

## Low pressure radio frequency plasma effects on the mould control, physical quality, nutritional value, mineral content and trace element content of brown rice snack bars

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### Summary

This study investigated the application of low pressure radio frequency (RF) plasma from 30 W to 160 W at exposure times from 5 min to 30 min on an *Aspergillus flavus* infected brown rice snack bar. Changes in the microbial quality (total yeast and mould), physical quality (colour and hardness), nutritional value, mineral content and trace element content of a brown rice snack bar before and after treatment (130 W for 30 min) were also studied. Low pressure RF plasma at 130 W for 30 min inhibited *A. flavus* on a brown rice snack bar the best. Plasma treatment at these conditions provided a 4 log CFU·g<sup>-1</sup> reduction of yeast and mould and kept the snack bar at an acceptable quality. Low pressure RF plasma did not affect the brown rice snack bar colour ( $L^*a^*b^*$ ), hardness quality, crude protein, fibre or ash ( $p < 0.05$ ). There were no significant changes in sodium, potassium, zinc, manganese and selenium ( $p > 0.05$ ). However, the contents of crude fat, calcium, magnesium and copper were significantly higher than before treatment ( $p < 0.05$ ). These results suggest that low pressure RF plasma can be effectively used as an alternative method for snack bar sterilization.

### Keywords

brown rice snack bar; low pressure radio frequency (RF) plasma; mould control; physical quality; mineral content; trace element content

Rice is one of the most important cereal crop foods. At about 9% of the total cultivated soil, this grain nourishes approximately 2.5 billion people [1], being mainly consumed as a white grain. In the last decade, more and more brown rice products have been produced in the health food market. The reason is that the outer coating of brown rice contains highly valuable amino acids [2] and bioactive compounds such as phytic acid,  $\alpha$ -tocopherol and  $\gamma$ -oryzanol [3]. These are beneficial for health. In 2011, KIM et al. [4] reported that the consumption of brown rice could decrease the waist circumference of type 2 diabetic patients.

Unfortunately, brown rice can be easily con-

taminated by moulds. *Aspergillus flavus*, *Penicillium viridicatum* and *Fusarium graminearum* have been reported to be found on rice grains and also could produce ochratoxin A or aflatoxins [5]. These toxins are among the most observed in food products including breakfast cereal [6, 7]. Suppressing mould growth by using chemical preservatives has been suggested by many researchers [8, 9]. Although chemical preservatives can reduce surface fungal contamination, they are often unacceptable for consumers [10].

Plasma is a partially ionized gas also known as a highly energized fourth state of matter that contains ions, electrons and reactive neutral spe-

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cies (radicals, excited atoms and molecules). With sufficient energy, plasma can break covalent bonds and/or initiate various chemical reactions [11]. An advantage of microbiological decontamination using plasma is the possibility, under appropriate conditions, of achieving such a process at a relatively low temperature ( $\leq 50^\circ\text{C}$ ) [12]. Therefore, if food is treated with low-temperature plasma, nutritionally important components might not be destroyed. The effects of plasma on various foods such as apples [13], chicken and meat [14] have been reported. However, only a limited number of studies have investigated the effect of low pressure plasma against mould growth on brown rice cereal in association with the quality of brown rice cereal after plasma treatment. The objective of this study was to investigate the effects of low pressure radio frequency (RF) plasma on yeast and mould contamination, hardness, colour, mineral and trace element content of a brown rice snack bar.

## MATERIALS AND METHODS

### Brown rice samples

Khai Mod Rin (NSRC950013) brown rice, a local rice grown in the Nakhon Si Thammarat province in Thailand, was selected for this study. It was obtained from the Nakhon Si Thammarat Rice Research Center (Nakhon Si Thammarat, Thailand). It was harvested in December 2011.

### Production of the brown rice snack bar

Experiments were conducted with 1.8l automatic rice cooker (SR-DG 182; Panasonic Management, Bangkok, Thailand). Three hundred grams of brown rice were soaked in a pot for 10 min with 900 ml of deionized water. The rice was cooked for 30 min. Then it was removed from the cooker and put into a stainless form that was 2 cm wide by 4 cm long by 0.5 cm deep. After cooling at room temperature, the cooked brown rice was put in a tray to dry at  $60^\circ\text{C}$  for 4 h. Next, it was taken out and put into an electric deep fryer (IF-836; Imarflex Industrial, Bangkok, Thailand) and kept at a temperature of  $190^\circ\text{C}$  for 20 s. Lastly, after the temperature of the samples decreased to room temperature, the brown rice snack bars were put in plastic bags. Rice snack bars ( $\sim 25\text{g}$ , 2 cm wide  $\times$  3 cm long) were prepared for all experiments.

### Low pressure radio frequency plasma apparatus

The low pressure radio frequency (RF) plasma system, developed at the Plasma Technology for Agricultural Application Laboratory at Walailak University (Nakhon Si Thammarat, Thailand), is shown in Fig. 1. The vacuum chamber was made of Pyrex glass that was 150 mm in diameter and 300 mm in length. Air was used as discharge gas. Low pressure could be obtained using a rotary pump and was monitored by a vacuum gauge. RF of adjustable frequency between 20–600 kHz

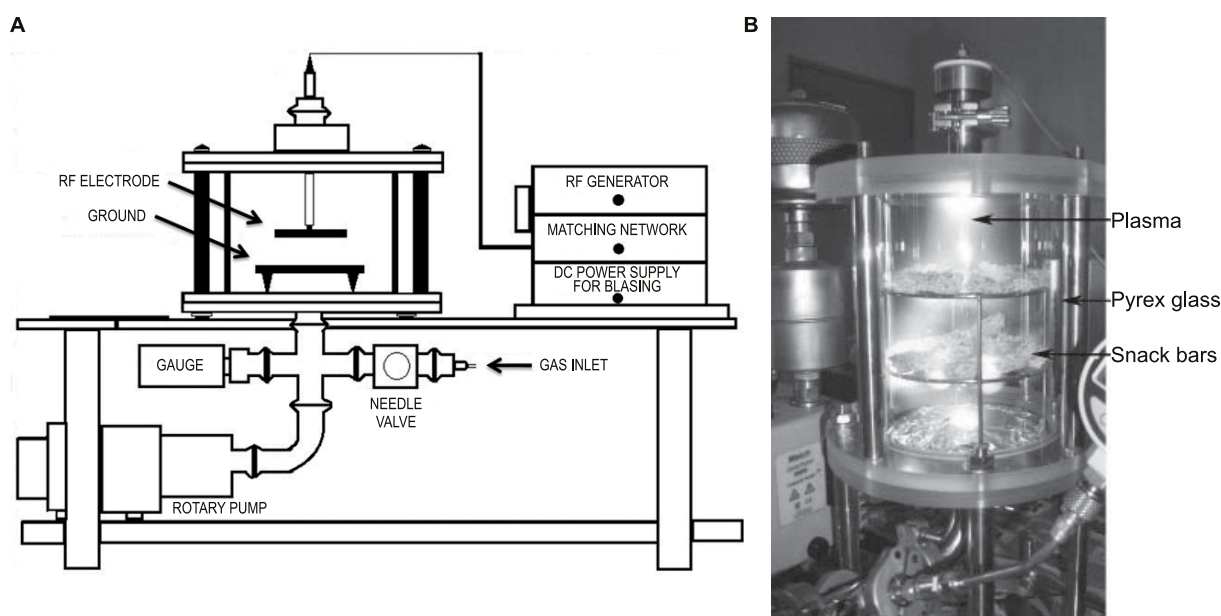


Fig. 1. Experimental setup.

A – Schematic diagram, B – Picture of snack bars under plasma treatment.

and maximum power of 160 W was used as power source. Plasma was produced by the capacitive coupling method. The copper RF electrode was 20 mm in diameter and the rack was grounded.

#### Preparation of inoculum

*Aspergillus flavus* WU 0211 was from the culture collection of the Food Microbiology Laboratory of the Center for Scientific and Technological Equipment at Walailak University, Nakhon Si Thammarat, Thailand. Spores were obtained from A mycelium grown on malt extract agar (MEA; Merck, Bangkok, Thailand) at 25 °C for 7 days and were collected by washing the surface of the plates with ~5 ml sterile saline solution (NaCl, 8.5 g·l<sup>-1</sup>) containing Tween 80 (0.1% v/v, Merck). After counting the spores using a hemocytometer, the suspension was standardized to a concentration of 10<sup>6</sup> spores per millilitre by dilution with sterile water. The viability was checked by the use of quantitative colony counts.

#### Application of low pressure RF plasma

An aqueous suspension of *Aspergillus flavus* spores (10<sup>6</sup> CFU·ml<sup>-1</sup>) was prepared and inoculated on brown rice snack bars. The specimens were air-dried in a safety cabinet (NuAire, Plymouth, Minnesota, USA) for 10 min. After that, the specimens were incubated at 25 °C for 8 h. Then, the surface viability was checked by the use of quantitative colony counts (limit: 10<sup>5</sup> CFU·g<sup>-1</sup>). Specimens were then exposed to the low RF plasma at 40 W, 70 W, 100 W, 130 W, and 160 W for 5, 10, 15, 20, 25 and 30 min of exposure time. The contaminated brown rice cereal bars without plasma treatment served as controls. In addition, pressure (80 Pa) without plasma treatment was also applied. All specimens were treated in three replicates and were incubated at 25 °C for 72 h before the colony count. The number of colonies from the brown rice cereal bars were assessed both before ( $C_b$ ) and after ( $C_a$ ) plasma treatment. The reduction factor ( $RF$ ) of spore germination was calculated as follows:

$$\log RF = \log C_b - \log C_a \quad (1)$$

#### Measurement of microbiological quality

The low pressure plasma (130 W) effect to reduce moulds on brown rice cereal bars was investigated using an exposure time of 30 min. Surface samples from brown rice snack bars (25 g each) were removed before and after plasma treatment by using a sterile scalpel, mixed with 225 ml of peptone water and homogenized using a stomacher (MIX 2; AES Laboratoire, Combourg, France)

for 3 min and diluted with peptone water to determine the microbial counts. Serial dilutions were analysed on potato dextrose agar plates at 25 °C for 72 h in three replicates and expressed as logarithm of colony forming units (CFU) per gram.

#### Microscopy

Structure of *Aspergillus flavus* before and after low RF plasma treatment at 130 W for 30 min was also examined by using an optical microscope (Carl Zeiss, Oberkochen, Germany). A loopful of *A. flavus* was placed on the clean glass slide containing few drops of lactophenol cotton blue stain. The slide was observed under the microscope and the image was photographed.

#### Measurement of colour

The MiniScan EZ (Hunter Associates Laboratory, Reston, Virginia, USA) was used to measure the degree of lightness ( $L^*$ ), redness-greenness (+ or -  $a^*$ ) and yellowness-blueness (+ or -  $b^*$ ). The measurements were performed in eight replicates per one treatment.

#### Measurement of hardness

Hardness as a texture parameter was measured using the Texture Analyzer (Lloyd Instruments, West Sussex, United Kingdom). Hardness was measured in terms of breaking strength, which is the force required for breaking the product. Measurements were made at a loading speed of 5 mm·s<sup>-1</sup> with a 5 kg load cell at a 30 mm distance. Eight replicates were performed for each treatment.

#### Change in nutritional value

The approximate composition of cooked brown rice both before and after plasma treatment at the power of 130 W for 30 min was determined after grinding the sample using a grinder (Philips, Bangkok, Thailand). The official methods of analysis (AOAC) [15] were employed for the analysis of moisture, crude fat, total ash, crude fibre, crude protein and carbohydrates. For the determination of moisture content, the sample was dried at 105 °C until constant weight, and then it was cooled and weighed. The weight loss was used to calculate the moisture content. For crude fat, the dried sample was extracted in a Soxhlet-type extractor with petroleum ether (boiling point 60–80 °C). The extract was dried for 30 min at 100 °C, cooled and then the residual fat was weighed. Total ash content was determined by weighing the residual ash obtained by combustion in a Muffle furnace at 550 °C. Crude fibre was measured after digesting a known amount of a fat-free sample in refluxing

1.25 g H<sub>2</sub>SO<sub>4</sub> per 100 ml distilled water and 1.25 g NaOH per 100 ml distilled water. Crude protein determination was done by Kjeldahl method. Finally, available carbohydrates was obtained by the difference method (subtracting the percent crude protein, fat, fiber and ash from 100% dry matter). All determinations were carried out in triplicate and reported on dry matter basis.

#### Mineral and elemental analysis

Elemental analysis was done by using inductively coupled plasma optical emission spectrometry (Perkin-Elmer model optima 3300 Dv, Perkin-Elmer, Norwalk, Connecticut, USA). Argon gas was used to induce plasma. All samples were digested in triplicate. Two hundred milligrams of each sample were placed in the vessel and 5 ml of concentrated HNO<sub>3</sub> were added and incubated overnight. Then, 1 ml of H<sub>2</sub>O<sub>2</sub> was added. The digestion was as follows: the samples were heated up at a temperature of 100 °C for 10 min, then at 150 °C for other 10 min and then at 250 °C for 30 min. The digestion was completed in about 60 min as indicated by the appearance of a transparent liquid mixture. The wet-digested solution was transferred to plastic bottles and the bottles were labeled and stored in the refrigerator for elemental determination (modified from HEINEMANN et al. [16]).

#### Statistical analysis

All variables were tested for normality by applying the Kolmogorov–Smirnov test. The homogeneity of variances was assessed by using Levene's test. Data transformation was done where necessary. All results were expressed as mean  $\pm$  standard deviation (SD). Data were statistically treated by a one-way ANOVA and Duncan's post hoc test

with  $p < 0.05$  considered to be statistically significant. The statistical analysis was performed using Statistica software (StatSoft, Tulsa, Oklahoma, USA).

## RESULTS AND DISCUSSION

#### *Aspergillus flavus* growth on the brown rice snack bar

The amount of *Aspergillus flavus* growth after low RF plasma treatments at 40 W, 70 W, 100 W, 130 W, and 160 W for 5, 10, 15, 20, 25 and 30 min is presented in Fig. 2. After being exposed to the plasma for 30 min at 130 W or 160 W, *A. flavus* colonies were not found on the bars examined. On the other hand, for the control without plasma and pressure treatment, *A. flavus* could grow on the brown rice snack bar surface at approximately 10<sup>5</sup> CFU·g<sup>-1</sup>. In this experiment, pressure was found to reduce mould growth by two orders of magnitude. The combined effect of plasma (40 W to 100 W) and pressure showed higher mould growth reduction by 2 to 3.5 orders of magnitude. Using higher plasma power at 130 W to 160 W for 30 min with low pressure could completely eliminate *A. flavus* on snack bars.

In this experiment, the structure of *A. flavus* after plasma treatment at 130 W for 30 min is shown in Fig. 3. It seems that low RF plasma could destroy the conidiophore bearing vesicles and conidiospores of *A. flavus*. Nevertheless, it should be noted that *A. flavus* is a mycotoxigenic fungus and the mycotoxin might be still found after plasma treatment over storage period, and may still be a problem to human health. Effect of plasma treatment on mycotoxins in brown rice cereal, however, warrants further investigation. It is well

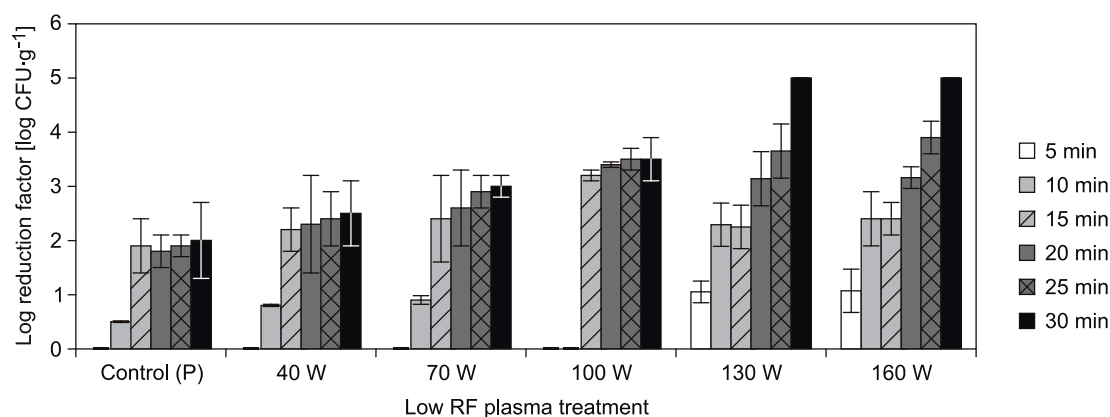
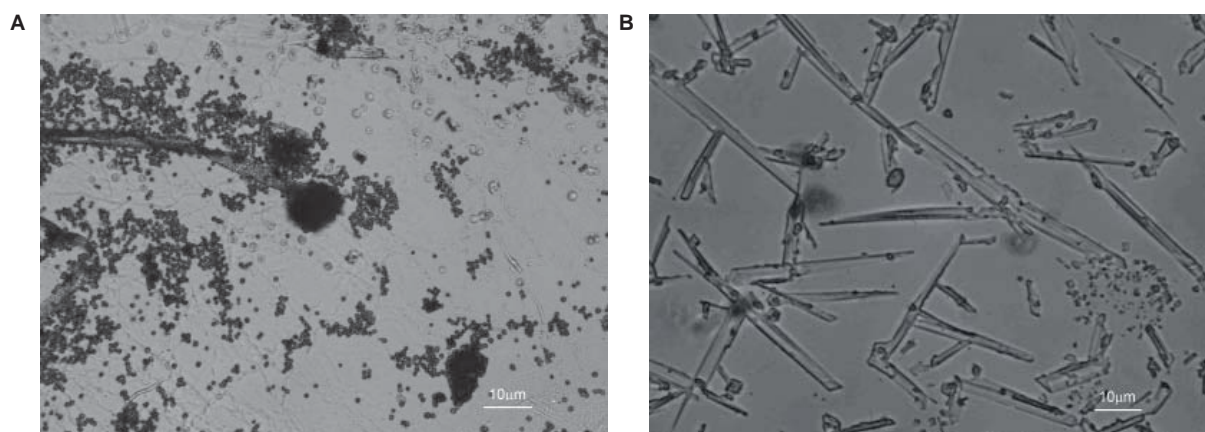


Fig. 2. Log reduction factor of *A. flavus* after low RF plasma treatment.





**Fig. 3.** Structure of *Aspergillus flavus* before and after low RF plasma treatment at 130 W for 30 min.

A – before treatment, B – after treatment.

known that charged plasma particles can break the cell protein and nucleic acid of microorganisms and this was further demonstrated in destruction of bacterial cells [17]. In addition, UV and activated free radicals generated during plasma treatment can damage the cell walls of microorganisms [18]. Nevertheless, most of reports demonstrated that the time needed to devitalize bacteria by plasma should be less than 75 s [19]. In comparison to this, a longer time ( $\geq 30$  min) is needed to inhibit mould growth. Moulds and bacteria are usually different in size and shape, yeasts cells and mould hyphae are much larger than bacteria [20]. Thus, moulds needs much more time to be devitalized.

#### Change in quality of brown rice snack bar after plasma treatment

##### Microbiological quality

While the total counts of yeasts and moulds on the control brown rice snack bars (without plasma or low pressure without plasma) reached  $10^4$  CFU·g<sup>-1</sup>, bars treated with low pressure RF plasma at 130 W for 30 min were free from yeasts and moulds. According to the Thai Community Product Standard (TCPS 743-2548) [21], total counts of yeasts and moulds on the surface of crispy rice must be  $\leq 1 \times 10^2$  CFU·g<sup>-1</sup>. From these results, the brown rice snack bars were safe to consume after a 30 min plasma treatment. Low pressure RF plasma can sterilize brown rice snack bars contaminated with yeasts and moulds.

##### Physical quality (texture and colour)

It is shown in Tab. 1 that the colour ( $L^*$   $a^*$   $b^*$ ) and hardness of the brown rice snack bars before and after the plasma treatment at 130 W for

30 min were not significantly different ( $p > 0.05$ ). These results show that cold RF plasma processing can preserve the colour and hardness quality of a brown rice snack bar.

It is well known that brown rice is rich in anthocyanins (dark purple colour), which are also found in berries. ARANCIBIA-AVILA et al. [22] found that loss of anthocyanins could be observed when using high temperature processing (100 °C for more than 20 min). In our experiments, temperature on the surface of brown rice after using cold plasma at 130 W for 30 min was lower than 50 °C. Our results confirm that this technology did not affect the colour quality of a brown rice snack bar.

##### Nutritional value of brown rice snack bar

The chemical composition of the snack bar is shown in Tab. 2. The results indicated that low pressure plasma did not affect the nutritional value (protein, ash, fibre and carbohydrates) of the brown rice snack bar ( $p > 0.05$ ) except for crude fat. After plasma treatment, the fat con-

**Tab. 1.** Effects of plasma treatment (130 W, 30 min) on colour and hardness of brown rice snack bars.

Quality parameter	Before plasma treatment	After plasma treatment
Colour		
$L^*$	$69.55 \pm 0.36^a$	$70.33 \pm 0.23^a$
$a^*$	$4.85 \pm 0.36^a$	$5.12 \pm 0.11^a$
$b^*$	$29.34 \pm 0.48^a$	$29.66 \pm 0.23^a$
Hardness [N]	$22.62 \pm 7.64^a$	$21.21 \pm 5.58^a$

Values in the same row with different superscripts are significantly different ( $p < 0.05$ ).

**Tab. 2.** Effects of plasma treatment (130 W, 30 min) on approximate composition of brown rice snack bars.

Assay	Before plasma treatment	After plasma treatment
Protein [g·kg <sup>-1</sup> ]	9.8 ± 3.5 <sup>a</sup>	10.9 ± 1.5 <sup>a</sup>
Crude fat [g·kg <sup>-1</sup> ]	240.3 ± 41.3 <sup>b</sup>	325.1 ± 51.2 <sup>a</sup>
Ash [g·kg <sup>-1</sup> ]	16.1 ± 1.2 <sup>a</sup>	15.2 ± 0.2 <sup>a</sup>
Fibre [g·kg <sup>-1</sup> ]	9.9 ± 1.2 <sup>a</sup>	10.4 ± 0.2 <sup>a</sup>
Carbohydrates [g·kg <sup>-1</sup> ]	740.0 ± 41.5 <sup>a</sup>	653.6 ± 53.2 <sup>a</sup>

Data are expressed per kilogram of dry matter and represent the mean of three replicates ± standard deviation. Values in the same row with different superscripts are significantly different ( $p < 0.05$ ).

tent of the brown rice snack bar was higher than before treatment ( $p < 0.05$ ). After reduced pressure in a plasma chamber, the boiling point of frying oil was lower since air may diffuse much faster into the porous space of the snack bar. In addition, after the treatment, low plasma temperature (40–50 °C) could affect free fatty acids. PARK et al. [23] indicated that fat acidity values of the rice stored between 30 °C and 40 °C were higher than those of rice stored at 4 °C and 20 °C. These changes could be from the hydrolysis of lipids to produce free fatty acids or from the oxidation of lipids to hydroperoxides and secondary products [24, 25]. However, oxidation of free fatty acids in rice snack bars requires further study.

#### Mineral and trace element composition

Changes in trace elements after the plasma treatment of brown rice snack bars are of importance because their presence may represent a qualitative parameter for food nutrition

**Tab. 3.** Effects of plasma treatment (130 W, 30 min) on elemental contents.

Element	Before plasma treatment	After plasma treatment
K [mg·kg <sup>-1</sup> ]	2801.40 ± 263.00 <sup>a</sup>	3706.48 ± 528.52 <sup>a</sup>
Mg [mg·kg <sup>-1</sup> ]	1195.17 ± 103.24 <sup>b</sup>	1573.23 ± 232.23 <sup>a</sup>
Ca [mg·kg <sup>-1</sup> ]	482.09 ± 38.31 <sup>b</sup>	1260.33 ± 282.97 <sup>a</sup>
Na [mg·kg <sup>-1</sup> ]	54.71 ± 9.42 <sup>a</sup>	80.62 ± 19.17 <sup>a</sup>
Zn [mg·kg <sup>-1</sup> ]	36.05 ± 1.99 <sup>a</sup>	54.11 ± 11.22 <sup>a</sup>
Mn [mg·kg <sup>-1</sup> ]	26.13 ± 2.50 <sup>a</sup>	35.06 ± 7.00 <sup>a</sup>
Se [mg·kg <sup>-1</sup> ]	1.75 ± 1.75 <sup>a</sup>	1.52 ± 0.26 <sup>a</sup>
Cu [mg·kg <sup>-1</sup> ]	2.97 ± 0.80 <sup>b</sup>	5.33 ± 1.05 <sup>a</sup>

Mean ± standard deviation values in the same row with different letters are statistically different ( $p < 0.05$ ).

and health. Some trace elements might be released or their contents decreased during the plasma treatment. The results of the trace element metal content (Na, K, Ca, Mg, Zn, Mn, Se, Cu) from the brown rice snack bar samples both before and after the plasma treatment are presented in Tab. 3. K, Mg, and Ca contents ranged from 2801.40 mg·kg<sup>-1</sup> to 3706.48 mg·kg<sup>-1</sup>, 1195.17 mg·kg<sup>-1</sup> to 1573.23 mg·kg<sup>-1</sup> and 482.09 mg·kg<sup>-1</sup> to 1260.33 mg·kg<sup>-1</sup>, respectively, with increasing Mg and Ca content in plasma samples ( $p < 0.05$ ). Na, Zn, Mn, and Se contents in these snack bars were from 54.71 mg·kg<sup>-1</sup> to 80.62 mg·kg<sup>-1</sup>, 36.05 mg·kg<sup>-1</sup> to 54.11 mg·kg<sup>-1</sup>, 26.13 mg·kg<sup>-1</sup> to 35.06 mg·kg<sup>-1</sup>, and 1.75 mg·kg<sup>-1</sup> to 1.52 mg·kg<sup>-1</sup>, respectively. Plasma-treated samples did not differ significantly ( $p > 0.05$ ) from non plasma treatment. However, Cu content before plasma treatment (2.97 mg·kg<sup>-1</sup>) was lower than that after plasma treatment (5.33 mg·kg<sup>-1</sup>). Increases in the contents of Ca, Mg, and Cu might be a result of sputtered elements from the cathode, which is made from of copper alloy. From Tab. 3, the mean contents of the trace elements analysed were similar to those found in the composition of brown rice [25–27] except for Ca and Zn. The snack bar produced from Khai Mod Rin (NSRC950013) brown rice had higher Ca and Zn than the other brown rice cultivars from previous reports [26–28]. The brown rice snack bar from our study might be a source of calcium and zinc for children between the ages of 1 to 8 according to the daily dietary reference intake set by the Institute of Medicine (Washington, D.C., USA), which set the intake at 500–800 mg per day for Ca and 3–5 mg per day for Zn [29].

Two types of plasma, namely thermal and non-thermal, can be defined according to the conditions in which it is created. Non-thermal plasma was used for this test. This plasma exhibited a moderate neutral gas temperature, which was either identical or close to room temperature. Consequently, ions and neutral gas atoms gained only a little energy and stayed cold [30]. Our results demonstrate that non-thermal plasma does not destroy minerals in the brown rice snack bars.

#### CONCLUSIONS

The experiments on the effect of cold plasma at 130 W using exposure times of up to 30 min gave good results. Low pressure RF plasma treatment could reduce *A. flavus* on the brown rice snack bars by approximately 5 orders of magnitude, and

reduced total yeasts and moulds on the brown rice snack bars to an acceptable quantity. No statistical difference in colour or hardness was observed in all samples. Most nutritional value parameters and trace elements in the brown rice snack bars were not affected by cold plasma. The results indicate that cold plasma can be used for treatment of rice snack bars in their production.

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