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Germinated VD20 rice – a local rice variety in Vietnam: Effect of

process conditions

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Abstract

Rice is one of important staple foods, which mainly consumed by human in daily life. One of the methods which could enhance the nutritional profile as well as functional of rice is germination process. VD20 rice, a local rice from Tien Giang province (Vietnam), was used in this study. This investigation was aimed to study the effect of ratio of rice to soaking solution (1:1-1:3), pH of soaking solution (distilled water, pH = 3-5), and time of soaking (3-6 hours) on the germination rate, moisture content of grain and total polyphenol content of germinated rice. Besides that, the impact of germination conditions, including incubation time (16-22 hours) and temperature (31-37°C), on the quality of germinated rice was also elucidated. The obtained results showed the ratio of soaking water to rice is 2.5:1 for the highest polyphenol content and germination rate. The longer the soaking time, the polyphenol content gradually decreases but the germination rate gradually increases over time. Soaking solutions with a pH of 4 for 6 hours were the most apporiate conditions for soaking VD20 rice. During the germination process, temperature and incubation time significant affected the polyphenol content and germination rate. At a temperature of 37°C for 20 hours, the polyphenol content was highest (33.85 mgGAE/100 g) and gradually decreases with incubation time, but the germination rate gradually increases. Germination process of VD20 enhance the nutrional quality of rice, which could be seen in the increasing of phenolic compounds content. However, it also affected by various factors, as know as, soaking conditions and incubation conditions, which could further study to ultilize and optimize. The obtained product also have the promising application as the nutraceutical food.

Keywords: germination, rice, phenolic compound, soaking, incubation

 Full length article
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1. Introduction

Rice is widely consumed by a large portion of the global population since it provides a substantial amount of nutrients and comes in many forms that cater to different preferences [1]. Whole rice contains higher levels of fiber, resistant starch, and a wider range of proteins and lipids compared to polished rice, which primarily consists of starch with small amounts of protein components. Certain colored rice types also possess chemicals that exhibit antioxidant properties, which have positive effects on health [2]. According to Dam [3], VD20 rice variety is a short-term fragrant rice variety, imported from Taiwan by the Ministry of Agriculture and Rural Development and transferred for testing in Tien Giang in 1996. The VD 20 variety was selected by the method of first-line selection and comparison of varieties. Promising lines and varieties were recognized as technically advanced varieties in 2004 according to Decision No. 2182 QĐ/BNN-KHCN dated July 29, 2004 [3]. VD20

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rice variety is not only known as a quality, specialty variety but also has good resistance to drought and salinity. This is one of the few specialty rice varieties that is both adaptable to harsh weather conditions and brings high economic efficiency to farmers thanks to its high quality, fragrant, sticky, delicious rice, suitable for the market [3]. Normally, the price of VD20 rice is 1,000 VND/kg higher than other rice varieties. People who produce this rice variety make a profit of about 40 million VND/ha/crop, 5-8 million VND higher than other rice varieties/ha [3]. Every year, this specialty rice variety is planted in the province with an area of over 11,000 hectares, including 300 - 400 hectares organically in coastal localities of the province such as Go Cong Tay and Go Cong Dong districts. and Go Cong town. VD 20 Rice is also known by the brand "Go Cong Rice". At the 5th Vietnam Rice Festival, VD20 Go Cong Rice won second prize in the contest: "Delicious Vietnamese brand rice". Currently, businesses and localities are continuing to develop the Go

Cong specialty rice product brand VD20 that meets this OCOP, contributing to maintaining the product's reputation and improving the lives of rice producers in Go Cong land, Tien Giang Province [3]. Despite the availability, the product from this type of rice is still limited. Germinated brown rice is created through the process of immersing brown rice grains in water until they undergo germination. During germination, the high-molecular-weight polymers break down, resulting in the production of bio-functional chemicals and enhancing the organoleptic qualities by softening the texture and increasing the number of taste components [4]. While the basic requirements for grain germination are same across different species, each species has its own distinct set of requirements. These requirements might vary in terms of time, temperature, intensity, and other elements such as light and nutrients [5]. The metabolic responses are directly affected by these conditions, leading to variations in the germination process [5, 6].

Germination happens when the grain is exposed to optimal conditions, including a moisture content ranging from 15% to 45%, a temperature over 4 °C, the lack of germination inhibitors, and the presence of atmospheric air. Certain seeds, such as rice, require light as it plays a crucial role in the generation of energy by plants and serves as a signal for numerous physiological reactions [6]. Thus, the initial phase of the germination process involves immersing the grains/seeds in water, allowing water to enter the seed through the pores and micropyle, and then being retained within the grain mostly through interactions with proteins and fibers. The water absorption index becomes stable during the second stage, which involves the breaking of the pericarp in the area of the germ, followed by the emergence of the radicle. During the third stage, the rate of water absorption increases once more, and the cells of the radicle undergo multiplication as they develop [6]. To the best of author knowledge, it still not have the current study on the VD20 rice. Therefore, this study aim to develop the process to produce the germinated rice, by afffecting of the volume of water and pH, time of soaking conditions and germnation conditions on the germination rate and polyphenol content of product.

2. Materials and methods

2.1 Material

VD20 rice was purchased from local farmer in Tien Giang province (Vietnam). The initial proximate compositions, polyphenol content, posstasim content, sodium content, saturated fat content and un-saturated fat content of raw material were analyzed.

2.2 Preparation of germinated brown rice

Rice grains were de-husked by a grain sheller (JGMJ8098, Shanghai Jiading Oils and Grains Apparatus Co. Ltd., China) and cleaned manually to remove disfigured and other extraneous materials.

2.3 Investigation of ratio of rice and water on quality of germinated VD20 rice

Germination of brown rice was conducted by soaking the brown rice grains in distilled water at different *Loan et al.*, 2024

ratio of rice and water as follow 1:1, 1:1.5, 1:2, 1:2.5, and 1:3. Soaking process was conducted at a temperature of 25° C in a incubator at the dark condition. The soaking water was changed every 4 h. After reaching the required germination period, the grains were divided into two parts, one part was used to analyse the germination rate and the left one was dried at 50°C by hot-air oven to a moisture content of $14\pm2\%$ to analyse the total polyphenol content. The rice grains after drying were ground into fine flour using a pin-mill. All samples passing through a 100-mesh sieve were packed in hermetically sealed plastic bags and stored at 4 °C until used.

2.4 Investigation of ratio of soaking conditions on quality of germinated VD20 rice

In this study, germination of brown rice was conducted by soaking the brown rice grains in distilled water at different ratio of rice and water that selected in the previous experiment. The effect of pH of soaking solution (distilled water, pH = 3, 4, 5) and soaking time (3, 4, 5, 6 hours) was investigated. Soaking process also was conducted at a temperature of 25° C in a incubator at the dark condition. Process of drying was operated as the same previous experiment. Sample was collected for further analysis.

2.5 Investigation of ratio of germination conditions on quality of germinated VD20 rice

Different conditions of germination of VD20 rice was studied, including germination temperature (31, 33, 35, 37°C) and germnation time (16, 18, 20, 22 hours). Rice was first soaking as the select rice:water ratio and soaking conditions in previous experiment. Then, it was germinated in the setting conditions. After germination, rice was then dried 50°C by hot-air oven to a moisture content of $14\pm2\%$ to analyse the total polyphenol content. The rice grains after drying were ground into fine flour using a pin-mill. All samples passing through a 100-mesh sieve were packed in hermetically sealed plastic bags and stored at 4 °C until used. The germination rate and total phenolic content were the parameters for this experiment.

2.6 Germination rate

The germination rate was determined by recording the number of sprouted kernels out of the total 100 kernels [7].

2.7 Quality of rice and germinated rice

The proximate composition was determined follow AOAC method (AOAC, 2005). The sodium, potassium, and calcium contents in the rice were determined using ion chromatography [8]. The total polyphenol content of germinated rice was analyzed followed the method of Shen *et al.* [9].

2.8 Statistical analysis

Three replicates of each sample were evaluated. The results underwent statistical analysis using one-way analysis of variance (ANOVA). The means were compared using the

Duncan multiple range test with a mean square error at a 5% probability level.

3. Results and Discussions

Raw materials are important components that determine product quality. Based on the data of Table 1, raw material's moisture, protein, lipid, carbonhydrate, ash, polyphenol contents were 10.11%, 7.39%, 0.39%, 81.84%, 5.58%, 28.39 mgGAE/100g, respectively. Brown VD20 rice also has relative high protein and polyphenol conten. VD20 rice also contains important minerals as well as un-saturated fat. Quality of product could affect the initial nutritional profile of raw material. Depending on the origin and type of rice, the chemical composition may vary. According to Ngo et al. [1], starch is the main nutrient in rice and accounts for approximately over 70%, and protein content only about 7%, but this is a very important source of nutrients. This is because rice protein contains many essential amino acids such as histidine, leucine, tryptophan, etc. High protein and polyphenol content in VD20 rice was comparable with the study of Ngo et al. [1] and Van Ngo et al. [10].

3.1 Effect of ratio of rice:water on germination rate, moisture content, appearance and polyphenol content of grain

The germination rate, moisture content and apperance of germinated VD20 rice showed significant different under differant ratio of soaking water to rice. When the ratio of rice to water is low, specifically the ratio of 1:1, the amount of soaking water is not enough to absorb to stimulate the germination process, thereby causing the sprouts to grow slowly or may do not germinate. It could be seen that the lowest germination rate (80.95%) and moisture content (28.15%) was found on the sample from ratio of rice:water is 1:1. When the ratio of rice and soaking water increased to 1:1.5 and 1:2, the amount of soaking water provides enough water to stimulate the germination process and the sprouting process occurs faster, as can be seen in the picture in Table 2. However, although the germination process has occurred with a relatively high germination rate (93.14%), it is still mixed with ungerminated grains. When increasing the soaking water ratio to 2.5 times of the rice weight, the grain moisture reaches its almost maximum and the rice germination rate is relative high (95.49%) and the moisture content of rice was 34.34%. Table 2 also showed that the germination rate and moisture content were significantly increased when the amout of soaking water rose. The results confirm that the germination rate increased and depended on the soaking water ratio, which led to differ on water absorption of rice. A higher soaking ratio can create better conditions for the rice grains to fully absorb water. However, if the water ratio is high and soaking for a long time can cause the sprouts to develop poorly and the seeds to crack. Therefore, the ratio of rice:water of 1:2.5 was choosen for germinating VD20 rice.

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Follow the increasing of germination rate, TPC content also raised, which could be seen that the highest content of TPC was found in sample from soaking ratio of 1:2.5. However, the polyphenol content gradually decreased from 31.62 mgGAE/100g to 28.51 mgGAE/100g after incubation when increasing the amount of incubation water from 2.5 times to 3 times. The non-significant different of the value of TPC in germinated rice when the rice was soaking with ratio of 1:1, 1:1.5 and 1:3.0. During the soaking phase, elevated moisture content not only stimulates and enhances the physiological and biochemical activity in the grain, but also triggers the transformation of useful chemicals as GABA and TPC (total phenolic compounds) in the grain through reproductive processes [13]. Table 2 also showed that the germination rate and moisture content were significantly increased when the amout of soaking water rose. The results confirm that the germination rate increased and depended on the soaking water ratio, which led to differ on water absorption of rice. A higher soaking ratio can create better conditions for the rice grains to fully absorb water. However, if the water ratio is high and soaking for a long time can cause the sprouts to develop poorly and the seeds to crack. Therefore, the ratio of rice:water of 1:2.5 was choosen for germinating VD20 rice.

Polyphenols are an important compounds, which are considered as an anti-inflammatory, antibacterial, antiallergic and anti-aging substance for humans. Many experimental results show that a diet rich in polyphenols will limit the occurrence of stress, potentially reducing stress and many related diseases [2]. Follow the increasing of germination rate, TPC content also raised, which could be seen that the highest content of TPC was found in sample from soaking ratio of 1:2.5. The results also was the same trend with the germination rate.

Table 1: Physicochemical content of VD20 rice

Parameters	Content		
Moisture content	10.11%		
Protein	7.39%		
Lipid	0.39%		
Carbohydrate	81.84%		
Ash	5.58%		
Polyphenol	28.39 mgGAE/100g		
Crude fiber	0.22 g/100g		
Calcium (Ca)	6.398 mg/100g		
Posstasium (K)	63.29 mg/100g		
Sodium (Na)	4.601 mg/100g		
Saturated fat	0.26 g/100g		
Un-saturated fat	0.111 g/100g		

Table 2: Germination rate, moisture content and apperance of grain under different ratios of rice to water

Ratio of rice:water	1:1	1:1.5	1:2	1:2.5	1:3.0
Germination rate (%)	80.95 ^d	87.82°	93.14 ^b	95.49 ^a	95.80ª
Moisture content (%)	24.15 ^c	30.33 ^b	32.50 ^{ab}	34.34 ^a	34.21 ^a
TPC (mgGAE/100g)	28.67°	29.08 ^{bc}	29.95 ^b	31.62 ^a	28.51°

Note: Values at the same row with different superscript letters represent statistically significant differences (p < 0.05).

Table 3:	Soaking	conditions	influence	on the	germination ra	ate, moisture	content an	d polypheno	ol content of rice
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Soaking time (hour)	pH of soaking water	Germination rate (%)	Moisture content (%)	Polyphenol content (mgGAE/100g)
3	Distilled water	88.73 ^g	29.21 ^f	27.21 ^h
	pH = 3	90.53 ^{efg}	29.56 ^f	27.87 ^{gh}
	$\mathbf{p}\mathbf{H} = 4$	92.12 ^{def}	30.64 ^e	28.56^{fg}
	$\mathbf{p}\mathbf{H}=5$	92.78 ^{cdef}	30.58 ^e	28.64^{fg}
4	Distilled water	90.37 ^{fg}	30.57 ^e	28.57^{fg}
	pH = 3	92.30 ^{def}	31.69 ^d	29.15 ^{ef}
	$\mathbf{p}\mathbf{H} = 4$	93.63 ^{bcde}	32.44 ^d	29.97 ^{de}
	pH = 5	94.07 ^{abcd}	31.91 ^d	30.07 ^d
	Distilled water	93.76 ^{abcd}	32.40 ^d	30.4 ^d
5	pH = 3	94.73 ^{abcd}	33.67°	30.62 ^d
	pH = 4	96.78 ^{ab}	34.18 ^{bc}	31.53°
	pH = 5	95.06 ^{abcd}	33.93°	31.96 ^{abc}
6	Distilled water	95.49 ^{abc}	34.34 ^{bc}	31.62 ^{bc}
	pH = 3	95.94 ^{abc}	35.58ª	32.1 ^{abc}
	$\mathbf{p}\mathbf{H} = 4$	96.86 ^a	35.98ª	32.92 ^a
	pH = 5	96.35 ^{ab}	35.08 ^{ab}	32.24 ^{ab}

Note: Values at the same column with different superscript letters represent statistically significant differences (p < 0.05).

Germination temperature (°C)	Germination time (hour)	Germination rate (%)	Polyphenol content (mgGAE/100g)
31	16	91.87 ^h	28.84 ^g
	18	92.78 ^{gh}	29.57 ^{gh}
	20	93.73 ^{fg}	30.32 ^{fg}
	22	94.45 ^{ef}	30.55 ^f
	16	94.43 ^{ef}	30.57^{f}
22	18	94.98 ^{def}	31.03 ^{de}
33	20	95.60 ^{cde}	31.54 ^d
	22	95.60 ^{cde}	31.77 ^d
	16	96.33 ^{bcd}	32.78 ^{bc}
35	18	96.53 ^{abc}	32.87 ^{bc}
	20	97.04 ^{abc}	33.51 ^{abc}
	22	97.08 ^{abc}	33.19 ^{abc}
37	16	96.47 ^{abcd}	32.75 ^c
	18	97.24 ^{ab}	32.84 ^{bc}
	20	97.94 ^a	33.85 ^a
	22	97.13 ^{ab}	33.64 ^{ab}

Table 4: Germination conditions affect on germination rate and polyphenol content of rice

Note: Values at the same column with different superscript letters represent statistically significant differences (p < 0.05)

Brown rice is considered a natural source of antioxidants, which contains high levels of phenol and flavonoids, ensuring the higher antioxidant capacity of brown rice [14]. Polyphenol content increases after germination due to the breakdown of cell walls and the bonding of phenolic compounds with other substances, which led to phenolicreleasing increased in the germinated rice [14]. However, the polyphenol content gradually decreased from 31.62 mgGAE/100g to 28.51 mgGAE/100g after incubation when increasing the amount of incubation water from 2.5 times to 3 times. The main reason could be that during soaking the enzyme oxidase can be activated, which degradation phenolic compounds. In addition, the leaching process also reduce the TPC in rice [15]. Moreover, some phenolic compounds are water soluble, during the incubation process, the polyphenol content decreases because the incubation machine automatically spray water for 5 minutes to prevent the rice from becoming dry and sour due to germination process, thus the polyphenols would be partially dissolved in water or decomposed by effect of oxygen.

3.2 Effect of pH and soaking time on the germination rate, moisture content, and polyphenol content of grain

Table 3 shows that the germination rate depends mainly on pH of soaking water. Soaking time also has a significant effect on germination rate. The highest germination rate was found when the rice was soaked in soaking solution for six hours. The germination rate of rice was low when soaking rice in distilled water and low pH solution (pH=3) when comparing at the same soaking time. The rate of germination in this pH was ranged from 88.73 to 92.78%. At pH = 6, the significant high of germination rate was found, which reached at value of 95.49-96.86%. During the germination process, in addition to the amount of water used for soaking, the pH of the soaking water also, is one of most influencial factors, which affects biological compounds and germination rate [16]. Soaking is a method used in household technology to hydrate seeds by immersing them in water for a few hours [17]. Because soaking at too low a pH would reduce the cell's pH buffering ability, this leads to a decrease in the seed's ability to germinate, causing a low germination rate [18]. Study of [19] also reported that acidity condition could reduce the germination rate of rice.

pH and soaking time affect grain moisture after soaking (Table 3). During the first 3 hours, the moisture of the rice grain increases rapidly due to the fast water absorption rate and high osmotic pressure. The difference between the moisture inside the grain and outside the grain is high, so moisture is quickly transferred into the grain. During the period from 4 hours to 6 hours, the water absorption rate of rice grains is lower than during the first 3 hours of soaking because at this time the equiriblum stage almost reach. After 6 hours of soaking, the moisture of the rice grain changes very little, reaching a moisture content of 30-35% suitable for germination. However, when the grain is soaked in water for a longer time, it might lose water-soluble nutrients. Benjamasuttikul and Naivikul [20] reported that in the first stage of soaking rice at 0-2 hours, water absorption increases rapidly due to absorption into the embryo of the grain. After 2 - 5 hours, the rice grain slowly absorbs water and reaches a state of equilibrium or saturation. The decreasing of pH of solution at the same soaking time has a significant impact in the moisture content of the germinated grains. The moisture content of seeds at pH of 4 was the highest value and it was lowest when grain was immersed in distilled water during at different soaking time. The above results show that at the soaking time was 6 hours and soaking water pH was 4, the germination rate and moisture content of rice reached maxium value, 96.86% and 35.98%, respectively. Under this condition, the polyphenol content in rice also reached the highest value (32.92 mgGAE/100g). Soaking is a very important process before seed germination. However, during the soaking process at different times and pH, it could be seen that the polyphenol content gradually decreases over the soaking time of 3-6 hours and decreases the most at pH 5. The main reason is due to soluble phenolic compounds, which leaching during process. The water should partially dissolve into the soaking solution [21]. Another reason is that during soaking, the oxidase enzyme can be activated by appropriate pH for this enzyme to reduced polyphenol content. Some studies also showed similar results on the decline of polyphenol content during the soaking process of some other cereals [22, 23].

3.3 Effect of germination temperature and time on the total polyphenol content and germination rate of rice

During germination process, biosynthetic activities in the seeds take place strongly, functional compounds increase sharply during this period [24]. From Table 4, its temperature and incubation time have an impact on the germination rate of seeds. Seed germination rate was lowest value at incubation temperature of 31°C and incubation time of 16 hours. However it The germination rate gradually increases with the temperature and incubation time of the rice. In the study by Chung et al. [25] after incubation of rice varieties, the seed germination rate increased with incubation time and the highest rate was 95.5%. Incubation temperature has an effect on polyphenol content in germinated. The polyphenol content of brown rice increases with incubation time and reaches the highest value at 20 hours of incubation. When incubated for 20 hours at 37 °C, the polyphenol content of rice is 33.85 mgGAE/100g, an increase compared to the original material of 28.39 mgGAE/100g. Nelson et al. (2013) showed that polyphenol content increased during the germination process of some cereal grains such as wheat, rice, rye, and sorghum. Polyphenol content increases after germination due to increased antioxidant activity that increases free radicals leading to cell breakdown releasing phenolic compounds (Ti et al., 2014). High content of antioxidant could also lead to reduction in digestion rate, which is beneficial to the diabetic patients (Ngo et al., 2022). Germination rate of 95.49% and total polyphenol content of 31.62 mgGAE/100g was observed when the VD20 rice was soaked in 2.5 times higher its weight. Besides, the pH of soaking solution of 4 and soaking period of 6 hours were the most suitable conditions. VD20 rice germinated well when it was incubation at 37°C for 20 hours. At these conditions, the germinated VD20 rice reached the content of polyphenol of 33.85 mgGAE/100g, which could be have further various applications. This study also is the fundamental knowledge for researchers to utilize the VD20 rice in the further based on their characteristic. Optimization of germination process also should be considered.

4. Conclusions

Germination rate of 95.49% and total polyphenol content of 31.62 mgGAE/100g was observed when the VD20 rice was soaked in 2.5 times higher its weight. Besides, the pH of soaking solution of 4 and soaking period of 6 hours were the most suitable conditions. VD20 rice germinated well when it was incubation at 37°C for 20 hours. At these conditions, the germinated VD20 rice reached the content of polyphenol of 33.85 mgGAE/100g, which could be have further various applications.

Conflicts of interest

There are no conflicts to declare.

Author Contributions

L.T.K.L: Conceived, designed performed the experiments, analysed the data and contributed funding administration. All authors wrote, reviewed, edited the paper and agreed to submit this version to the journal.

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