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Effect of Microwave Heat Treatment on Inhibition of Corn Seed Germination

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Abstract

Purpose: Corn is a major commercial crop targeted for genetic modification owing to its high consumer demand as a foodstuff for humans and livestock, as well as its other industrial applications. However, the safety of genetically modified (GM) crops is controversial. Indeed, several countries have banned the importation of GM seeds that can germinate. Therefore, development of effective, convenient, and nondestructive methods to inhibit seed germination is required. **Methods:** This study aimed to examine the efficacy of microwave heat treatment for inhibition of germination of corn kernels and for optimization of power and exposure time required for effective aging treatment. Artificial inhibition was induced in corn kernels using microwave heat treatment. Seven power levels were examined (400, 500, 600, 700, 800, 900, and 1000 W) at each of the four exposure times (0.5, 1.0, 1.5, and 2.0 min). **Results:** Corn kernels could be aged effectively after heating for 0.5~1.0 min at powers greater than 800 W, with increasing efficacy observed at higher powers. Further analysis showed that the most effective inhibition of germination was observed at 1000 W for 40 s. This setting did not cause any physical damage to the corn kernels. **Conclusions:** Optimal inhibition of corn kernel germination was achieved using higher power for shorter times, which may be useful for industrial corn seed treatment.

Keywords: Genetically modified corn, Germination inhibition, Microwave heat treatment, Process optimization

Introduction

Advances in biotechnology and genetic engineering have led to the development of genetically modified (GM) organisms, defined as organisms whose genetic material (i.e., DNA and RNA) has been altered in a way that is not possible in nature through mating or natural recombination. Since the inception of this technology in the mid-1990s, food producers in various countries have targeted high-demand cash crops such as corn, soybean, rice, and wheat for genetic modification to improve yields and food quality. Various countries use corn and corn products as food sources for humans and livestock. Indeed, James (2012) reported that cultivation of GM crops already account for more than 170 million hectares of farmland worldwide, approximately 33% of which represents maize cultivation.

The main objectives of genetic modification of plants include increasing herbicide tolerance, pest and pathogen resistance, and product quality. For example, feed corn is genetically modified such that it can tolerate various herbicides and express a protein from *Bacillus thuringiensis* (Bt), which is inhibitory to certain insect pests (Albajes et al., 2009). Genetic engineering firms and seed companies have promoted the use of GMOs in agricultural systems, advocating their resistance to pests and diseases, high yield potential, and therefore having high commercial value. However, other scientific studies have indicated that most GMOs possess undesirable biological attributes that can be harmful to closely related indigenous species, the health of humans and animals that consume them, and the ecosystem in general (Vijayakumar et al., 2009; Albajes et al., 2009; Domingo and Bornodaba, 2011). For example, the higher abundance of GM seeds and products

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increases the possible risk of admixture with natural seed varieties, which may occur intentionally or inadvertently during transfer and/or spillage during transport. In one such incident in the Republic of Korea, several GM maize plants were discovered along the roadside in 2006. This unexpected finding was reported in a survey of GM soybean and maize at a grain receiving port and around cultivated fields conducted from 2003 to 2006 (Kim et al., 2006; Lee et al., 2009).

Environmental concerns with GM crops involve the potential transfer of genes to non-target species, which may result in crossbreeding with weeds, leading to the development of pesticide-resistant "hybrid super weeds" that withstand the very herbicides designed to kill them (Powles, 2008). Other possible risks often associated with GM cropping include the toxic effects of Bt protein in non-target natural organisms such as butterflies and bees, which are key organisms for pollination, and gradual extinction of these transgenic species through gene flow to wild relatives (Ellstrand et al., 1999). In some countries where GMO experimentation is underway, such as the Netherlands, appropriate isolation distance between GM corn and organic corn varieties is a crucial requirement in agricultural systems to avoid gene flow between the species (Van De Wiel et al., 2009). The minimum distance between corn fields is stipulated as 250 m.

On the other hand, some studies e.g., that by Chrenkova et al. (2002), have indicated that the biochemical composition of GM corn does not differ significantly from that of organic/conventional corn kernels. The controversy surrounding the benefits and risks of GMOs continues to be complex in terms of both the science of GMOs and how they are viewed from a cultural/societal perspective. Therefore, campaigns to improve awareness of the public about the health concerns of GMOs, as well as bans on the importation of GM seeds and other products in the active form, have been introduced in many countries. Although the cultivation of GM crops is not permissible in several countries, the importation of GM crops for food and foraging purposes continues to increase (Park et al., 2010).

As such, producers and seed market businesses that focus on export must first inactivate and properly inspect the seed batches before export to avoid legal penalties both locally and from the recipient countries.

According to Meulen et al. (2000) and Sveinsdo´ttir et al. (2009), the loss of germination capability of aged corn seeds is related to decreased cell division and expansion in the primary root (radicle). Seed membrane cell division and expansion is catalyzed by H^+ ATPase, an ATP-driven enzyme that transforms the energy of ATP hydrolysis into electrochemical potential differences of protons across diverse biological membranes via the primary active transport of H^+ (Beyenbach and Wieczorek, 2006). Aging treatment of corn kernels significantly reduces enzyme activity, consequently leads to loss of seed viability.

Germination inhibition can be achieved by several methods, including natural aging, (time dependent) and artificial aging treatment (e.g., water bath, chemical treatment, and heat treatment). Aging treatment is necessary to "kill" the GM corn feed kernels without significantly affecting their physical, chemical, and nutritional attributes.

However, artificial aging treatment methods suitable for industrial use may be costly and/or time consuming. Chemical methods are mainly used on seeds that are meant for research purposes or weed control rather than consumption. Therefore, chemical treatment is not appropriate for corn kernels meant to feed animals or humans. The water bath method is commonly used, particularly by small-scale producers and researchers to carry out seed aging in order to inhibit seed germination. However, in this method, one cycle of treatment takes at least 168 h (Kim et al., 2014); this time-consuming process also requires a constant power supply to maintain the water bath temperature at at least 50°C, which may increase operational costs. In addition, given the batch-type nature of its operation, this method is not suitable for large-scale applications. Alternatively, microwave heat treatment is a promising method for inhibition of corn seed germination; this method is expected to require less time and is suitable for online operations. However, no optimal input parameters such as exposure time and power have been established for effective corn seed aging treatment by using microwave heat. Therefore, the aim of this study was to determine the optimal combination of input microwave power and seed exposure time for effective inhibition of corn kernel germination.

Materials and methods

Microwave oven calibration

A high-frequency (2450 MHz) laboratory microwave oven (Korea Microwave Instrument Co., South Korea) with an adjustable power range between 0 and 1000 W

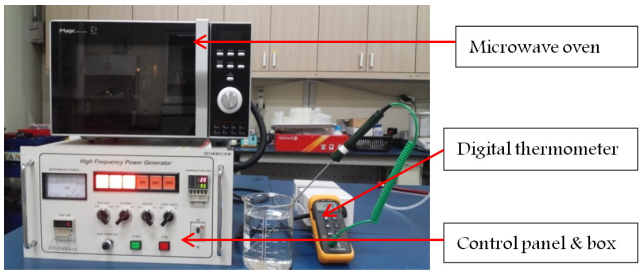

Figure 1. Experimental microwave oven.

was used for the corn aging treatment (Figure 1). This microwave oven had a chamber size of 305 × 305 × 205 mm, and the power could be adjusted manually by turning the knob on the control panel. Additionally, the operation time could be set, and the microwave oven would automatically turn off after the time elapsed.

A standard calorimetry procedure was used to calibrate the power output of the microwave oven at each input power value. Next, 1000 g of tap water was collected in a diamond glass beaker, and its initial temperature (T_1) was measured before placing the beaker into the microwave oven for heating. The heated water was removed from the oven immediately after the specified heating time elapsed, and its final temperature (T_2) was measured. T_1 and T_2 were measured using a digital thermometer with a sensing steel thermocouple. The power output, of the oven was calculated using the proposed equation (1).

$$P_o = \frac{((m_w \times c_w) + (m_g \times c_g)) \times \triangle T}{t} \quad (1)$$

Where, m_w is the mass of water (1000 g),
m_g is the mass of the glass beaker (289.4 g),
c_w is the specific heat capacity of water (4.186 $J \cdot g^{-1} \cdot °C^{-1}$),
c_g is the specific heat capacity of the glass beaker (0.84 $J \cdot g^{-1} \cdot °C^{-1}$),
$\triangle T$ is $T_2 - T_1$, and t is the heating time (s)

The input power levels considered for calibration were 400, 500, 600, 700, 800, 900, and 1000 W. The data were tabulated using Microsoft Excel, and the average power output was calculated at each power input level.

Microwave power optimization for GM corn aging treatment

In order to discern the optimal microwave power and time exposure for seed treatment, seven input power levels were tested (400, 500, 600, 700, 800, 900, and 1000 W). Four exposure times (0.5, 1.0, 1.5, and 2.0 min) were tested for corn treatment at each power level. Fifty kernels were used for each treatment period at each power level.

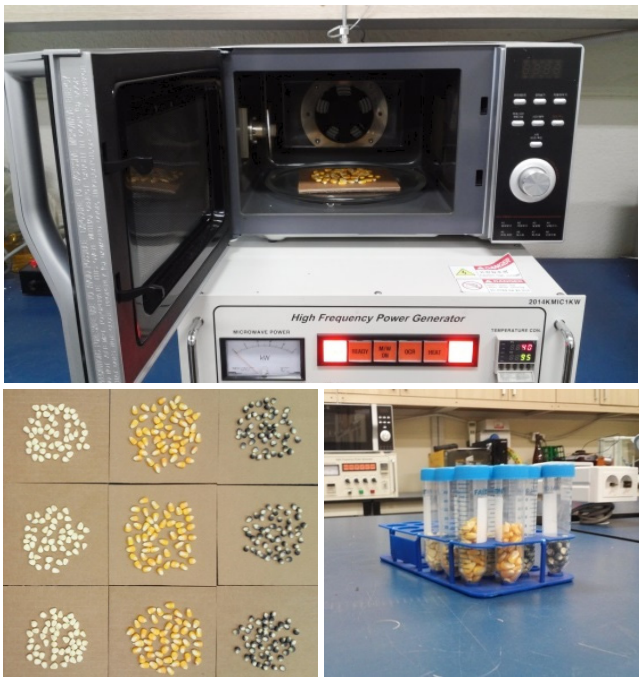

Figure 2. Sequence of microwave corn aging treatment.

Seed selection and treatment

Healthy and intact kernels from three categories of corn, i.e., white, yellow, and purple, were selected from random cultivars. All the kernels from each category had fairly uniform moisture content, within the limit of 5%. The moisture content was determined by drying the kernels in an oven at 105°C ± 1°C for 17 h (Mollazade et al., 2009), and then calculating it on dry weight basis. The kernels were uniformly spread on a square paper board and then placed on the turn table inside the microwave (Figure 2). After the set treatment time elapsed, the kernels were immediately removed and allowed to cool completely before storing them in polypropylene conical tubes.

Germination test

The treated and untreated (control) kernels were subjected to germination tests to investigate the effects of the microwave heat treatment at particular power levels and for the indicated exposure time intervals. The International Seed Testing Association seed germination procedure

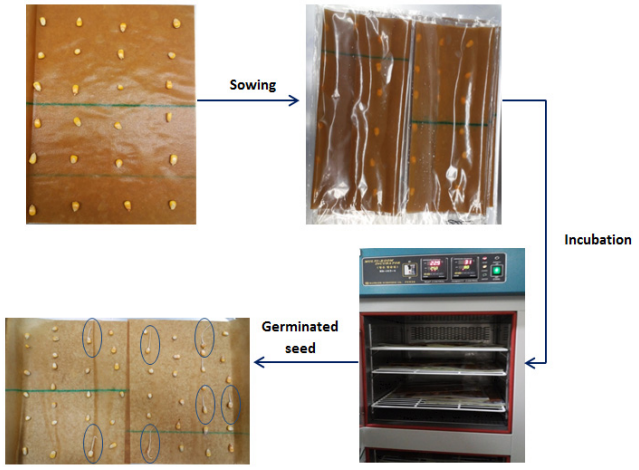

Figure 3. Corn germination test sequence.

was adopted for this experiment, and the between paper method was used. Test seeds (treated and untreated) were placed in line on the germination paper, which was then neatly folded to cover the seeds before carefully placing them into plastic bags. Next, 50 mL distilled water was added to each plastic bag containing test seeds to raise the seed moisture content to approximately 25%, a level suitable for germination (ISTA, 1985).

The bags containing the seeds were placed in a controlled germination incubator (Hanbaek Scientific Co., Korea) at 25°C and relative humidity at 80% (Figure 3). The incubation period was 7 days; thereafter, the germination data were collected as percentages based on the number of germinated kernels in each sample. Kernels with germinated primary roots measuring at least 2 mm in length were considered to have germinated. The experiment was independently replicated twice.

Statistical analysis

The collected data were tabulated and summarized using Microsoft Excel. Data were then further analyzed using SAS 9.0 software to determine the significance of the differences between mean germination percentages of different experimental treatments. The results are presented as mean ± standard deviation (SD), and Duncan's multiple range test was performed to compare mean values. Differences in mean germination percentages between the experimental units were considered statistically significant when the probability (p) of the result (assuming a null hypothesis) was less than 5%. The power level with absolute zero germination and minimal or no physical damage (burns and/or rupture) in the shortest time was considered the optimal value and was further used to determine the optimal treatment time.

Table 1. Microwave input power versus output

Nom. P(Y)	Observed P(Ŷ)	e = ‖Ŷ-Y‖	Efficiency (%)
400	293.12	106.88	73.28
500	366.22	133.78	73.24
600	458.19	141.81	76.37
700	525.14	174.86	75.02
800	581.93	218.07	72.74
900	639.35	260.65	71.04
1000	714.19	285.81	71.42
		Avg. Efficiency	73.3

Optimization of microwave exposure time

The result of the power optimization test indicated that 1000 W was the most effective for corn treatment, with the exposure time ranging between 0.5 and 1 min. Therefore, this power level was tested for 30, 40, 50, and 60 s to determine the optimal exposure time. The treatment time with absolute zero germination and minimal or no physical damage was considered optimal. The optimal combination of microwave power and exposure time was used to treat corn kernels; these treated kernels were later tested for viability using spectroscopy.

Results and Discussion

Calorimetry test for microwave calibration

Results from the calorimetry test indicated that the observed output power of the microwave oven was lower than the input power (Table 1), with an average efficiency of 73.3%. The microwave efficiency increased at lower input powers and then decreased steadily at higher power levels (Figure 4). The differences between input power and output may be attributed to the magnetron inside the microwave, which often degrades faster than other components (Khraisheh et al., 1997). Additionally, permanent magnets induce the accumulation of heating effects that may cause a reduction in magnetic field, thereby reducing voltage and power. The microwave oven used in this experiment was custom-made and delivered with all components intact, just a few weeks after manufacture; therefore, the latter point (i.e., accumulation of heating effects) probably led to the power drop. Power reduction of household

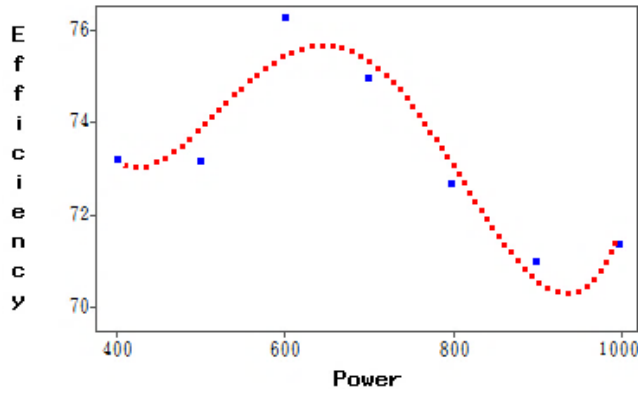

Figure 4. Microwave power efficiency.

microwave ovens during operation may be limited to 17.3% for the first 30 min (Swain et al., 2007). This value may however be higher depending on the brand, and the age of the microwave oven.

Optimum microwave power and exposure time

The germination test procedure helped assess the behavior of the microwave oven with regard to various combinations of input power and seed exposure time in order to obtain the optimal conditions for effective corn aging treatment. In general, the percentage of kernel germination for all corn categories reduced with increase in microwave exposure time (Figure 5). The test results indicated that corns treated for 0.5 min at any microwave power level showed relatively higher germination and vigor than those treated for 1.0, 1.5, and 2.0 min. Absolute zero germination was achieved for all corn kernels treated for 2.0 min at all power levels tested.

On the other hand, the germination percentage significantly decreased as microwave power increased for all categories of corn subjected to equivalent exposure times. Corn kernels treated at 400 W exhibited the highest germination percentage and vigor, whereas those treated at 1000 W exhibited the least germination and vigor for each treatment time for each category (Table 2).

During the microwave heat treatment of corn kernels, physical damage to kernels, i.e., rupture and/or burns was observed. The rate and extent of physical damage increased with increased exposure time and power level. No corn seeds were damaged after 0.5 min of exposure, even at 1000 W. However, 2 min of exposure resulted in the most damage at each power level tested.

Inhibition of germination is considered effective when the treated seeds have zero germination potential and with minimal physical damage. The preliminary results of this study indicated that complete inhibition of germination can be achieved by microwave heat treatment for 0.5~1.0 min using an input power of 800~1000 W, with increasing inhibition at higher power levels. These data implied that effective germination inhibition could be achieved in the shortest time using a power of 1000 W or higher. Shorter treatment times are expected to enhance the overall operational efficiency in terms of time reduction and, consequently, business optimization. Therefore, we further examined the optimal time for microwave heating at 1000 W.

A similar trend of germination with regard to time was observed as the germination percentage decreased with

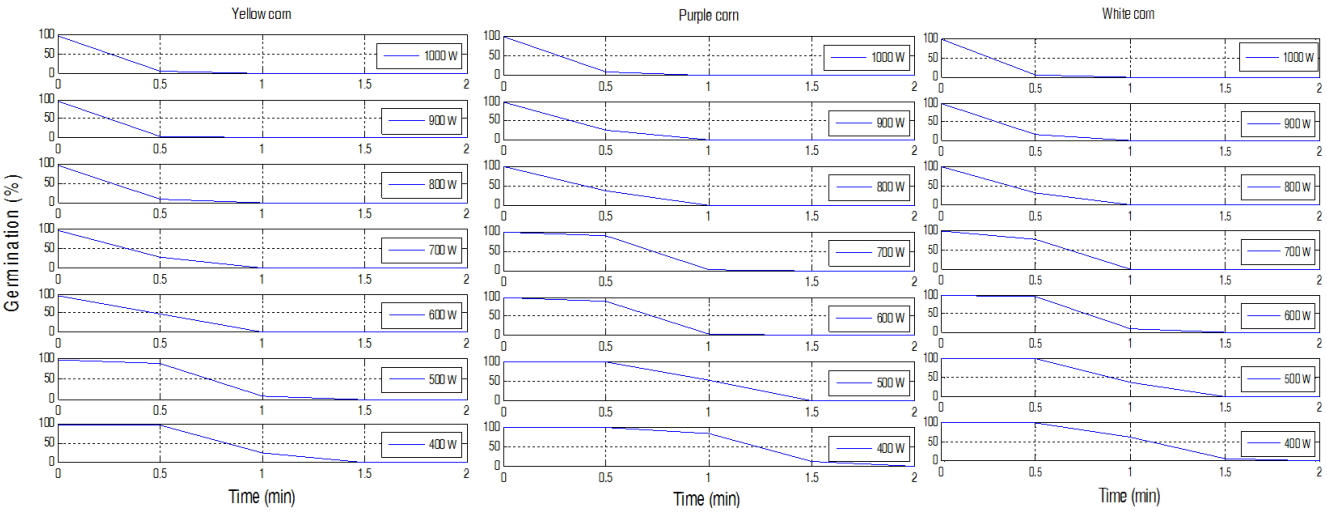

Figure 5. Germination response of corn kernels to microwave heat treatments with different input powers and exposure times.

Table 2. Multiple mean comparison test

		Power (Watts)						
	Exposure time (min)	400	500	600	700	800	900	1000
White corn	0.0	98±0.00^{Aa}	98±0.00^{Aa}	98±0.00^{Aa}	98±0.00^{Aa}	98±0.00^{Aa}	98±0.00^{Aa}	98±0.00^{Aa}
	0.5	99±1.41^{Aa}	99±1.41^{Aa}	94±2.83^{Ab}	78±28.28^{Aa}	29±18.38^{Bb}	15±4.24^{Bb}	6±0.00^{Bb}
	1.0	62±11.31^{Ab}	35±18.38^{Bb}	8.0±0.00^{Cc}	0.00±0.00^{Cb}	0.00±0.00^{Cc}	0.00±0.00^{Cc}	0.00±0.00^{Cc}
	1.5	4.0±5.66^{Ac}	0.00±0.00^{Ac}	0.00±0.00^{Ad}	0.00±0.00^{Ab}	0.00±0.00^{Ac}	0.00±0.00^{Ac}	0.00±0.00^{Ac}
	2.0	0.00±0.00^{Ac}	0.00±0.00^{Ac}	0.00±0.00^{Ad}	0.00±0.00^{Ab}	0.00±0.00^{Ac}	0.00±0.00^{Ac}	0.00±0.00^{Ac}
Yellow corn	0.0	95±4.24^{Aa}	95±4.24^{Aa}	95±4.24^{Aa}	95±4.24^{Aa}	95±4.24^{Aa}	95±4.24^{Aa}	95±4.24^{Aa}
	0.5	94±5.66^{Aa}	88±5.66^{Aa}	48±28.28^{Bb}	27±15.56^{Bb}	7±4.24^{Cb}	2±0.00^{Cb}	5±1.41^{Cb}
	1.0	24±5.66^{Ab}	6±0.00^{Bb}	0.00±0.00^{Cc}	0.00±0.00^{Cc}	0.00±0.00^{Cb}	0.00±0.00^{Cb}	0.00±0.00^{Cb}
	1.5	0.00±0.00^{Ac}	0.00±0.00^{Ab}	0.00±0.00^{Ac}	0.00±0.00^{Ac}	0.00±0.00^{Ab}	0.00±0.00^{Ab}	0.00±0.00^{Ab}
	2.0	0.00±0.00^{Ac}	0.00±0.00^{Ab}	0.00±0.00^{Ac}	0.00±0.00^{Ac}	0.00±0.00^{Ab}	0.00±0.00^{Ab}	0.00±0.00^{Ab}
Purple corn	0.0	100±0.00^{Aa}	100±0.00^{Aa}	100±0.00^{Aa}	100±0.00^{Aa}	100±0.00^{Aa}	100±0.00^{Aa}	100±0.00^{Aa}
	0.5	100±0.00^{Aa}	100±0.00^{Aa}	89±12.73^{Aa}	89±9.90^{Aa}	37±4.24^{Bb}	24±5.66^{Bb}	8±0.00^{Cb}
	1.0	83±18.38^{Aa}	53±32.53^{Ab}	2±2.83^{Bb}	1.0±1.41^{Bb}	0.00±0.00^{Bc}	0.00±0.00^{Bc}	0.00±0.00^{Bc}
	1.5	11±1.41^{Ab}	0.00±0.00^{Bc}	0.00±0.00^{Bb}	0.00±0.00^{Bb}	0.00±0.00^{Bc}	0.00±0.00^{Bc}	0.00±0.00^{Bc}
	2.0	0.00±0.00^{Ab}	0.00±0.00^{Ac}	0.00±0.00^{Ab}	0.00±0.00^{Ab}	0.00±0.00^{Ac}	0.00±0.00^{Ac}	0.00±0.00^{Ac}

[1]Mean values (germination percentages) with different superscript letters (A, B, C) in each row for each corn category indicate significant differences at $p<0.05$ by Duncan's multiple mean comparison test
[2]Means with different superscript (a, b, c, d) in each column for each corn category are significantly different at $p<0.05$ by Duncan's multiple mean comparison test

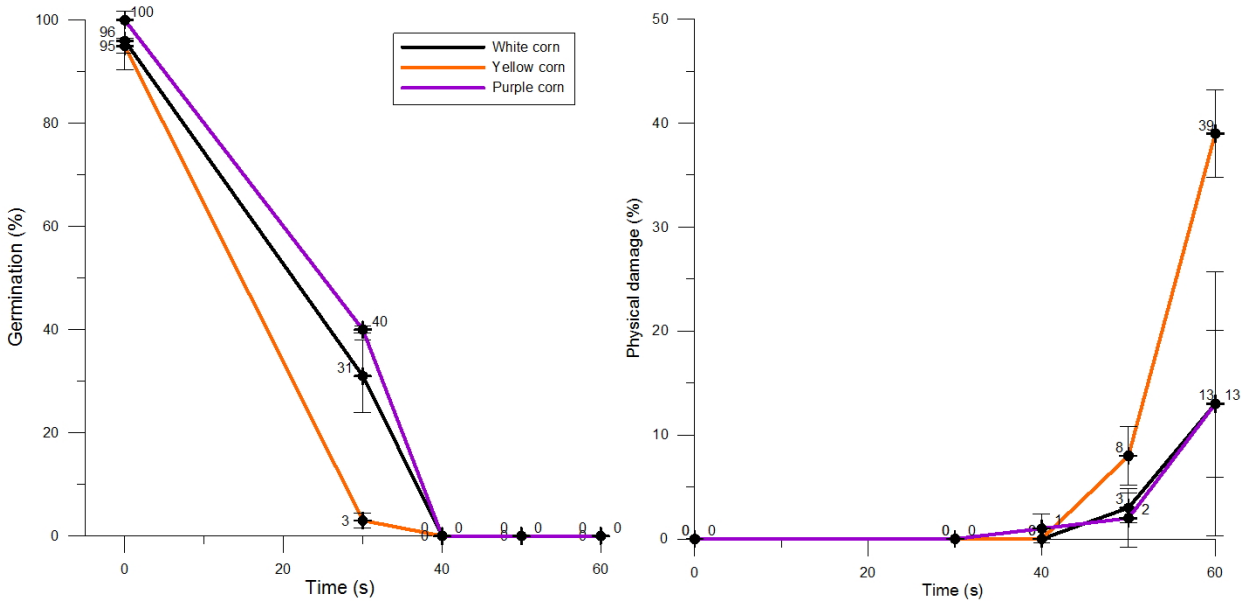

Figure 6. Optimal conditions for microwave aging treatment of corn kernels.

increase in seed exposure time. All three categories of corn exhibited some germination at an exposure of 30 s and zero germination at 40 s or more (Figure 6). The minor differences in germination percentages between yellow, white, and purple corn kernels could be attributed to the extent of microwave heat treatment, which may vary with seed size and color. Additionally, the percentage of physical damage was the highest at 60 s and negligible at 40 s. Therefore, 1000 W and 40 s were considered as the optimal conditions for effective GM corn aging treatment.

Conclusions

This study revealed that inhibition of corn kernel germination can be achieved using microwave heat treatment, with the microwave oven set at an optimum power of 1000 W for 40 s. Non-optimized parameters of the microwave lead to ineffective germination inhibition and/or excessive nutrient degradation and physical damage to the seeds. Our data demonstrated that the relationship between seed exposure time and microwave input power was inversely proportional with regard to their effect on seed germination, allowing researchers or industrial manufacturers to decide the most suitable trade-off between process time and power cost for their specific application. Moreover, microwave power calibration is critical and may be used to ascertain the quantity of usable output power, which is often lower than input power by at least 17%. In this study, the seed treatment was executed on a laboratory scale, which may not fully represent the industrial setting. Therefore, future research may be conducted with a larger number of seeds and an online microwaving system to assess the variable effect of microwave heat treatment on germination inhibition of corn seeds.

Conflict of Interest

The authors have no conflicting financial or other interests to declare.

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