

Research Article

Reduction in antioxidant properties lost during processing of a powdered beverage from young organic rice plants

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Abstract

Young rice plants have high potential as raw material for health drinks. Unfortunately, fresh young rice plants have a short self-life so a drying process could extend the shelf-life at ambient temperature. This study was designed to optimize the drying conditions for a minimal loss in antioxidant properties as well as iron binding affinity.

To extend the raw material shelf-life, drying the rice plants in a hot air oven was studied. Drying process under optimum conditions in a hot air drier was then compared to both freeze drying and vacuum-microwave drying. In order to obtain a sample extract from the best drying process, extraction in hot water was investigated. In order to study the ability of *in vitro* binding affinity for ferric (Fe³⁺) and ferrous (Fe²⁺) ion, the sample was extracted under 80°C for 10 min.

The optimum age of young rice plants used in this study is 12-15 days after germination. The best drying condition was 50°C for 2 hours, resulting in the least reduction in antioxidant properties, total polyphenols and chlorophyll. In comparison, the sample from vacuum-microwave drying showed the least reduction in antioxidant properties and total polyphenol content, but significantly caused the highest loss in chlorophyll (p<0.05). Higher temperatures and times caused higher extraction resulting in higher antioxidant properties and total polyphenols in the drink extracts. The extract of young rice plants dried under 50°C for 2 hours in a hot air oven had the highest iron binding affinity, whereas the activity for Fe²⁺ was higher than Fe³⁺.

Keywords: *Oryza sativa*, hot air dryer, freeze drying, vacuum-microwave drying, polyphenol, chlorophyll, iron binding, shelf life, rice grass, Thailand.

Introduction

Young leaves of monocotyledon plants such as wheat, barley, oats, rye and rice are rich in vitamins, minerals, dietary fibre, antioxidants, superoxide dismutase (SOD) enzyme [1], and other bioactive substances, such as tyrosinase and phosphodiesterase inhibitor [2]. They have received attention for

their usability as materials for health food products that provide effects of improving intestinal environment, suppressing absorption of cholesterol and preventing the postprandial sudden rise in blood sugar [1]. Nutrient contents in the cereal plants depend on where they are grown, soil qualities, average rainfall and harvesting techniques [3]. It is known the highest concentrations of the plant nutrients are present for just a few critical days [4].

Young rice plants (*Oryza sativa* L.) germinated at an early stage normally produce a fragrant aroma. Chen *et al.* [2] found that rice plant protein extracts may show immuno-modulation activity. In addition, water-soluble extracts showed regulation of blood glucose levels and an inhibitory effect against xanthine oxidase. However, the fresh leaves are difficult to store and have a short shelf-life due to microorganism contamination and enzyme activities. High temperature, such as in a particular drying process could extend their self-life, but possibly affect the beneficial nutrients including antioxidant properties and polyphenol contents.

Iron overloading in β -thalassaemia patients is caused by an increase of iron absorption or multiple blood transfusions. An excessive non-transferrin-bound iron can catalyze free radical formation leading to damage of cell membranes and organs in the body [5]. Deferoxamine is an injection drug treatment used in iron-overload patients. It is an iron chelator as the excessive iron is bound and excreted in urine and feces. However, the drug treatment possibly causes many side-effects; for example, skin discolouration, muscle cramping and pulmonary toxicity [6]. Natural herbs and natural food products, including young organic rice plants, are being researched as an alternative method for treatment.

In processing to produce a powdered beverage from young organic rice plants, heat treatment is applied to remove moisture content resulting in a substantial loss in beneficial properties of the product. This research aimed to study antioxidant properties, polyphenol and chlorophyll contents in young rice plants during germination, as well as an optimum drying method, with respect to the best retention of antioxidant properties, polyphenol content and iron (Fe^{2+} and Fe^{3+}) binding efficiency of young rice plant extract.

Materials and Methods

Materials

Paddy rice and young rice plants (*Khao Hom Daeng* Sukhothai 1) were provided by Natural Rice Co., Ltd., Sukhothai Province. The plants were stored under vacuum conditions at -20°C until studied. Prior to drying, young rice plants were thoroughly washed with water then submerged in 100 ppm sodium bicarbonate solution for 15 minutes for dirt removal and a microbial reduction.

Methods

Germination of young rice plants

Dry paddy rice was soaked in water for 12 hours then drained and matured in a damp bag for an additional 24 hours. The seed was transferred to a soil bed under natural light for further growth. Leaf samples were collected every 3 days for 18 days and packed in aluminum foil bags under vacuum conditions prior to storage at -20°C until analysis. The optimum germination day was determined as being the highest in antioxidant activity and total polyphenol content in the leaves.

Drying process of young rice plants

The young rice plants at optimum age were collected and divided into approximately 100 g per sample, then dried in a hot air oven (Termaks, Norway) under assigned temperature (50, 60, 70 and 80°C) and time (2, 3 and 4 hours). The best oven drying (HAD) condition was then compared to vacuum-microwave drying (VMD) and a freeze drying (FD). The vacuum-microwave dryer

(Marchcool, Thailand) was equipped with three pairs of magnetrons and a 360° rotating load basket. Sample load for the vacuum-microwave drying was approximately 200 g per batch and dried under microwave power levels of 960W (VMD 960), 1920 W (VMD 1920) and 2880 W (VMD 2880) with a -9.33×10^{-4} Pa control pressure. A vertical freeze dryer (Heto, Power Dry PL3000, USA) was operated under 40 Pa with -50°C condenser temperature. All dried samples were packed in aluminum foil bags and stored under -20°C until analysis.

Water extraction of young rice plant powder

Dried rice plants were pulverized and packed in a teabag with 1.50 g each. Water extraction was conducted with a continuous mechanical agitation under 80, 90°C and boiling water for 3, 6 and 9 minutes. The extracts were adjusted to 150 ml then stored at -20°C until analysis.

Determination of antioxidant activity (AOA)

Fresh and dried samples, 1 g dry matter (DM), were extracted with 50 ml of 95% ethanol using a shaker bath for 12 hours at room temperature. Each extracted sample was filtered through a filter paper, kept in a closed container and stored at -20°C before analysis.

ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) assay

Radical scavenging activity was determined using the method of Re *et al.* [7], with some modifications. In brief, the $\text{ABTS}^{\cdot+}$ radical cation was generated by the interaction of ABTS (7 mM, 5 ml) and $\text{K}_2\text{H}_2\text{O}_8$ (2.45 mM, 7.5 ml). The mixture was incubated at room temperature for 12 hours and dark green solution was obtained. Before analysis, the solution was diluted with distilled water to reach the absorbance of 0.7-0.9 at 730 nm using a UV-VIS Spectrophotometer (Perkin Elmer, Germany). For measurement, 2 ml of $\text{ABTS}^{\cdot+}$ solution was mixed with 20 μl of the extracts and incubated at room temperature for 3 min then measured for absorbance at 730 nm. Trolox solution was used to generate a standard line and activity was reported as trolox equivalent antioxidant capacity in mg/1g DM (mg TEAC/1g DM).

FRAP (Ferric reducing ability power) assay

The ferric reducing property of the extracts was determined using the method of Benzie and Strain [8], with some modifications. In brief, 1.2 ml of FRAP reagent (300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, 20 mM $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$, ratio 10:1:1) was mixed with 40 μl of the extracts and incubated at room temperature for 4 min then measured for absorbance at 593 nm. FeSO_4 solution was used to generate a standard line. Results were expressed as FeSO_4 equivalent in mg /1 g DM.

Determination of total polyphenol contents (TPC)

Fresh and dried rice plant samples, 1 g DM, were refluxed with 95% ethanol (50 ml) at 80°C for 1 hour. Each extracted sample was filtered through a filter paper then adjusted to 50 ml before storage under -20°C until analysis.

TPC was determined using the method of Pinsirodom and Changnoi [9], with some modifications. Briefly, 500 μl of the extracts, 9.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent were thoroughly mixed in a test tube and incubated at room temperature for 5 min. Two millilitres of 10% (w/v) sodium bicarbonate were added and incubated for 10 min before an absorbance measurement at 734 nm. Gallic acid solution was used to generate a standard line. Results were reported as gallic acid equivalent in mg/1 g DM (mg GAE/1g DM).

Determination of chlorophyll content

Chlorophyll content was determined using the method of Intarakosol [10], with some modifications. Fresh and dried samples, 0.1 g DM, were extracted in 50 ml of 80% acetone until decolourized. The extracts were then filtered through filter papers and measured absorbance ($\lambda = 643$ and 665 nm).

In vitro iron binding affinity

Spectral analysis

Iron binding affinity was determined by the method of Srichairatanakool *et al.* [11]. Young rice plants (2.0 g DM) were extracted in 98 ml of 80°C water for 10 minutes then filtered through a filter paper. The extract was incubated with ferric citrate (Fe^{2+} -citrate) and ferric nitriloacetate (Fe^{3+} -NTA) (25, 50, 100, 200 and 400 μM), pH 7.0 at room temperature for 15 minutes. Absorbance measurement was conducted at 400-700 nm using a scanning UV-VIS spectrophotometer. The wavelength with maximum absorbance was chosen for dose-response analysis.

Dose-response analysis of iron binding complex

Crude extract of young rice plant was incubated with Fe^{2+} -citrate and Fe^{3+} -NTA (25, 50, 100, 200 and 400 μM) at room temperature for 15 minutes before an absorbance measurement at an optimum wavelength from 7.1.

Statistical analysis

The data were analyzed using ANOVA (SPSS version 16.0 for Windows) and mean comparisons were run by Duncan's new multiple-range test. Significant difference was defined at $p \leq 0.05$.

Results and Discussion

Effects of germination age on AOA and TPC

AOA and TPC in the young rice plants were rapidly increased within 9 days after planting ($p < 0.05$) and gradually accumulated afterward through to days 12-18 (Table 1). Optimum germination ages was observed during 12-15 days as the AOA and TPC were 25.17-26.68 mg TEAC/g DM, 45.17-54.10 mg FeSO_4 /g DM and 14.44-14.47 mg GAE/g DM, respectively. The 15 days of germination age, therefore, was chosen for further studies. Randhir and Shetty [12] reported that polyphenol in young corn leaves at an early germination stage was in a soluble form and had more antioxidant properties in nature. However, in the late germination, lower polyphenol was investigated as was being partitioned in a polymerization process; lignification into lignans and lignins. Kohler [13] found that fibre in oat leaves increased with germination age but vitamins, protein, fat and chlorophyll decreased. In addition, Liu *et al.* [14] reported an increase of polyphenol in young leaves of pigeon pea in an early germination stage and a decrease with longer germination ages. Cevallos-Casals and Cisneros-Zevallos [15] found that AOA and TPC in wheat with 7 germinated days was higher than that in dry and imbibed seed. Higher polyphenol was synthesized at an early stage of the plant growth for protecting young leaves from environmental oxidative reactions.

Effects of drying process on AOA, TPC and chlorophyll content

Hot air drying caused significant loss in AOA, TPC and chlorophyll compared to the fresh sample ($p < 0.05$). The lowest loss in TEAC, FRAP, TPC and chlorophyll, observed under a drying condition at 50°C for 2 hours, were 41.81, 25.71, 34.21 and 8.53%, respectively (Table 2). A decrease of AOA was correlated to a reduction of TPC since the polyphenol was mainly attributed to its antioxidant activity by donating a hydrogen atom from the aromatic hydroxyl group to free radical [16]. Other evidence showed the same trend as AOA and TPC during the drying process of mulberry leaves [17], and dried peanut skins [18]. The process possibly caused a reduction of natural antioxidants in raw material from plants [19]. Intense or prolonged thermal treatment may

be responsible for a loss of natural antioxidants, as most of these compounds are unstable [20]. In addition, in the absence of water or in a dried form, the plant cell components adhere together leading to some difficulty in solvent extraction during analysis [21]. Furthermore, Maharaj and Sankat [22] reported that drying temperature elevated from 40 to 70°C decreased chlorophyll content in dasheen leaves. This is in an agreement with results reported by Ahmed *et al.* [23], drying of coriander leaves in low temperature air drying gave the product with the highest chlorophyll content.

Table 1. TEAC, FRAP and TPC in young rice plants at different germination ages.

Germination ages (day)	Antioxidant activity (AOA)		Total polyphenol content (TPC) (GAE/g DM)
	ABTS ⁺ (mg TEAC/g DM)	FRAP (mg FeSO ₄ /g DM)	
3	11.79 ± 5.40 ^c	16.60 ± 1.16 ^c	5.44 ± 0.10 ^c
6	14.91 ± 6.12 ^{bc}	26.82 ± 3.70 ^c	7.89 ± 1.24 ^b
9	22.70 ± 10.02 ^{ab}	44.87 ± 12.75 ^b	12.26 ± 2.91 ^a
12	25.71 ± 11.01 ^a	45.17 ± 8.89 ^b	14.47 ± 4.01 ^a
15	26.68 ± 11.30 ^a	54.09 ± 18.53 ^{ab}	14.44 ± 2.76 ^a
18	26.71 ± 10.39 ^a	62.27 ± 15.89 ^a	14.10 ± 1.09 ^a

Values are mean±SD (n=10).

^{a-c} Values within columns with different letters are significant difference according to Duncan's new multiple range test (p<0.05).

Table 2. Reduction (%loss) of TEAC, FRAP, TPC and chlorophyll content in dried young rice plants (15 germination days) processed in a hot air oven under different drying temperature and time.

Treatment	%Loss compared to fresh young rice grass			
	ABTS ⁺ * (mg TEAC/g DM)	FRAP* (mg FeSO ₄ /g DM)	Total polyphenol content (TPC)* (GAE/g DM)	Chlorophyll** (mg/g DM)
50°C				
2 hours	41.82±11.71 ^f	25.71±3.83 ^f	34.21±5.38 ^c	8.53±0.52 ^e
3 hours	44.49±5.82 ^{def}	28.46±2.48 ^f	35.45±5.76 ^{de}	7.79±1.02 ^e
4 hours	43.83±3.51 ^{ef}	27.99±2.69 ^f	36.18±6.97 ^{de}	11.49±1.01 ^c
60°C				
2 hours	43.36±10.99 ^{ef}	31.93±1.88 ^e	42.29±4.20 ^{bc}	10.30±0.40 ^d
3 hours	45.03±7.54 ^{def}	32.75±1.15 ^e	42.52±9.23 ^{bc}	9.65±0.61 ^d
4 hours	49.35±4.89 ^{cde}	32.69±1.53 ^e	40.26±6.70 ^{cd}	11.93±1.09 ^c
70°C				
2 hours	53.58±5.49 ^{abc}	33.51±2.54 ^{de}	44.20±5.58 ^{bc}	11.39±0.10 ^c
3 hours	54.70±4.36 ^{abc}	36.30±2.47 ^{cd}	46.35±3.70 ^b	11.92±0.73 ^c
4 hours	50.60±5.57 ^{bcd}	38.86±3.43 ^{bc}	44.70±1.84 ^{bc}	13.28±0.96 ^b
80°C				
2 hours	56.08±4.40 ^{ab}	40.30±6.78 ^b	42.97±5.36 ^{bc}	13.13±0.38 ^b
3 hours	56.05±2.97 ^{ab}	46.68±0.95 ^a	47.54±2.96 ^b	13.89±0.10 ^b
4 hours	57.82±3.44 ^a	45.76±4.09 ^a	53.26±6.89 ^a	16.46±0.15 ^a

* Values are mean±SD (n=10).

** Values are mean±SD (n=6).

^{a-f} Values within columns with different letters are significant difference according to Duncan's new multiple range test (p<0.05).

Vacuum-microwave drying (VMD) is a rapid method and more energy efficient compared to hot air drying (HAD). With low temperature and fast mass transfer conferred by the vacuum, combined with a rapid energy transfer by microwave heating, generates very rapid dehydration [24]. During VMD, the drying times for microwave power of 960, 1290 and 2880 W were 30, 15 and 9 minutes, respectively, which resulted in sample moisture contents approximately of 7%. As shown in Table 3, dried young rice plant samples from VMD showed a lower loss in AOA and TPC than that from HAD (50°C, 2 hour) (p<0.05). Losses in AOA under 2880 VMD were not significantly different from FD (p≥0.05), except for the loss of chlorophyll content for which a higher loss was shown compared to the FD. The results agree with dried blueberries [25], dried oregano leaves [26], and dried Thai red curry powder [27] processed under VMD, HAD and FD. The higher the power level of microwave applied, the higher values of AOA and TPC preserved in the samples [26, 27].

Disruption to plant tissue increased with an increase of microwave power resulting in more polyphenols being liberated [27].

Table 3. Reduction (%loss) of TEAC, FRAP, TPC and chlorophyll content in dried young rice plants (15 germination days) processed under different drying methods.

Treatment	%Loss compared to fresh young rice plants			
	ABTS ⁺ * (mg TEAC/g DM)	FRAP* (mg FeSO ₄ /g DM)	Total polyphenol content (TPC)* (mg GAE/g DM)	Chlorophyll** (mg/g DM)
freeze dry	15.70±4.64 ^b	24.14±5.17 ^c	23.55±3.35 ^d	20.43±0.18 ^{cd}
hot air dry				
50°C 2 hours	27.68±5.86 ^a	39.70±5.65 ^a	43.52±3.68 ^a	19.37±0.65 ^d
vacuum-microwave dry				
960W	19.28±5.01 ^b	31.01±6.91 ^b	30.99±5.39 ^b	21.71±1.65 ^{bc}
1920W	16.99±6.04 ^b	25.86±5.78 ^c	26.94±3.38 ^c	23.08±2.32 ^b
2880W	15.82±4.49 ^b	21.58±4.45 ^c	18.82±3.66 ^c	26.76±0.41 ^a

* Values are mean±SD (n=10).

** Values are mean±SD (n=6).

^{a-d}Values within columns with difference letters are significant difference according to Duncan's new multiple range test (p<0.05).

In the iron binding study, young rice plant extracts (2% DM) were incubated with various concentrations of Fe²⁺-citrate (25, 50, 100, 200 and 400 µM). Reactivity of iron with crude extracts was detected by measuring the optical density (OD) in a range of 400-700 nm. When Fe²⁺ bound into the compounds in the extracts, a complex was formed and exhibited a maximum absorption at 415 nm (Fig.1). Spectrum of the formed complex was dependent upon the iron concentration added. In addition, young rice plant extracts were able to bind with the iron in a form of Fe²⁺ rather than Fe³⁺ where as the extract from hot air dried sample was able to bind Fe²⁺ and Fe³⁺ more efficiently than the others (Fig.2).

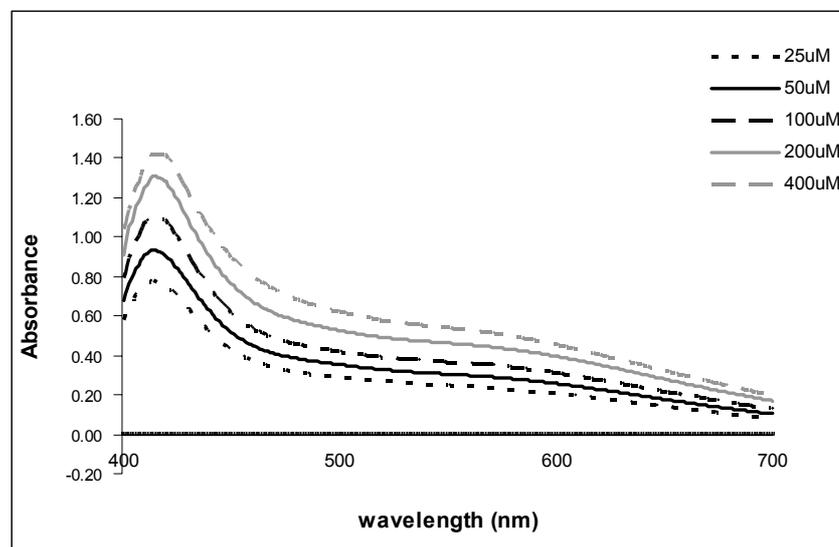


Figure 1. Complex forming spectrums of young rice plant crude extract incubated with differing Fe²⁺ concentrations.

Effect of water extraction

Based on the least reduction in AOA and TPC as discussed previously, vacuum-microwave drying at 2880W was proposed for the production of a young rice plant powdered beverage. In water extraction of the beverage, AOA and TPC increased with higher temperature and longer extraction time (Table 4). Su *et al.* [28] reported that AOA and TPC of oolong tea solution increased with an

increase of extraction temperature and soaking time. Higher level of AOA in young rice plant beverage exposed to high temperatures and long extraction times can be revealed as a formation of maillard reaction products associated with a formation of heat induced antioxidants [29].

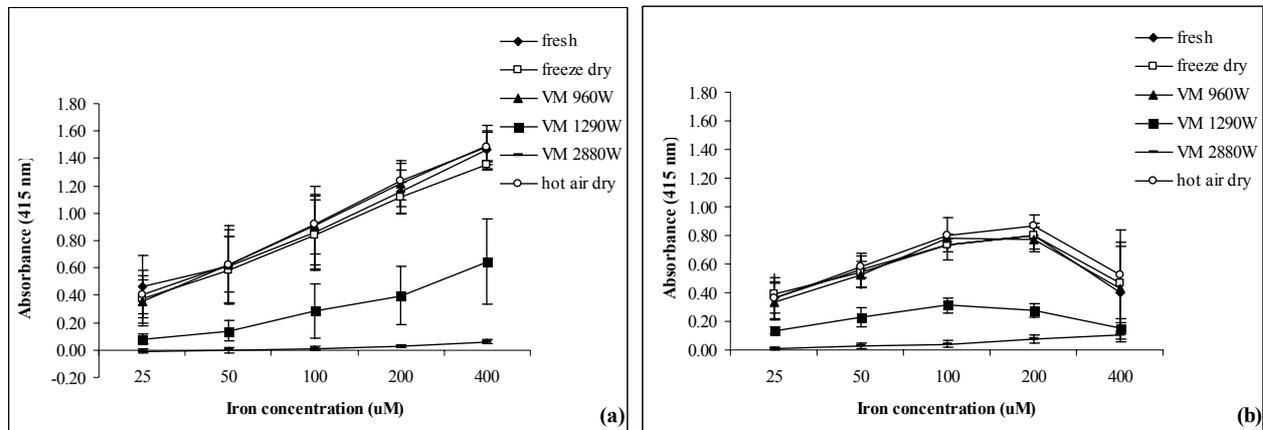


Figure 2. Dose-response of iron binding capacity in young rice plants (15 germination days) crude extract prepared from fresh, freeze dried, vacuum-microwave dried and hot air dried with Fe²⁺ (a) and Fe³⁺ (b).

Table 4. TEAC, FRAP and TPC in young rice plants (15 germination days) product as extracted under different temperature and time.

Treatment	Antioxidant activity (AOA)		Total polyphenol content (TPC) (GAE/ml)
	ABTS ⁺ (mg TEAC/ml)	FRAP (mg FeSO ₄ /ml)	
80°C			
3 minutes	0.07±0.02 ^d	0.02±0.01 ^e	0.01±0.01 ^f
6 minutes	0.11±0.01 ^d	0.04±0.03 ^{de}	0.02±0.02 ^{ef}
9 minutes	0.10±0.02 ^d	0.06±0.04 ^d	0.03±0.02 ^{de}
90°C			
3 minutes	0.09±0.03 ^d	0.03±0.02 ^d	0.02±0.01 ^{ef}
6 minutes	0.29±0.07 ^{bc}	0.10±0.02 ^c	0.05±0.01 ^{bc}
9 minutes	0.30±0.05 ^b	0.14±0.01 ^b	0.06±0.01 ^{ab}
Boiling water			
3 minutes	0.26±0.05 ^c	0.09±0.04 ^c	0.04±0.02 ^{cd}
6 minutes	0.34±0.05 ^a	0.14±0.04 ^b	0.06±0.02 ^b
9 minutes	0.37±0.04 ^a	0.18±0.03 ^a	0.08±0.02 ^a

Values are mean±SD (n=10).

^{a-f} Values within columns with difference letters are significant difference according to Duncan's new multiple range test (p<0.05).

The results supported a study of white cabbage juice which reported higher AOA in a heated sample than that in an unheated one. The observed property increased over a longer heating time [30]. Labbé *et al.* [31] reported that solubilization of green tea polyphenol highly depended on extraction time and temperature. In addition, TPC in green tea extracts significantly increased with higher extracting temperature (60-100°C) and time (3-30 minutes) [32].

For best efficacy in iron binding characteristics, young rice plants from hot air drying (50°C 2 hours) were studied. Sample extracts under higher temperature and longer time were able to form more complexes with the Fe²⁺-citrate, especially at boiling water for 6-9 min as shown in Fig 3. According to Yoshimura *et al.* [33], Maillard reaction products from glucose and glycine heating were able to chelate with Fe²⁺. Melanoidins were polymeric brown compounds formed in the last stage of the Maillard reaction that had a negative net charge and were able to bind metallic ions [34].

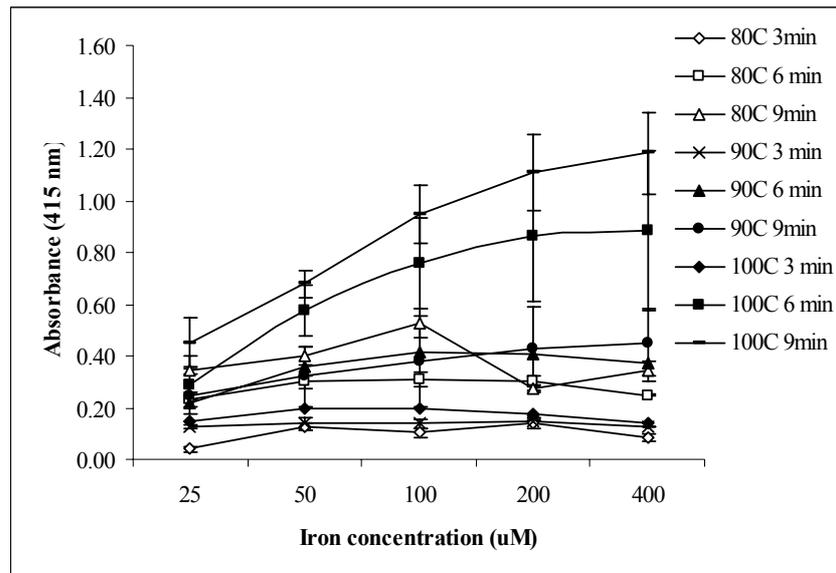


Figure 3. Dose-response of iron (Fe^{2+}) binding capacity in young rice plant (15 germination days) crude extracts under different extraction conditions.

Conclusion

Germination ages had a significant impact on the antioxidant activity and total polyphenol content in young rice plants, especially during the 12th to 15th days. The young rice plants, rich in polyphenols possibly stimulated antioxidant function. Vacuum-microwave drying (2880 W) was shown to be effective in preserving antioxidant activity and total polyphenols, while hot air drying (50°C, 2 hrs.) showed the best iron binding characteristics. Higher water extracting temperature and time lead to higher antioxidant activity and total polyphenols in the extracts, as well as the iron binding capacity. Using young rice plant extract for β -thalassaemia therapy should be further studied.

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