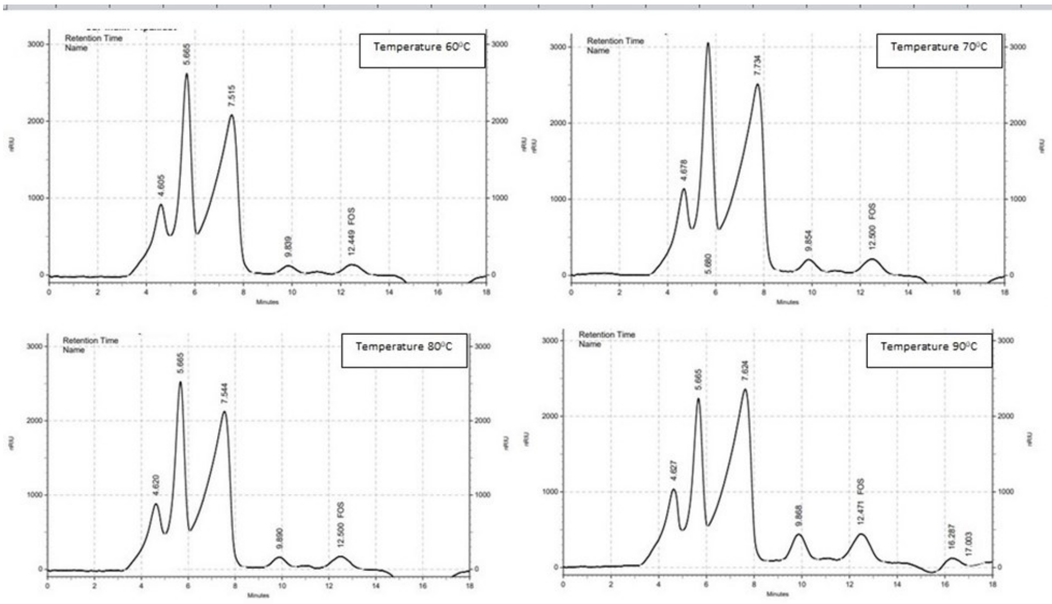

Figure 4. FOS content of inulin of mangrove apple

Nystose (R_f = 0.32) and oligomers. The FOS content gradually increased along with the extraction temperature, ranging from 60°C to 90°C. The result of the study indicated that the FOS in inulin from mangrove apple extract was 0.28%, 0.38%, 0.34%, and 0.66%. The FOS content of inulin of mangrove apple content was found lower than that of reported Sunu *et al*. (2019) showing that FOS content in garlic was 3.34% w/w.

The percentage of FOS increased at the extraction temperature of 90°C (compared with other treatments). The FOS content of inulin from mangrove apple extract at the temperature of 80°C was higher than that of the temperature of 70 and 60°C. The inulin from mangrove apple extract in the extraction temperature 70°C had a higher FOS content than the one in the extraction temperature at 60°C. The highest FOS content was found in the extraction temperature at 90°C although all of them had almost the same FOS content. It might be due to the changes of the extraction temperature hydrolysis process into the short-chain fructans. The presence of a higher temperature caused the inulin content to be shorter resulting in different FOS content. FOS had a degree of polymerization ranging from 2-10. According to Gholami *et al*. (2018) the degree of polymerization and the length of chain, inulin can be divided into categories: Fructooligosaccharide (DP = 2-10 unit) and inulin with long chains (DP= 10-65 unit). High temperature also affected hydrolysis, where the inulase enzyme hydrolyzes the fructose polymer to form the smaller fructose units as fructooligosaccharides. Maryati *et al*. (2016) reported that FOS was broken down by an enzyme (3-fructosidase) to produce glucose and fructose molecule.

4. Conclusion

Inulin could be obtained from mangrove apples at different temperatures during the extraction process. The characteristics gradually increased with the increasing extraction process at temperature 90°C. They were relatively higher total yield, inulin content, solubility, soluble dietary fibre, FOS, but lower in the total sugar and reduction sugar. The study indicated that temperatures during the extraction process could improve the characteristics of inulin from mangrove apple.

Conflict of interest

The authors declare no conflict of interest.

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