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Effects of extraction conditions on soluble oxalate content of the germinated paddy rice (*Oryza sativa* L.)

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Abstract

In this study, effects of extraction conditions on soluble oxalate of paddy rice *(Oryza sativa* L.cv IR 4625*)* were analyzed using UHPLC chromatography. Extraction pH, sample-to-solvent ratios, extraction time and temperature were studied. The results showed that extraction in acidic condition (pH 3.0 – 6.0) led to a higher extracted soluble oxalate than other conditions (pH 7.0 and 8.0). The lowest soluble oxalate content was recorded at the 1:5 sample- solvent ratio (12.77 ± 0.07 mg/100g DW), while there were insignificant differences between other ratios. Moreover, the soluble oxalate content was insignificantly different at extraction temperatures and durations. The recorded data maybe useful for developing appropriate conditions for extraction of oxalate in paddies and may become databases for further study.

Keywords: pH, sample-to-solvent ratio, extraction time, extraction temperature, UHPLC

Introduction

Oxalates, salts of oxalic acid, have been researched in both basic and applied sciences for decades (Hodgkinson, 1977) [1]. They are widely found in many plants (Noonan and Savage, 1999) [2] and existing in two forms: soluble and insoluble ones. Depending on the plant cultivars and species, amounts of each form are different (Nguyễn and Savage, 2013) [12]. Oxalate content in plants such as spinach, beetroot, Cocoa were reported to be higher than others (Savage *et al*., 2000) [4]. High oxalate contents are also found in tropical plants such as taro and sweet potato (Noonan and Savage, 1999) [2]. In nature, oxalates often present in soluble oxalates as potassium, sodium, and ammonium oxalates or insoluble oxalates as calcium oxalates (Holloway *et al*., 1989) [5]. Among these forms, soluble fraction is the most concerned one as it can form chelates with dietary calcium, consequently making the complex molecule unavailable for absorption (Altunay and Gürkan, 2016) [6]. Frequently consumption of soluble oxalate-rich foods can increase the formation of calcium oxalate crystals and inhibit the absorption of minerals including calcium into the bone (Boontaganon *et al*., 2009, Ruan *et al*., 2013) [7, 8] leading to kidney stones, hyperoxaluria, mineral deficiencies and low bone density (Nguyễn *et al*., 2018) [9]. It is reported that oxalate daily consumption is ranged 50-200 mg per day (Noonan and Savage, 1999) [2]. Moreover, for patients suffered with kidney stone problems, dietary oxalate intake should be less than 40-50 mg per day (Ruan *et al*., 2013) [8].

Rice (*Oryza sativa* L.) is a monocotyledon plant and a major staple food consumed by over half seven billion people in the world, especially Asian people (Mohanty, 2013, Izawa and Shimamoto, 1996) [10, 11]. According to Ruan *et al*. (2013) [8], black glutinous rice (*Oryza sativa* L. var. glutinosa Matsum.), glutinous rice (*Oryza sativa* L. var. glutinosa Matsum.), and rice (Oryza sativa L. subsp. indica Kato) contained certain amounts of oxalates. However, depending on extraction conditions, the amounts of oxalates detected would be different (Nguyễn and Savage, 2013) [3, 12]. Therefore, accurately measuring soluble oxalate contents in paddy rice is crucial because of its effect on human health.

Materials and methods

Sample preparation

Rice paddies (*Oryza sativa* L.cv. IR 4625) were supplied by Long An Agricultural Extension Center, Long An province, Vietnam. The seeds were stored at room temperature until the experiments could take place.

The germination process was followed a method of Cáceres *et al*. (2014) [13] with little modifications. In brief, paddy rice was immersed in deionized water at 35ºC for 48 hrs, then germinated at 28ºC for 3 days in the absence of light using a seed sprouting machine (KG-262, Kangaroo, Hanoi, Vietnam). After that, the seeds were dried at 40ºC by an oven air dryer (SLW 53 ECO, Pol-eko, Wodzislaw Śląski, Poland) until their moisture contents decrease below 10%. The dried seedlings were powdered and stored at -20ºC for further experiments.

Extraction of soluble oxalates

The extraction procedure was altered from the method of Kanauchi *et al*. (2009) [14]. In detail, a mixture of 10 g of powdered seedling and a certain volume of buffers that would be identified in **2.2.2 section** was shaken at 0ºC for an identified duration using a shaking incubator (KS 4000 IC, IKA, Germany). Then, the mixture was centrifuged at 4000 x g, 4ºC for 10 min (Universal 320R, Hettich, Tuttlingen, Germany). The supernatant was collected and stored at 4ºC for further analysis. In this current study, concerned variables of extraction conditions were pH, the raw material to buffer ratios, extraction duration and extraction temperature.

Effects of extraction pH on soluble oxalate content

The samples were extracted by buffer with the adjusted pH

at 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 using succinate and Tris HCl buffers (Sigma-Aldrich Co., St. Louis, MO, USA). This experiment was conducted with the sample-buffer ratio at 1:5 (w/v) at 0°C for 1 hr.

Effects of the seedling powder to solvent ratio (w/v) on soluble oxalate content

The most suitable pH was chosen from the previous experiment. In this experiment, 10 g of seedling powder was extracted with solvent at chosen pH in the ratios ranging from 1:1 to 1:10 (w/v) at 0°C for 1 hr.

Effects of extraction time and temperature on soluble oxalate content

After choosing a pH value and a sample-solvent ratio (w/v) from above experiments, impacts of two concern variables including extraction time (15, 30, 45, 60, and 75 min) and extraction temperatures (0 °C, 4 °C, 20 °C, and 30 °C) were studied.

Measurement of soluble oxalate contents

Sample preparation for measurement of soluble oxalate content

A method by Nguyễn *et al*. (2018) [9] was followed with some modifications to measure soluble oxalate contents in the extracts and after mixing with oxalic acid. To measure soluble oxalate contents in the seedling extract, 5 mL of the extract was mixed with 45 mL of nano-pure water before incubating at 80 °C for 20 min (Nguyễn *et al*., 2018). The mixture was centrifuged at 3500 x g for 15 min (Universal 320R, Hettich, Tuttlingen, Germany). The supernatant was filtered through 0.45-µm cellulose acetate filters (CA syringe filter, Membrane Solutions, Kent, WA, USA) and analyzed using the UHPLC system (Dionex Ultimate 3000 HPLC System, USA).

UHPLC analysis

The UHPLC system was used to analyze soluble oxalate contents in the germinated paddy rice. Chromatographic separations were performed on a 300 x 7.8 mm Rezex ROA ion exclusion organic acid column (Phenomenex Inc., Torrance, CA, USA) attached to a cation H^+ guard column (Bio-Rad, Hercules, CA, USA). The system was running at 30°C. The mobile phase was 25 mM H_2SO_4 (Merck, USA) with flow rate was 0.6 mL/min. Oxalic acid (99.999% pure, Sigma-Aldrich Co., St. Louis, MO, USA) was used as a standard and the absorbance was recorded at 210 nm every 0.02 min by a UV/VIS detector already equipped in the system. The limit of detection (LOD) of the system was 0.01 mg oxalic acid/100 mL water.

Statistical analysis

All data was collected, analyzed, and expressed as means ± standard deviation (SD). One-way ANOVA and two-way ANOVA were performed to analyze data using Minitab software version 18 (Minitab Inc., State College, PA, USA).

Results and discussion

Effects of extraction pH on soluble oxalate content of the germinated paddy rice

Table 1 near here

To investigate influence of extraction pH on the amounts of soluble oxalates in the seedlings, extraction was employed at different pH values, ranging from 3.0 to 8.0. Table 1 illustrates that soluble oxalate content of the seedlings when extracted with pH 3.0 to 6.0 were significantly higher ($p < 0.05$) than that at pH 7.0 and 8.0. To be specific, soluble oxalate content at pH 3.0 was 13.06 ± 0.18, pH 4.0 was 13.32 ± 0.38, pH 5.0 was 13.11 ± 0.22, and pH 6.0 was 13.32 ± 0.17 mg/100g DW. Meanwhile, the lowest soluble oxalate content was on pH 7.0 and 8.0 which stood at 12.64 ± 0.17 and 12.3 ± 0.15 mg/100g DW, respectively. According to Kusuma *et al*. (2016), the acidic conditions had shown the positive results in the increment of oxalate content extracted from spinach. In conclusion, the results in Table 1 showed that soluble oxalate content was greater than when extracted at acidic pH (pH 3.0 to 6.0).

Table 1: Effects of extraction pH on soluble oxalate content (mg/100g DW) of the germinated paddy rice.

Extraction pH	Soluble oxalate content (mg/100g DW)
3.0	13.06 ± 0.18^{a}
4.0	13.32 ± 0.38^{a}
5.0	13.11 ± 0.22^{a}
6.0	13.32 ± 0.17^{a}
7.0	12.64 ± 0.17^{b}
8.0	12.3 ± 0.15^{b}

The values were means ± SD of three independent experiments. Data with the same superscript letters within a column are not significantly different (P<0.05).

Effects of sample-solvent ratios (w/v) on soluble oxalate content of the germinated paddy rice

Table 2 near here

The effect of sample-to-solvent on the soluble oxalate content of germinated paddy rice was examined in **Table 2**. In this study, the sample- solvent ratio was ranging from 1:1 to 1:10 and succinate buffer (pH 4.0) was used as the extraction solvent. It is clear that the lowest soluble oxalate value was obtained at the ratio of 1:5 with 12.77 ± 0.07 mg/100g DW. On the other hand, no significant differences in soluble oxalate content were found in other ratios. According to Table 2, sample extracted in 1:10 ratio solvent obtained 28% higher total oxalate content than that of 1:6.

Table 2: Effects of different sample-to-solvent ratios on soluble oxalate content (mg/100g DW) of the germinated paddy rice

Sample-solvent ratios (w/v)	Soluble oxalate content (mg/100g DW)
1:1	13.12 ± 0.32^{bc}
1:2	13.13 ± 0.10^{bc}
1:3	13.00 ± 0.23^{cd}
1:4	13.11 ± 0.13^{bc}
1:5	12.77 ± 0.07^{d}
1:6	13.39 ± 0.17^{ab}
1:7	13.14 ± 0.19^{bc}
1:8	13.11 ± 0.17^{bc}
1:9	13.59 ± 0.01^{ab}
1:10	13.16 ± 0.23^{bc}

The values were means ± SD of three independent experiments. Data with the same superscript letters within a column are not significantly different (P<0.05).

Effects of incubation time and temperature on the oxalate content of the germinated rice paddy crude extracts

Table 3 near here

The soluble oxalate of germinated rice paddy was extracted at four temperatures, 0ºC, 10ºC, 20ºC, and 30ºC, and at five extraction time, 15, 30, 45, 60, and 75 min shown in Table 3.

It could be seen that the mean soluble oxalate content was insignificantly different at the extraction temperatures. Indeed, the mean soluble content of the samples extracted at the ranges of studied temperatures were 13.32 ± 0.32, 12.87 ± 0.28, 13.11 ± 0.41, and 13.16 ± 0.18 mg/100g DW for 0ºC, 10ºC, 20ºC and 30ºC, respectively. Al-Wahsh *et al*. (2012) also studied about effects of extraction temperature on the determination of oxalate contents of 50 dry herb and 10 fresh fruits. Similarly, regarding effect of extraction time, it showed that there were insignificantly variables on soluble oxalate contents extracted at different extraction duration. After extracting at 0ºC, the mean oxalate amounts were 13.43 ± 0.01 mg/100g DW, 13.43 ± 0.14, 13.39 ± 0.13 mg/100g DW, 13.21 ± 0.04 mg/100g DW and 13.16 ± 0.23 mg/100g DW after extraction for 15 min, 30 min, 45 min, 60 min and 75 min, respectively. It could be concluded that the soluble oxalate content was insignificantly different at the different extraction duration.

Table 3: Effects of extraction time and extraction temperature on soluble oxalate content (mg/100g DW) of the germinated paddy rice

Extraction temperature	Extraction time (mins)	Soluble oxalate content (mg/100g DW)
0ºC	15	13.43 ± 0.01^{a}
	30	13.43 ± 0.14^{a}
	45	13.39 ± 0.13^{ab}
	60	13.21 ± 0.04^{ab}
	75	13.16 ± 0.23^{b}
10ºC	15	12.98 ± 0.15^{a}
	30	12.88 ± 0.08^{ab}
	45	12.99 ± 0.17^{a}
	60	12.71 ± 0.15^{b}
	75	12.78 ± 0.09^{ab}
20ºC	15	12.85 ± 0.24^{b}
	30	13.22 ± 0.23^{a}
	45	13.04 ± 0.09^{ab}
	60	13.24 ± 0.17^{a}
	75	13.22 ± 0.14^{a}
30ºC	15	13.05 ± 0.02^{a}
	30	13.23 ± 0.15^{a}
	45	13.14 ± 0.14^{a}
	60	13.17 ± 0.15^{a}
	75	13.21 ± 0.21^{b}

The values were means ± SD of three independent experiments. Data with the same superscript letters within a column are not significantly different (P<0.05).

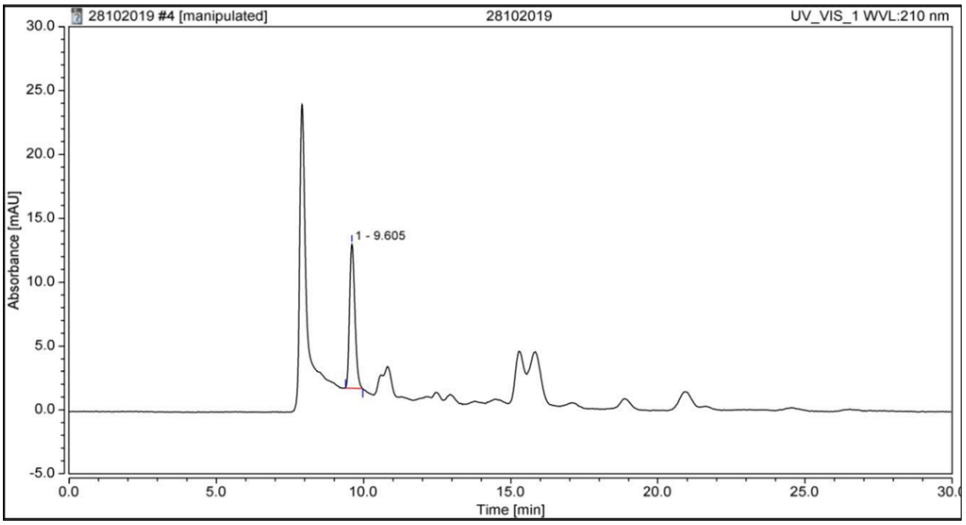

Fig 1: UHPLC peak of soluble oxalate content in the germinated paddy rice.

Conclusions

Overall, for extracting soluble oxalate content from the germinated paddy rice, the acidic conditions (pH 3.0 – 6.0) resulted in higher amounts of soluble oxalates than the alkaline conditions. In terms of sample-solvent ratio, except for the ratio 1:5, there was insignificant difference on soluble oxalate content. The extraction temperature and extraction duration studied in this work seems to have insignificant effects on the extraction of soluble oxalates from the germinated paddy rice.

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