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Application of Oxygen-argon Plasma as a Potential Approach of Improving the Nutrition Value of Pre-germinated Brown Rice

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Abstract This is the first study to apply plasma technology for improving nutrition value of cereal products. Cold plasma, a mixture of oxygen and argon plasma, was applied to rice seeds before pre-germination process. Plasma condition of 10 watts, 5 seconds, and 5 millimeters distance and 10 watts, 5 seconds, and 8 millimeters distance created high germination rates of pre-germinated brown rice. The latter conditions gave the grains with longer roots and bodies. Plasma treatment could result in increasing contents of total phenolics and γ-aminobutyric acid. Gas chromatography-mass spectrometry revealed 13 identifiable compounds: simple phenolic compounds; pyrans; furan; quinone; and fatty acids, in which biosyntheses of these 13 compounds were likely to be promoted by the plasma processing. These findings suggest that plasma technology could provide better quality pre-germinated brown rice.

Keywords: *plasma, pre-germination brown rice, gas chromatography-mass spectrometry, phenolics, γ-aminobutyric acid*

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1. Introduction

Pre-germinated brown rice (PBR) is known as a cereal product with ultimate health benefits. The process of seed germination enhances productions of functional nutritive compounds including γ-aminobutyric acid (GABA), lysine, dietary fiber, niacin magnesium, vitamin B1, vitamin B6, γ-oryzanol, and vitamin E [1,2]. Many studies have demonstrated the health advantages of consuming PBR. For instance, the intake of PBR boosts the immune system, ameliorates the hyperglycemia, and assists treating anxiety disorders [3,4,5]. Nowadays, PBR and related products, such as yogurts, drinks and cosmetics, are becoming more popular in many countries such as Japan, Korea, Thailand and some European countries. Due to this increased market demand, technologies or procedures that can furtherimprove and sustain nutritive values of PBR products should be of great interest.

Plasma, which is one of the fourth fundamental states of matter (besides solid, liquid, and gas), is clean and environmentally-friendly. It has been introduced as an approach useful in modification of the surface materials, decontamination of micro-organism, and treatment of diseases [6,7,8]. Recently, plasma has been applied to accelerate seed germination [9]. For instance, air plasma treatment changes the wetting properties of seeds due to oxidation of their surface that leads to faster germination and greater yield of wheat and oats [10]. Plasma treatment can have a variety of effect on the morphological and sowing characteristics of seeds due to its complex interaction with organic materials and living cell [11]. On the basis of those findings, plasma has shown potential promise as a tool for developing physical growth and the appearance of plants. However, there, seemingly, has been no study that has focused on the nutritional application of plasma to edible plant grains.

Therefore, this is the first attempt to apply plasma in a nutritional perspective. Cold plasma, a mixture of oxygen and argonplasmas, was applied to rice seeds before the pre-germination process. The changes in the rice seed's functional components were evaluated using UV/Vis spectrophotometer, high performance liquid chromatography with photodiode array detector (HPLC-DAD), and gas chromatography-mass spectrometry (GC-MS). The knowledge gained from this study will be useful and should expand the perception of scientists, engineers and researchers concerning the potential role of plasma in the food industry.

2. Materials and Methods

2.1. Rice Samples and Pre-germination

Rice seed samples of *Oryza sativa* L. (cultivar Riceberry) used in this study were from the Phitsanulok Rice Research Center in Phitsanulok province, Thailand. Pre-germination of the seeds was done by soaking the seeds in tab water for 120 hr. During this time, the soaking water was changed every 12 hr to prevent fungal deterioration. Before analysis, the pre-germinated seeds were dried and kept in a cool place.

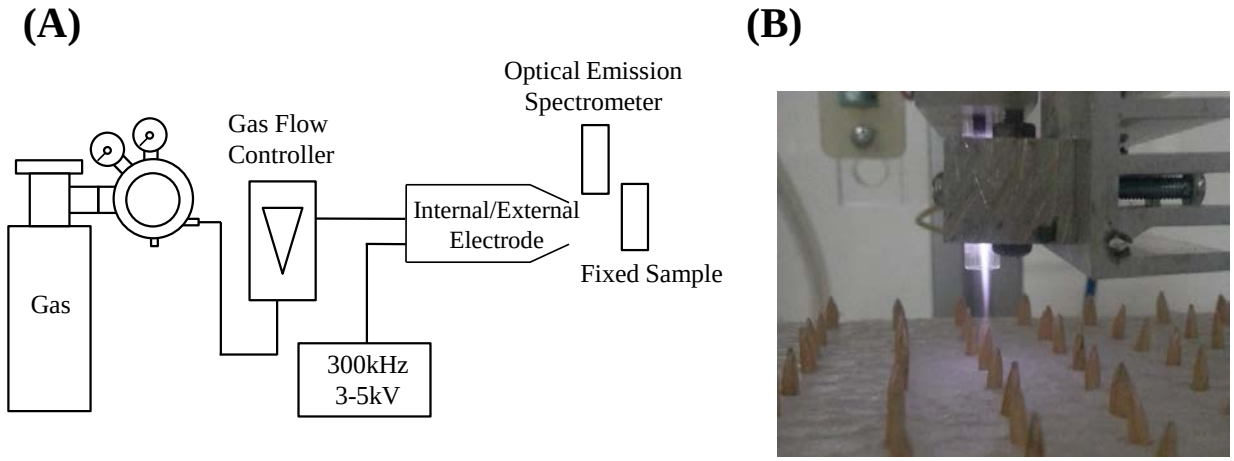


Figure 1. Application of plasma. (A) Scheme of atmospheric plasma jet system. (B) Illustration of rice seed plasma treatment

2.2. Reagents and Chemicals

Methanol (analytical and HPLC grades) was purchased from Merck (Darmstadt, Germany). Folin-Ciocalteu's reagent, sodium carbonate, gallic acid, 2,6-dimethylpyridine (internal standard for GC-MS), and sodium sulphate anhydrous were analytical grade purchased from Sigma-Aldrich Chemical Co. (St. Louis, Mo, USA).

2.3. Atmospheric Plasma Jet and Plasma Application to Rice Seeds

A laboratory-made plasma jet (Figure 1) was used to generate cold plasma under atmospheric pressure, in which argon (5.0 mL) was used as the carrier gas and oxygen (100.0 mL) as the reactive gas. The inner electrode of this jet was covered with a quartz tube and was centered at the axis of the outer electrode.It was connected to a 400 kHz, 3–5 kV voltage source whereas the outer electrode was grounded. Plasma was generated by applying an electric current between the two electrodes at certain radio frequency. This allowed the argon carrier gas and the oxygen reactive gas to be ionized to an excited state, thus creating plasma. This plasma was to have high energy and conductive properties similar to that of some metals.

Rice seeds were fixed with a foam plate on which turned up seed buds had direct exposure to the plasma. The power for plasma operation was 10 to 14 watts, the distance between the outlet and rice seeds was 5 or 8 mm, and the exposure time was 5 to 10 sec.

2.4. Determination of Total Phenolic Content

The total phenolic content of the samples was determined usinga Folin-Ciocalteau assay.Briefly, 100 µL of methanolic rice extract was added to 250 µLof Folin-Ciocalteu reagent, 250 µLof 7% sodium carbonate solution, and 1400 µL of water. Total phenolics were spectrophotometrically determined at 760 nm and were expressed as gallic acid equivalence.

2.5. Determination of γ-Aminobutyric Acid (GABA)Using High Performance Liquid Chromatography (HPLC)

Rice GABA was determined using HPLC after derivatization with hydroxynaphthaldehyde (HN). Briefly, 500 µL of ethanolic rice extract was mixed with 500 µL of water and 1000 µL of 0.3% HN at 80°C for 10 min. The HPLC system consisted of an Agilent HPLC 1100 connected to a DAD detector (Model G1315 A, Agilent Technologies, Palo Alto). The separations of GABA was carried out using a Vertical-C18 column (4.6 × 250 mm, 5 µm) at 40°C with a methanol/water (70/30, v/v) flow rate of 1.0 mL/min. The UV detection was at 330 nm.

2.6. GC-MS Analysis of Chemical Profile

Each 3.00 g rice sample was extracted with 15.00 mLof methanol, and 2,6-dimethylpyridine (0.3µL) was used as the internal standard. After the addition of sodium sulphate anhydrous followed by filtering, each mixture was vacuum dried to adjust to a final volume to 1.00 mL.GC-MS separation was done using an Agilent 6890 gas chromatograph (Agilent Technologies, Polo Alto) using a capillary column AT-5MS (30 m× 0.25 mm I.D. and 0.25 µm film thickness (Deerfield, IL)). The helium flow rate was 1.0 mL/min. The oven temperature was 45 to 250°C (ramping up by 5°C/min). An Agilent 5973A mass spectrometer utilizing electron impact ionization was used as a chromatographic detector. Identification of the components was performed by matching their spectra with the reference spectra in the NIST 11 Mass Spectral Library. The relative quantity of an identified compound was calculated by dividing its peak area with that of the internal standard.

2.7. Statistical Analysis

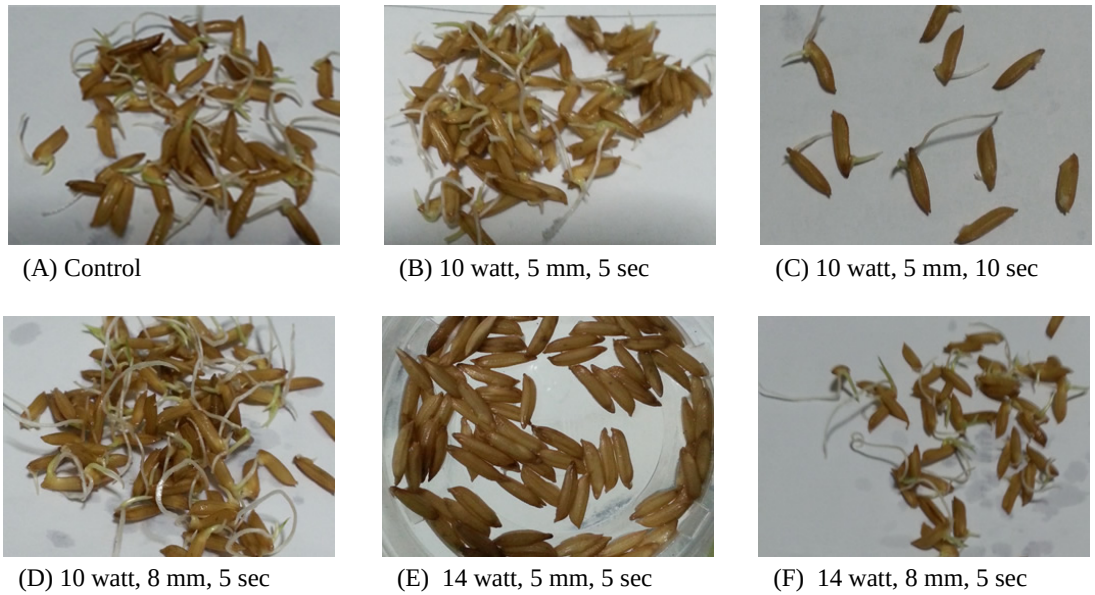
The quantitative data are expressed as the mean ± standard deviation (n=3). Statistical analysis in this study

was analyzed using one-way ANOVA. The statistical software used in the study was GNU PSPP (version 0.7.9). Differences are statistically significant at $P<0.05$.

3. Results and Discussions

3.1. Germination of Rice Seeds

Plasma application affected the germination rate and physical appearance of the rice seeds. Germination rate of the plasma-untreated rice seeds was 97%, whereas those of the plasma-treated seeds differed from 0-93% being dependent upon the plasma conditions. The plasma conditions giving the highest germination rate were 10 watts, 5 sec, and 8 mm (93 % germination), and 10 watts, 5 sec, and 5 mm (91% germination). As for physical appearance, seeds treated with plasma at10 watts, 5 sec, and 5 mm had similar appearance as the untreated seeds, and seeds treated with plasma at 10 watts, 5 sec, and 8 mm showed longer roots and body (Figure 2). Other treatment conditions gave lower germination rates and inferior physical characters (Figure 2). Therefore, the two conditions with highest germination rates were selected for ongoing examination on chemical seed changes. Preliminary observation suggested that plasma could affect germination process of rice seeds. These results are supportive to a previous study investigating the effect of plasma processing and seed surface modification [9]. The earlier study suggested that plasma could remove thin lipid layers and also reduce biopolymer chains that make up the seed coat, thus allowing better water transportthrough the seed coat that would improveseed germination.



(A) Control (B) 10 watt, 5 mm, 5 sec (C) 10 watt, 5 mm, 10 sec

(D) 10 watt, 8 mm, 5 sec (E) 14 watt, 5 mm, 5 sec (F) 14 watt, 8 mm, 5 sec

Figure 2. Physical appearance of PBR after 96hr germination period. (A) Control (B) 10 watts, 5 mm, 5 sec (C) 10 watts, 5 mm, 10 sec (D) 10 watts, 8 mm, 5 sec (E) 14 watts, 5 mm, 5 sec (F) 14 watts, 8 mm, 5 sec

3.2. Total Phenolics and GABA

Phenolic compounds are a class of antioxidant agents which act as free radical terminators [12]. The amounts of total phenolic compounds in the germinated seeds are presented in Figure 3A. The control groups contained 0.38–0.39 mg gallic acid equivalent/g dry wt during 48 hr to 96 hr of pre-germination. After 48 hr of pre-germination, total phenolic content of both plasma groups was greater than that of the control groups, which were 0.43 mg/g for the 5 mm group and 0.46 mg/g for the 8 mm group. However, after 72 hr and 96 hr of pre-germination, the phenolic content of the plasma groups had gradually decreased while that of the control groups remained unchanged (Figure 3A). The decreasing of phenolic compounds in the pre-germinated brown rice is in agreement with some previous studies [13,14]. A study by Tian *et al.* (2005) reported the decreasing tendency of major soluble phenolic compounds 6'-*O*-(*E*)-feruloylsucrose and 6'-*O*-(*E*)-sinapoylsucrose during germination process, while the levels of free ferulic acid and sinapinic acid increased [13]. It has been speculated that during germination seed coat is injured by oxidation and microorganism infiltration. The process induces saccharolytic enzymes to hydrolyze starch that causes production of free phenolic compounds from hydroxycinnamate sucrose esters (6'-*O*-(*E*)-feruloylsucrose and 6'-*O*-(*E*)-sinapoylsucrose) to have more antioxidant activity [14]. In this study, plasma processing might influence the activity of those saccharolytic enzymes, thereby increasing production of free phenolic compounds that were partly dissolved and lost into soaking water during pre-germination process. Accordingly, total phenolic compounds in the PBR samples were detected decreased. From the results (Figure 3A), it is suggested that rice seeds pre-germinated for 48 hr after plasma application would be a good source of phenolics.

The γ-aminobutyric acid (GABA), which is a non-protein amino acid known as an inhibitory neurotransmitters in the sympathetic nervous system [15] and is useful for reduction of anxiety, providing pain relief, and treating attention deficit-hyperactivity disorder (ADHD) [16,17], was quantitatively investigated (Figure 3B). The control had 1.3-1.6 μg GABA/g dry wt. The GABA content of both groups of seeds treated with plasma

increased from 1.2 and 1.2 μg/g after 48 hr to 1.8 and 1.7 μg/g after 96 hr for the plasma groups of 5 mm and 8 mm, respectively. The results clearly show the improvement of GABA through 96 hr of plasma application.

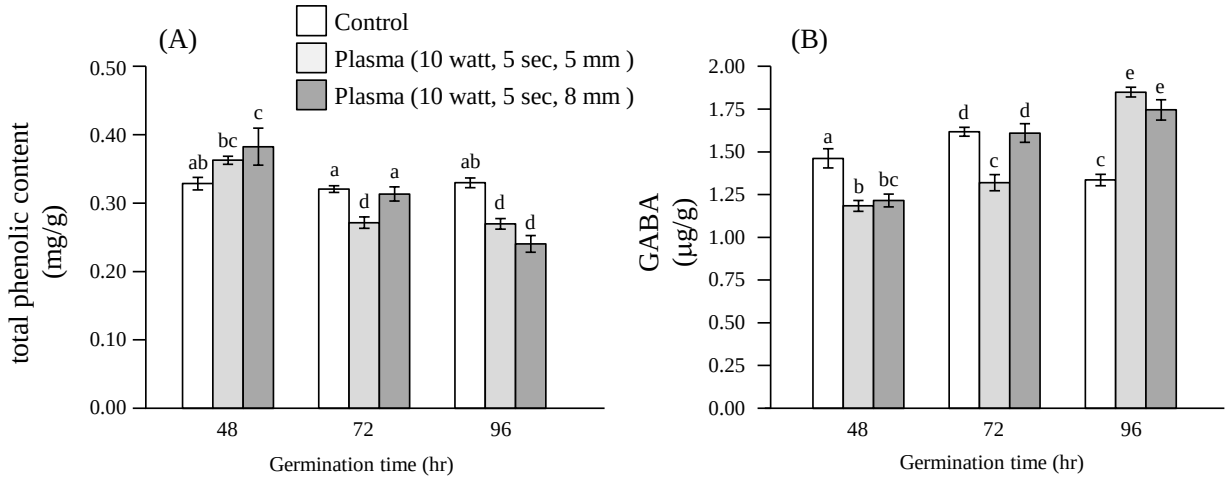


Figure 3. Contents of (A) total phenolic content and (B) γ-aminobutyric acid in PBR samples. Each value is the mean±SD (n = 3). Means without a common letter differ, $P< 0.05$

3.3. GC-MS Analysis for Other Chemical Profiles

Since it is obvious that plasma treatment could affect both physical and chemical properties of PBR (Figure 2 and Figure 3), further investigation of some chemical and functional components were employed using GS-MS analysis. Thirteen compounds were identified in the pre-germinated seed samples, and these compounds were simple phenolic compounds (2-methoxyphenol, 2-methoxy-4-vinylphenol, and 4-hydroxy-2-methoxy benzaldehyde), pyrans(2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one and 3-deoxy-*d*-mannoic lactone), quinine (2,6-dimethyl-3-(methoxymethyl)-*p*-benzoqui none), furan (5-methoxymethyl-2-furancarboxaldehyde), and fatty acids (tetradecanoic acid, 9-octadecanoic acid, hexadecanoic acid, 9,12-octadecanoic acid, 8,11-octadeca dienoic acid, and octadecanoic acid) (Table 1). There were noticeable changes in the relative quantities of the compounds after 72 hr and 96 hr of pre-germination. After 72 hr, relative quantities of all detected compounds of the 5 mm plasma group were greater than those of the control, while only 2-methoxyphenol of the 8 mm plasma group was higher than those of the control (Table 1). Furthermore, after 96 hr of pre-germination, the relative quantities of both plasma groups were greater than those of the control (Table 1). These results suggest that plasma treatment could enhance biosynthesis as well as metabolism of chemicals in pre-germinated rice seeds, thereby yielding a greater content of the chemicals analyzed by GC-MS. These detected phytochemicals have been reported to possess beneficial biological activities. Phytochemicals 2-methoxyphenols and 2-methoxyl-4-vimylphenol are known to have various physiological effects in humans, such as preventing oxidative damage of lipid and low-density lipoproteins, inhibiting platelet aggregation and reducing the risk of coronary heart disease [18]. 5-Hydroxymethyl-2-furan carboxaldehydeis evidenced for its anti-platelet activity [19].

The role of plasma on seed chemical properties would be explained as the plasma having an influence on cells inside the seed. When a seed is treated with plasma, the plasma energy and active particles apparently transfer and penetrate through the seed coat because of the plasma's ability to penetrate through porous materials [20]. The interaction of cells with plasma might stimulate growth, alter protein structure, enhance enzymatic activities [21,22], thereby producing more functional metabolites. Plasma technology was also reported to increase the concentration of free radicals in seeds that play an important role in acceleration of seed metabolism [23]. However, further clarification on the effect of different types of plasma and radicals onto the cells would be required to achieve the most benefit from plasma treatment.

We also performed another set of plasma experiment on another variety of rice (KhaoJowHawmPhitsanulok 1) using the same plasma conditions. Germination rate and survival ratio of this rice's PBR was similar to those of Riceberry, but the rice's total phenolics and GABA were somewhat different (data not shown). These differences would be partly due to differences in physical and morphological properties of the different kind of seeds (i.e., rice hull thickness, and seed dimensions). This implies that plasma conditions must be specifically optimized for each variety of rice.

Since this study was the first investigation of the nutritional perspectives of plasma technology, with rice seeds being individually bombarded with plasma beams, it was a time-consuming process. Therefore, process modification and development of plasma instrumentation should be considered in order to effectively transfer the knowledge and the applications to match industrial requirements. Simultaneously, chemical analysis using advanced chromatographic methods (such as liquid chromatography-mass spectrometry (LC-MS) and liquid chromatography tandem-mass spectrometry (LC-MS/MS)) would be done in order to assess the change of functional compounds (such as phenolic compounds or other antioxidants) at molecular species level, thereby clarifying the detailed mechanism of plasma stimulation in PBR samples.

Table 1. Relative quantities of chemicals in pre-germinated brown rice extracts analyzed using GC-MS

Peak	Time (min)	Compound	48hr		
			Control	Plasma (5mm)	Plasma (8mm)
1	4.22	2,6-Dimethylpyridine (Internal standard)	1.00	1.00	1.00
2	7.25	2-Methoxyphenol	9.32×10^{-3}	1.57×10^{-3}(↓)	3.72×10^{-3}(↓)
3	8.08	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	4.56×10^{-2}	1.67×10^{-2}(↓)	7.74×10^{-3}(↓)
4	9.17	5-(Methoxymethyl)-2-furancarboxaldehyde	7.31×10^{-2}	3.69×10^{-2}(↓)	1.26×10^{-2}(↓)
5	9.30	Unknown	6.06×10^{-2}	1.96×10^{-2}(↓)	8.11×10^{-3}(↓)
6	10.16	Unknown	3.04×10^{-2}	3.64×10^{-2}	1.87×10^{-2}(↓)
7	10.48	2-Methoxy-4-vinylphenol	5.56×10^{-2}	2.87×10^{-2}(↓)	9.66×10^{-3}(↓)
8	11.65	4-Hydroxy-2-methoxybenzaldehyde	7.05×10^{-4}	3.37×10^{-3}(↑)	7.01×10^{-3}(↑)
9	12.86	Unknown	1.4	7.04×10^{-1}(↓)	2.53×10^{-1}(↓)
10	13.68	2,6-Dimethyl-3-(methoxymethyl)-p-benzoquinone	4.97×10^{-3}	3.04×10^{-2}(↑)	1.13×10^{-2}(↑)
11	14.82	3,5-Dihydroxy-6-(hydroxymethyl)oxan-2-one	5.40×10^{-1}	3.21×10^{-1}(↓)	1.29×10^{-1}(↓)
12	15.79	Hexadecanoic acid	5.42×10^{-2}	3.01×10^{-1}(↑)	1.96×10^{-1}(↑)
13	17.89	Hexadecanoic acid	3.14×10^{-2}	1.21×10^{-2}(↓)	1.64×10^{-2}(↓)
14	19.12	8,11-Octadecadienoic acid	2.52×10^{-2}	7.85×10^{-3}(↓)	1.09×10^{-2}(↓)
15	19.17	9-Octadecanoic acid	2.12×10^{-2}	6.75×10^{-3}(↓)	9.35×10^{-3}(↓)
16	19.56	9,12-Octadecanoic acid	4.21	1.79(↓)	13.92(↑)
17	19.75	Octadecanoic acid	7.30×10^{-2}	3.47×10^{-2}(↓)	5.11×10^{-2}(↓)
Peak	**Time (min)**	**Compound**	**72hr**		
			Control	**Plasma (5mm)**	**Plasma (8mm)**
1	4.22	2,6-Dimethylpyridine (Internal standard)	1.00	1.00	1.00
2	7.25	2-Methoxyphenol	4.93×10^{-3}	5.27×10^{-3}(↑)	6.57×10^{-3}(↑)
3	8.08	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	6.43×10^{-2}	1.93×10^{-2}(↓)	5.76×10^{-2}(↓)
4	9.17	5-(Methoxymethyl)-2-furancarboxaldehyde	3.19×10^{-2}	1.79×10^{-2}(↓)	3.94×10^{-2}(↑)
5	9.30	Unknown	4.02×10^{-2}	1.06×10^{-2}(↓)	2.74×10^{-2}(↓)
6	10.16	Unknown	6.93×10^{-2}	3.31×10^{-2}(↓)	9.25×10^{-2}(↑)
7	10.48	2-Methoxy-4-vinylphenol	2.82×10^{-2}	1.71×10^{-2}(↓)	3.57×10^{-2}(↑)
8	11.65	4-Hydroxy-2-methoxybenzaldehyde	1.22×10^{-2}	1.08×10^{-2}(↓)	1.37×10^{-2}(↑)
9	12.86	Unknown	5.94×10^{-1}	3.57×10^{-1}(↓)	9.01×10^{-1}(↑)
10	13.68	2,6-Dimethyl-3-(methoxymethyl)-p-benzoquinone	2.47×10^{-2}	1.99×10^{-2}(↓)	3.86×10^{-2}(↑)
11	14.82	3,5-Dihydroxy-6-(hydroxymethyl)oxan-2-one	3.16×10^{-1}	1.60×10^{-1}(↓)	4.04×10^{-1}(↑)
12	15.79	Hexadecanoic acid	7.46×10^{-2}	6.88×10^{-2}(↓)	1.27×10^{-1}(↓)
13	17.89	Hexadecanoic acid	4.18×10^{-2}	3.09×10^{-2}(↓)	5.21×10^{-2}(↑)
14	19.12	8,11-Octadecadienoic acid	3.08×10^{-2}	2.27×10^{-2}(↓)	3.41×10^{-2}(↑)
15	19.17	9-Octadecanoic acid	2.76×10^{-2}	2.09×10^{-3}(↓)	3.66×10^{-2}(↑)
16	19.56	9,12-Octadecanoic acid	5.25	4.13(↓)	7.13(↑)
17	19.75	Octadecanoic acid	1.89×10^{-1}	1.39×10^{-1}(↓)	2.89×10^{-1}(↑)
Peak	**Time (min)**	**Compound**	**96hr**		
			Control	**Plasma (5mm)**	**Plasma (8mm)**
1	4.22	2,6-Dimethylpyridine (Internal standard)	1.00	1.00	1.00
2	7.25	2-Methoxyphenol	5.77×10^{-4}	1.88×10^{-3}(↑)	1.41×10^{-3}(↑)
3	8.08	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	6.39×10^{-2}	1.35×10^{-1}(↑)	9.51×10^{-2}(↑)
4	9.17	5-(Methoxymethyl)-2-furancarboxaldehyde	1.42×10^{-3}	9.33×10^{-2}(↑)	3.32×10^{-2}(↑)
5	9.30	Unknown	7.61×10^{-2}	8.19×10^{-2}(↑)	8.18×10^{-2}(↑)
6	10.16	Unknown	3.75×10^{-2}	1.80×10^{-1}(↑)	1.03×10^{-1}(↑)
7	10.48	2-Methoxy-4-vinylphenol	7.44×10^{-3}	6.15×10^{-1}(↑)	2.27×10^{-2}(↑)
8	11.65	4-Hydroxy-2-methoxybenzaldehyde	2.20×10^{-3}	2.10×10^{-2}(↑)	8.59×10^{-3}(↑)
9	12.86	Unknown	6.71×10^{-2}	8.65×10^{-1}(↑)	3.12×10^{-1}(↑)
10	13.68	2,6-Dimethyl-3-(methoxymethyl)-p-benzoquinone	4.98×10^{-3}	5.54×10^{-2}(↑)	1.66×10^{-2}(↑)
11	14.82	3,5-Dihydroxy-6-(hydroxymethyl)oxan-2-one	9.90×10^{-2}	5.14×10^{-1}(↑)	3.24×10^{-1}(↑)
12	15.79	Hexadecanoic acid	2.78×10^{-2}	1.99×10^{-1}(↑)	8.13×10^{-2}(↑)
13	17.89	Hexadecanoic acid	1.06×10^{-2}	8.25×10^{-2}(↑)	3.28×10^{-2}(↑)
14	19.12	8,11-Octadecadienoic acid	8.34×10^{-3}	8.73×10^{-2}(↑)	1.98×10^{-2}(↑)
15	19.17	9-Octadecanoic acid	8.69×10^{-3}	5.27×10^{-2}(↑)	2.16×10^{-2}(↑)
16	19.56	9,12-Octadecanoic acid	1.97	13.4(↑)	4.71(↑)
17	19.75	Octadecanoic acid	6.09×10^{-2}	5.70×10^{-1}(↑)	1.78×10^{-1}(↑)

Besides plasma processing, some other studies have shown the influence of environmental factors that promote biologically active compounds in PBR. For instance, a study by Komatsuzaki*et al.* (2007) reported that water soaking and gaseous treatment could enhance GABA levels [24], and another study (Zhang*et al.*, 2014) demonstrated that germination conditions of temperature and time also affected GABA content in japonica and

indicaPBR [25]. Accordingly, integration of available technologies and knowledge should be done in order to achieve the most benefit from PBR products.

4. Conclusion

Plasma processing could enhance and promote germination of rice seeds by improving physical and nutritional value when the conditions were optimal. Plasma treatment caused significant changes in several chemical compositions. Quantitative analysis of total phenolic content and GABA showed that the content of these compounds was greater than that of control samples at some points of time during the 48 hr to 96 hr of pre-germination. GC-MS analysis revealed 13 identifiable compounds in the pre-germinated seed samples, these being three simple phenolic compounds, two pyrans, one furan, one quinone, and six fatty acids. It appeared likely that the biosyntheses of these 13 compounds were accelerated in the plasma-treated groups due to the increasing tendency of the relative quantities of those compounds. These findings suggest that plasma technology would be a potential approach to provide better quality of PBR for health purposes.

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References

[1] Imsanguan, P., Roaysubtawee, A., Borirak, R., Pongamphai, S., Douglas, S. and Douglas, P. L. "Extraction of α-tocopherol and γ-oryzanol from rice bran," *LWT Food Science and Technology*, 41(8). 1417-1424. Nov. 2008.

[2] Yodpitak, S., Sookwong, P., Akkaravessapong, P. and Wongpornchai, S. "Changes in antioxidant activity and antioxidative compounds of brown rice after pre-germination," *Journal of Food and Nutrition Research*, 1(6). 132-137. Nov. 2013.

[3] Wu, F., Chen, H., Yang, N., Duan, X., Jin, Z. and Xu, X. "Germinated brown rice enhances antioxidant activities and immune functions in aged mice," *Cereal Chemistry*, 90(6). 601-607. Nov. 2013.

[4] Torimitsu, M., Nagase, R., Yanagi, M., Homma, M., Sasai, Y., Ito, Y., Hayamizu, K., Nonaka, S., Hosono, T., Kise, M., Seki, T. and Ariga, T. "Replacing white rice with pre-germinated brown rice mildly ameliorates hyperglycemia and imbalance of adipocytokinelevels in type 2 diabetes model rats," *Journal of Nutritional Science and Vitaminology (Tokyo)*, 56(5). 287-292. Jan. 2010.

[5] Mamiya, T., Kise, M., Morikawa, K., Aoto, H., Ukai, M. and Noda, Y. "Effects of pre-germinated brown rice on depression-like behavior in mice," *Pharmacology Biochemistry and Behavior*, 86(1). 62-67. Jan. 2007.

[6] Chu, P.K., Chen, J.Y., Wang, L.P. and Huang, N. "Plasma-surface modification of biomaterials," *Materials Science and Engineering: R: Reports*, 36(5-6). 143-206. Mar. 2002.

[7] Winter, T., Winter, J., Polak, M., Kusch, K., Mäder, U., Sietmann, R., Ehlbeck, J., van Hijum, S., Weltmann, K.D., Hecker, M. and Kusch, H. "Characterization of the global impact of low temperature gas plasma on vegetative microorganisms," *Proteomics*, 11(17). 3518-3530. Sep. 2011.

[8] Isbary, G., Shimizu, T., Li, Y.F., Stolz, W., Thomas, H.M., Morfill, G.E. and Zimmermann, J.L. "Cold atmospheric plasma devices for medical issues," *Expert Review of Medical Devices*, 10(3). 367-377. May 2013.

[9] Dhayal, M., Lee, S.Y. and Park, S.U. "Using low-pressure plasma for *Carthamustinctorium*L. seed surface modification," *Vaccum*, 80 (5). 499-506. Jan. 2006.

[10] Šerá, B., Špatenka, P., Šerý, M., Vrchotová, N. and Hrušková, I. "Influence of plasma treatment on wheat and oat germination and early growth," *IEEE Transactions on Plasma Science*, 38(10). 2963-2968. Oct. 2010.

[11] Filatova, I., Azharonok, V., Kadyrov, M., Beljavsky, V., Gvozdov, A., Shik, A. and Antonuk, A. "The effect of plasma treatment of seeds of some grain and legumes on their sowing quality and productivity," *Romanian Journal of Physics*, 56(Supplement). 139-143. Jan. 2011.

[12] Shahidi, F. and Wanasundara, P.K.J.P.D. "Phenolic antioxidants," *Critical Reviews in Food Science and Nutrition*, 32(1). 67-103. 1992.

[13] Tian, S., Nakamura, K., Cui, T. and Kayahara, H. "High-performance liquid chromatographic determination of phenolic compounds in rice," *Journal of Chromatography A*, 1063(1-2). 121-128. Jan. 2005.

[14] Tian, S., Nakamura, K. and Kayahara, H. "Analysis of phenolic compounds in white rice, brown rice, and germinated brown rice," *Journal of Agricultural and Food Chemistry*, 52(15). 4808-4813. Jul. 2004.

[15] McCormick, D.A. "GABA as an inhibitory neurotransmitter in human cerebral cortex," *Journal of Neurophysiology*, 62(5). 1018-1027. Nov. 1989.

[16] Möhler, H. "The GABA system in anxiety and depression and its therapeutic potential," *Neuropharmacology*, 62(1). 42-53.2012 Jan. 2012.

[17] Eddent, R.A.E., Crocetti, D., Zhu, H., Gilbert, D.L. and Mostofsky, S.H. "Reduced GABA concentration in Attention Deficit/Hyperactivity Disorder," *Archives of General Psychiatry*, 69(7) 750-753. Jul. 2012.

[18] Jeong, J. B. and Jeong, H. J. "2-Methoxy-4-vinylphenol can induce cell cycle arrest by blocking the hyper-phosphorylation of retinoblastoma protein in benzo[a]pyrene-treated NIH3T3 cells," *Biochemical and Biophysical Research Communications*, 400(4). 752-757. Sep. 2010.

[19] Pyo M.K., Jin, J.L., Koo, Y.K. and Yun-Choi, H.S. "Phenolic furan type compounds isolated from Gastrodiaelata and their anti-platelet effects," *Archives of Pharmacal Research*, 27(4). 381-385. Apr. 2004.

[20] Krentsel, E., Yasuda, H., Miyama, M. and Yasuda, T. "Penetration of plasma surface modification into porous media. III. Multiple samples exposed to CF4 and C2F4 low temperature cascade arc torch," *Journal of Polymer Science Part A: Polymer Chemistry*, 33(17). 2887-2892. Dec. 1995.

[21] Volin, J.C., Denes, F.S., Young, R.A. and Park S.M.T. "Modification of seed germination performance through cold plasma chemistry technology," *Crop Science*, 40(6). 1706-1718. Oct. 1999.

[22] Henselová, M., Slováková, L., Martinka, M. and Zahoranová, A. "Growth, anatomy and enzyme activity changes in maize roots induced by treatment of seeds with low-temperature plasma," *Biologia*, 67(3). 490-497. Jun. 2012.

[23] Stoffels, E., Sakiyama, Y. and Graves, D.B. "Cold atmospheric plasma: charged species and their interactions with cells and tissues," *IEEE Transactions on Plasma Science*, 36(4). 1441-1451. Aug. 2008.

[24] Komatsuzaki, N., Tsukahara, K., Toyoshima, H., Suzuki, T., Shimizu, N. and Kimura, T. "Effect of soaking and gaseous treatment on GABA content in germinated brown rice," *Journal of Food Engineering*. 78(2).556-560. Oct. 2007.

[25] Zhang, Q., Xiang, J., Zhang, L., Zhu, X., Evers, J., van der Werf, W. and Duan, L., "Optimizing soaking and germination conditions to improve gamma-aminobutyric acid content in japonica and indica germinated brown rice," *Journal of Functional Foods*, 10. 283-291. Sep. 2014.