Accumulation of the Unknown Possible Saccharide’s Derivative Compound during Soybean Seed Germination

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Abstract: The effect of temperature on oligosaccharides profile during germination process of soybean seed was studied to enrich the basic knowledge on the producing method of soybean sprout with high functionality. In the experiment, the germination temperature conditions were set at 20 ºC and 30 ºC. Stachyose and raffinose as the main oligosaccharides were determined using the high-performance liquid chromatography with charged aerosol detector. Both stachyose and raffinose were changed depending on germination temperature; they decreased rapidly under both temperature conditions. Moreover, it also observed the accumulation of a unknown compound during germination process. Since the detection of this compound has present on oligosaccharides chromatographic system and there is no report has informed the accumulation of saccharides derivative compounds during soybean seed germination, this finding need to be further clarified.

Keywords: identification, oligosaccharides, soybean sprouts, unknown compound

1. Introduction
The degradation of oligosaccharides during soybean sprout cultivation was suggested to be used as an energy source in certain state of growing [1]. For starting the germination, the degradation of oligosaccharides cannot be avoided because germination process will be inhibited without it [2]. Therefore, to produce soybean sprout with high amount of oligosaccharides, an effort to control the degradation of oligosaccharides during growing process should be made. Soybean seed germination process is influenced by various environmental factors such as temperature, light and salinity. Particularly, temperature is a major limiting factor [3]. Since only a little information on relationship between germination temperatures and the profile of oligosaccharides degradation in soybean sprout germination were available and the measurement of oligosaccharides in soybean sprouts is quite difficult due to trace concentration. In general, the use of mass spectrometers combined with liquid chromatography or gas chromatography is common for detecting metabolites in plants [4,5], but these apparatus have the disadvantage of being expensive prices. Therefore, a charged aerosol detector (CAD) was used in this study as one of the most powerful, versatile and low-cost representative analyzes of oligosaccharides [6].
2. Materials and methods

2.1 Materials
Soybean seeds ‘cv. BS501’ were used in this study as sample material. A 10 g soybean seeds was dipped into 70 °C of water for 10 s to sterilize. Then the seeds were transferred to 30 °C of water and left for 8 h in order to stimulate the germination. After soaking, the seeds were separated and moved to each germination chamber at 20 °C and 30 °C with 70-80 % RH. The seeds were watered by 100 mL of water, twice a day (10 am and 4 pm). Germinated sprouts were sampled daily until 5 days of germination and divided into 2 groups; cotyledon and hypocotyl.

2.2 The extraction of the unknown compound
The unknown compound in the lyophilized soybean sprout samples were extracted by 70% (v/v) of ethanol and analyzed using a HPLC system for sugar analysis (Ultimate 3000, Thermo Fisher Scientific, Massachusetts, USA). A 10 µL sample was loaded onto an amino bond column (Shodex, Asahipak NH2P-50 4E, 250 x 4,6 mm id.,5 µm) through an auto-sampler. The mobile phase were water (solution A) and acetonitrile (solution B). The programmed elution was performed in various isocratic and step gradient polarity (SGP) mobile phase condition. The flow rate of mobile phase was 0.8 mL/min. The diode array detector and charged aerosol detector (CAD) were used for detection.

3. Results and Discussion
In this study, CAD was used for oligosaccharides detection with SGP separation technique. Generally, refractive index detection (RID) has been used for oligosaccharides analysis but its need a long time analysis. Moreover, RID is also less preferable as it less sensitive, room temperature depend, doesn’t permit work with gradients which can accelerate the analysis time [7]. Figure 1 shows the degradation profile of oligosaccharides during soybean sprouts germination. Similar trend of raffinose and stachyose degradation has observed during soybean sprouts germination. Oligosaccharides in cotyledon present in the higher amount compared to in hypocotyl. Moreover, at the end of germination, the level of oligosaccharides have present in the lower amount both in cotyledon and hypocotyl.

![Figure 1](image_url)

**Figure 1.** The changes of oligosaccharides during soybean seed germination
During germination process of soybean seed ‘cv. BS501’, the accumulation of unknown compound was observed both on 20°C and 30°C germination temperature treatments (Fig. 2). The unknown compound seemed also be influenced by the temperature. The unknown compound was detected on liquid chromatographic method for sugar measurement with using an amino column for separation and charged aerosol system for detection.

![Figure 2](image)

**Figure 2.** The changes of unknown compound during soybean seed germination.

The unknown compound was present at various retention time (RT) during measurement both at isocratic or SGP mobile phase condition. In a basic isocratic condition at 70 % of acetonitrile against water, the unknown compound has presence between raffinose and stachyose RT with time of 14,3 min and 23,2 min respectively. However, the unknown compound has a shifting trend with keep moving forward to early retention time (Figure 3).

![Figure 3](image)

**Figure 3.** Chromatogram of detected oligosaccharides and unknown compound during soybean sprouts germination under isocratic condition.

In addition, in SGP condition with rapid increasing of water concentration, the presence of the unknown compound can be maintained at after stachyose’s retention time with shifting trend become moving backward to delay retention time (Figure 4). This situation was not good for sugar analysis itself, because there is a possibility that the retention time of the unknown compound will be overlap with targeted sugar for measurement. Referring to the RT, the unknown compound has the polarity similar as oligosaccharides; however, the column cannot retain the compound tightly. Therefore, it can be suggested that the unknown compound might be as saccharides derivatives that bound to lipid or...
protein. The suggestion might relate to the utilization of CAD can be widely used for the determination of nonvolatile or semi-volatile compounds, including: lipids, oligosaccharides, carbohydrates, proteins, steroids, surfactants, polymers, peptides, and other [8]. Moreover, based on characteristic analysis, it noted that the unknown compound has maximum UV absorption at around 200 nm, high solubility in water, insoluble in methanol, has m/z 505 by LC-MS measurement by positive ionization mode.

Figure 4. Chromatogram of detected oligosaccharides and unknown compound during soybean sprouts germination under SGP condition

4. Conclusion
The unknown compound is interesting to be investigated due to the noticeable change of amount occurring during seeds germination. Elucidation structure research needs to be conducted to identify and characterize the structure of the unknown compound so that its functionality might also be identified.

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