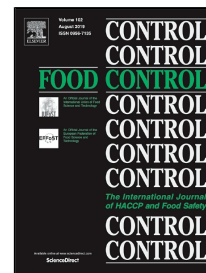


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**The application of slightly acidic electrolyzed water in pea sprout production to
ensure food safety, biological and nutritional quality of the sprout**

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Abstract

Slightly acidic electrolyzed water (SAEW) with available chlorine concentrations (ACC) of 35 and 70 mg/L is used instead of regular production water for soaking pea (*Pisum sativum* L.) seeds and spraying the sprouts during seed sprouting. Sodium hypochlorite (NaOCl) with the same ACC and tap water are used as a control in this study. The population of total bacteria, coliform, yeast and mold are determined at day 2, day 5, day 8, and day 11, respectively during seed sprouting. The biological indicators, nutritive indicators, and nitrite content after the sprouts are harvested are measured as well. The results indicate that when treated with SAEW, the counts of total bacteria, coliform, yeast and mold are reduced by 0.99~1.58 log CFU/g, 0.57~1.02 log CFU/g, and 1.01~1.22 log CFU/g respectively, compared to tap water treatment. Fresh weight, length, and edible rate of the sprouts significantly improve when treated with SAEW ($p < 0.05$). No evident adverse effects are observed in the nutritive indicators after SAEW treatment. In fact, a slight improvement (soluble sugar, flavonoid) was evident. Moreover, after a storage period of 7 d, the nitrite content of the sprouts was significantly lower in the SAEW samples than in any of other treatments. Therefore, SAEW could be a promising application in the production of pea sprouts to ultimately improve food safety.

Keywords: slightly acidic electrolyzed water, sprout, production, natural microbiota

1. Introduction

Germination has been widely employed to produce young edible seeds and sprouts for daily consumption, since many edible seeds do not take long to grow and provide improved nutritional value through simple germination procedures (Gan, et al., 2017). Seed sprouts, such as alfalfa, broccoli, mung bean, buckwheat, pea, and radish seeds, are commonly consumed raw in salads and sandwiches due to their nutritional value. However, consumption of sprouts have been associated with infections from a range of foodborne pathogens, particularly *Salmonella* and *Escherichia coli* O157:H7, as well as other Shiga-toxin-producing *E. coli* and *Listeria monocytogenes* (Callejon, et al., 2015; Chen, et al., 2018; Crowe, Mahon, Vieira, & Gould, 2015; Sadler-Reeves, et al., 2016). In 2011, a large outbreak of the Shiga-toxin-producing *E. coli* O104:H4 infection (caused by contaminated fenugreek sprouts) resulted in 3842 medical cases and 53 deaths in Germany (Muniesa, Hammerl, Hertwig, Appel, & Brussow, 2012). Sprouts that are usually consumed raw present a substantial food safety concern because the conditions in which they are produced (temperature, water activity, pH, and available nutrients) favor the growth of bacterial pathogens if present.

Since raw sprouts have become increasingly popular, a considerable rise in the outbreaks of foodborne illnesses are evident that presents significant challenges to the sprout industry. Therefore, appropriate action should be taken to eliminate or control microorganisms and pathogens found on sprouts. However, no single sanitation measure is entirely effective in removing pathogens from dry seeds, germinating seeds, and sprouts. In 1999, the National Advisory Committee on Microbial Criteria for Foods

recommended that just before sprouting, seeds should be subjected to “one or more” treatments able to “reduce or eliminate” pathogens, and 20,000 mg/L calcium hypochlorite was suggested as an intervention. However, treatment with high concentration of free chlorine was found to be inadequate since pathogens were not completely removed from the seeds and significantly decreased the germination rate and yield (Fransisca, Zhou, Park, & Feng, 2011). This ineffectiveness was theorized to be partly the result of cracks, abrasions, and crevices that might harbor and protect bacteria from the direct contact with the chemicals (Taormina & Beuchat, 1999). Sprout-associated foodborne disease outbreaks are becoming an increasing concern, and however, chemical residue, sprout properties and nutrition level should be taken into consideration while disinfectant was used. Therefore, it is imperative to find effective, safe, pollution-free methods to enhance food safety.

Researchers have developed technologies designed to decontaminate seeds and sprouts by eliminating bacteria and improving food safety. In accordance with microbiological safety assurance, studies have examined the efficacy of chemical disinfectants such as chlorine, ethanol, hydrogen peroxide, ozone, radio-frequency, and natural antimicrobials such as organic acid, essential oils, and medium fatty chain acids (Ling, Ouyang, & Wang, 2019; Pajak, Socha, Galkowska, Roznowski, & Fortuna, 2014; Yang, et al., 2013). Electrolyzed oxidizing (EO) water, developed as an environmentally friendly technology based on electrochemistry, has become a popular alternative to harsh chemical disinfectants due to its Generally Recognized as Safe (GRAS) status and environmentally friendly nature (Huang, Hung, Hsu, Huang, &

Hwang, 2008). Slightly acidic electrolyzed water (SAEW) with a pH of between 5.0 and 6.5 is produced by the electrolysis of hydrochloric acid with or without sodium chlorite in a chamber without a membrane. SAEW has been widely applied to inactivate or eliminate various foodborne pathogens on foods, such as fruits, vegetables and meat (Li, et al., 2018; Sheng, Shu, Tang, & Zang, 2018; Tango, et al., 2017; Wang, et al., 2019). Additionally, EO water, which was administered mainly by its available chlorine concentration (ACC) was used instead of tap water in mung bean production during seed soaking and sprouting. This process resulted in a reduced microorganism population and an increase in their growth rate (Liu, Hao, Liu, & Li, 2011; Liu & Yu, 2017). SAEW with different ACC levels was used for the disinfection of *E. coli* O157:H7 and *Salmonella enteritidis* inoculated onto mung bean sprouts (Zhang, et al., 2011). Although SAEW is considered safe and effective to use as a disinfectant in the food industry, studies mostly focused on the inactivation characteristics of SAEW on inoculated pathogens. Therefore, more consideration should be given to reducing the natural microbiota on seed sprouts since contamination can occur before and after harvest, as well as during the germination process.

The seed germination strategies can be carried out using several simple procedures, mainly including sterilization, soaking, and sprouting. Furthermore, the germination strategies may vary for different types of seeds (Gan, et al., 2017). To inhibit the growth of microbes, sterilization is mostly performed before seed soaking. In this study, SAEW instead of tap water was used for seed soaking and sprouting during the production of pea sprouts, and the population levels of total natural bacteria, coliform, yeast and mold

were determined during this period. Moreover, the biological indicators, nutritive indicators, and nitrite content were also evaluated after the sprouts were harvested. The aim of this research is to use SAEW instead of regular production water to produce safer and healthier sprouts.

2. Materials and methods

2.1. Preparation of the treatment solutions

SAEW was produced by the electrolysis of a 6% hydrochloric acid (Analytical pure, Sichuan Xilong Chemical Engineering Co. Ltd., China) solution using a SAEW generator (HD-240L, Shanghai, China). The physicochemical properties of SAEW were measured immediately after preparation. The ACC was determined using a colorimetric method and a digital chlorine kit (Chlorometer Duo, Palintest Co., UK). The pH and oxidation-reduction potential (ORP) were measured using a dual scale pH/ORP meter (Five Easy Plus FE28, Mettler Toledo, China) with a pH probe (FE438) and an ORP probe (FE510). NaOCl (Analytical pure, Guangdong Guanghua Sci-Tech Co., Ltd., China) with the same ACC and tap water were used as a control in this study. The physicochemical properties of the treatment solutions presented in Table 1.

2.2. Seed soaking and sprouting

Pea (*Pisum sativum* L.) seeds (weight of 227.92 ± 3.12 g/1000 grains) were purchased from a commercial seed supplier (Yangling Huaxing seed Co., Ltd, Shaanxi, China). Uniform seeds based on shape, size and its health status were selected for this study. Seed soaking and sprouting was in phytotron (Temperature: 25 °C during the

day, and 22 °C during the night; Relative humidity (RH): 80% during the day and 60% during the night; appropriate ventilation). One hundred and sixty grams of seeds were gently washed with 800 mL of their soaking solution and repeated three times. The seeds were then immersed in 800 mL of soaking solution to soak for 24 h. Following the soaking process, the seeds were washed three times with 800 mL of treatment solution to remove the mucus on the seed surface. The soaked seeds were placed in clean seedling-raising trays (32 cm×23 cm×5 cm) covered with a single layer of sterilized gauze. The trays were placed in phytotron for germination and sprouting. Fresh treatment solution was manually sprinkled over the seeds for approximately 20 s. This process was repeated for 2~3 times every day to maintain the humidity. All procedures involving the seeds were kept consistent during germination and sprouting. When the seed sprouts were about 3~4 cm long, they were exposed to weak LED light for 8 h every day until sprouts were harvested. The 11-day-old sprouts were harvested and stored at 4 °C for no more than 7 d before analysis.

2.3. Microbiological analysis

The microbial contaminants of pea sprouts were detected using general and selective growth media, and a viable count was enumerated using the standard plate count method. During the seed sprouting period, 10 g of sprouts from each tray was randomly selected and placed in individual sterile stomached bags before being sprayed with water to determine the population of total bacteria, coliform, yeast and mold at day 2, day 5, day 8, and day 11, respectively. Samples were manually mixed with 50 mL sterilized saline solution (0.85% sodium chloride) and vortexed to fully elute the

natural microbiota on the sprouts. One milliliter of the suspension was extracted and serially diluted using saline solution. The total bacteria count was analyzed and enumerated by plating 0.1 mL of the appropriate dilution onto sterile Plate Count Agar (PCA) (Land Bridge, Beijing land bridge technology Co., Ltd.). The coliform count was determined by pouring 0.1 mL of the diluted solution onto Violet Red Bile Agar (VRBA) (Land Bridge). The PCA and VRBA plates were incubated at 37 °C for 48 h before enumeration. The yeast and mold counts were detected using Rose Bengal Medium (Land Bridge), and the plates were incubated at 28 °C for 48 h before enumeration. The microbial count was recorded as the mean of the triplicate determinations and expressed as log colony forming units per gram (log CFU/g).

2.4. Biological properties of the sprouts

Length of sprouts: Fifty sprouts were randomly selected from each seedling tray to measure the length of the sprouts (including stem and leaf) using a calibrated scale (minimum scale is 0.5 mm). The length of sprouts was expressed as an average value.

Fresh weight of a hundred strains: One hundred strains of the sprouts in each seedling tray were randomly selected to measure its fresh weight using an electronic scale (resolution 0.01g, PTT-A2000, Fuzhou Huazhi Instrument Co. Ltd., China). If the root, stem, and leaf were included, the sample was marked as fresh weight 1, while it was marked as fresh weight 2 if only the stem and leaf were included.

Dry weight: Ten grams of the sprouts without roots were measured using a balance scale (resolution 0.0001g, AL204, Mettler Toledo, China). The sample was placed on silver paper at 105 °C for 30 min, and then at 80 °C until it had dried sufficiently to

reach a constant weight.

$$\text{Edible rate} = (\text{Fresh weight 2} / \text{Fresh weight 1}) \times 100\%.$$

2.5. Determination of the nutrient content

Randomly selected sprouts were removed the water from the surface, and homogenized by manually grinding the samples as required. The indicators were assayed using the spectrophotometric method by commercially available diagnostic kits (Nanjing Jiancheng Bioengineering Institute, China). The levels of all indicators were determined following the manufacturer's instructions, and the results were presented as mg/g and $\mu\text{g/g}$ in fresh weight.

2.6. Determination of nitrite content

Sprouts were stored at 4 °C for 7 d, after which random samples were selected for analysis to establish the nitrite content using commercially available diagnostic kits (Nanjing Jiancheng Bioengineering Institute, China). The nitrite content was determined following the manufacturer's instructions, and the results were presented as $\mu\text{mol/kg}$ in fresh weight.

2.7. Statistical analysis

The data in this study were obtained from three independent repeated trials and presented as the mean value \pm standard deviation (SD). Statistical analysis was performed using SPSS (IBM SPSS Statistics 24). A one-way analysis of variance (ANOVA), followed by a Least Significant Difference Test were used for multiple comparisons at the 0.05 probability level.

3. Results and discussion

3.1. The effect of SAEW on the *elimination* of natural microbiota during seed sprouting

The primary sources of microbial contamination in ready-to-eat sprouts are contaminated seeds that are improperly disinfected before sprouting. Seed germination is the first critical step of the plant life cycle and the foundation of agricultural production (Puligundla, Kim, & Mok, 2017), while it is also the beginning of bacteria growth and reproduction. Microbiota attached to seeds could grow and reproduce quickly under the ideal conditions of high temperature and humidity that is required for seed germination. Estimates of the risk of foodborne illness generally depend on the number of microorganisms present in food at the time of consumption (Ross & McMeekin, 2003).

SAEW with ACC of 35 and 70 mg/L was used in this study to reduce microbiota and supply water for seed germination. The bactericidal effect of SAEW was determined at day 2, day 5, day 8, and day 11, respectively during seed sprouting, and the results are shown in Figs. 1-3. It is clear that the population of total bacteria slightly increased from day 2 to day 11 during seed germination (Fig. 1). The total bacteria count on the sprouts treated with SAEW 70 was consistently lower than that in any of the other treatments. Contrarily, the total bacteria count in the samples subjected to tap water treatment (control) remained higher than any other group. The total bacteria count was 8.31, 7.32, 6.73, 7.71, and 7.53 log CFU/g at day 11 in the control group, SAEW 35, SAEW 70, NaOCl 35, and NaOCl 70, respectively. The high temperature and humidity that were supplied for seed germination was also the ideal conditions for

microorganism growth and proliferation. In practice, it was challenging to significantly reduce the microorganisms on seeds and sprouts with a single disinfection procedure. Inoculated *E.coli* cells survived in the chlorine soak with ACC of 20,000 mg/L, and even grew to 6 log CFU/g on the sprouts after a 72 h sprouting period (Fransisca, et al., 2011). Therefore, SAEW with proper chlorine concentrations coupled with other disinfection methods will be more useful in substantially inhibiting microorganisms during seed sprouting. The levels of foodborne pathogens on naturally contaminated seeds are generally low but can grow significantly during sprout production to exceed the initial density. Within 2 d of seed sprouting, microbial density on the seed sprouts reached approximately 7~8 log CFU/g (Splittstoesser, 1983). SAEW was applied to the overall process of seed sprouting. Additionally, the initial population of the total bacteria, coliform, yeast and mold on the seeds were determined, but only some counts could be observed on the plates. The reason may be that microorganisms attached to seeds were dormant and required more time to be activated and reproduce to reach levels that could be regularly detected. Therefore, the bacterial count shown in Figs.1-3 only started on day 2. SAEW 70 showed more effective bactericidal efficacy than NaOCl 70 with the same level of ACC. Moreover, SAEW 35 even showed a slightly higher antimicrobial effect than NaOCl 70 some time. A previous study reported that the degree of inactivation of the *B. subtilis* spore by chlorine solution treatment varied with different pH (5.6 in contrast 8.2) levels. Because, in the chlorine solution at a pH of 5.6, most of the ACC will be in the form of hypochlorous acid (HOCl), whereas in a solution at pH 8.2, most of the ACC will be in the form of hypochlorite ion (OCl⁻) (Cho,

Kim, & Yoon, 2006). The bactericidal activity of HOCl is 80 times greater than that of OCl⁻ for deactivating *E.coli* at the same chlorine density and response time (Anonymous, 1997). Findings from this study were consistent with results from a previous study since SAEW in the current study at pH of 5.46~5.57 contained ACC that mostly presented in the form of HOCl, while NaOCl at pH 8.88~9.31 may exhibit ACC mostly in the form of OCl⁻. Previous studies have indicated that the specific form of ACC can undergo modifications as the pH changes, which is primarily related to the antimicrobial activity of EO water (Xiong, Li, Guo, Li, & Liu, 2014; Xiong, Liu, & Li, 2012).

Seeds growing close to the soil might be contaminated with various types of microbiota such as *E.coli*, *Salmonella*, *Listeria*, *Staphylococcus*, fungi, and even spore-forming bacteria. These microorganisms were responsible for outbreaks of foodborne illnesses associated with sprouts (Beuchat, 1996). Contamination can occur before and after the seeds are harvested, as well as during seed germination since the conditions are optimal for the growth of many types of microorganisms (Yang, et al., 2013). Therefore, coliform, yeast and mold counts were also detected on the sprouts in this study and the results are shown in Figs. 2 and 3.

The increment trend of the bacterial counts in Figs. 2 and 3 were similar to those in Fig. 1, while the counts of coliform, yeast and mold appeared to be slightly less than that of total bacteria. Similar results were obtained for the disinfection efficacy of SAEW 70 for coliform, yeast and mold, in which bacterial counts were lower than in any of the other treatments. Compared to the control group, SAEW 35, SAEW 70, NaOCl 35, and NaOCl 70 reduced the counts of coliform by 0.57, 1.02, 0.47, and 0.61

log CFU/g, respectively by day 11 (harvest day). The yeast and mold count displayed respective reduced levels of 1.01, 1.22, 0.31, and 0.81 log CFU/g, over the same period. As shown in Figs. 1-3, ACC-dependent **elimination** in all the detected microorganism counts were observed in sprouts exposed to SAEW, while this treatment exhibited a positive effect on the microbiological density on the sprouts. A reduction was evident in the total bacteria count on the sprouts treated with SAEW 70 displayed individual levels of 1.62, 1.56, 1.46, and 1.58 log CFU/g, compared to the samples treated with tap water at day 2, day 5, day 8 and day 11, respectively. Similarly, respective reductions levels of 1.58, 0.89, 0.69, and 1.02 log CFU/g were evident for coliform, while levels of 1.86, 1.28, 0.97, and 1.22 log CFU/g were observed for yeast and mold on the same day. In a previous study, EO water with a pH of 2.76 and ACC of 70 mg/L was used to soak commercial mung bean sprouts for 10 min, and the reduction of total bacteria, coliform, yeast and mold was 2.20, 1.92, and 1.93 log CFU/g, respectively (Liu & Yu, 2017). The difference between the previous results and the results of this study might be attributed to the treatment method and time applied to the sprouts. During a previous study, the sprouts were soaked for 10 min, while they were only sprayed for about 20 s in the current study. During the sprouting period, the seeds and sprouts were watered every day to maintain a relatively high humidity to support their growth. Frequent water changes occurred twice to three times a day, and some metabolites and microbes on the germinated seeds were partly removed. Therefore, natural microbiota counts remained at a relatively stable level during seed sprouting. Alternative results indicated that the population of natural microbiota on radish sprouts

were inhibited by SAEW soaking and spraying to remain at a stable level during seed germination (Zhang, Cao, Hung, & Li, 2016). However, it is incredibly challenging to ensure that no microbiota are present in food. Therefore, it is crucial to maintain a low level of contamination.

SAEW instead of tap water was used in sprout production due to its high bactericidal effect, and it was proven capable of reducing total bacteria, coliform, yeast and mold counts on pea sprouts during seed germination. SAEW with higher ACC (70 mg/L) was more effective regarding bactericidal activity, while these properties in SAEW with lower ACC (35 mg/L) was similar to, and even higher than NaOCl with higher ACC (70 mg/L). Microbial contamination was inhibited by disinfectant soaking and spraying. Therefore, these methods were the safer options to utilize for the production of seed sprouts. Besides microbial safety, the growth state and nutritional value for the sprouts as a vegetable should be considered.

3.2. The effect of SAEW on the biological properties of sprouts

Pea seeds were germinated and sprouted in a phytotron at a temperature of 22~25 °C and RH of 60~80% for 11 days. SAEW 35 and SAEW 70 were applied to soak the seeds and spray the sprouts during the procedure of sprout production and sprouts were harvested after 11 d. The properties, including fresh weight, length, dry weight, and edible rate were determined, and the results are presented in Table 2. There was no significant difference in fresh weight 1, but some changes were evident in fresh weight 2 during different treatments. The fresh weight 2 of sprouts treated with SAEW 35, SAEW 70, and NaOCl 70 was significantly higher than that treated with NaOCl 35 and

tap water. Regarding economic yield, a higher fresh weight 2 subjected to SAEW 35, SAEW 70, and NaOCl 70 treatments should be chosen for sprout production. The SAEW 35 used to soak the seeds and spray the sprouts significantly enhanced the length of the sprouts, compared to those treated with tap water ($p<0.05$). A previous study indicated that different treatments led to variations in sprout length (Li, et al., 2018). The presence of a microorganism population on the sprouts treated with tap water, negatively affected seed germination and resulted in a shorter sprout length. It is possible that the microorganisms consumed some of the nutrients necessary for seed germination and causing this result. Dry weight is an important parameter for evaluating biomass concentration, productivity, and percentage for cell components (Zhu & Lee, 1997). The dry weight was significantly higher when treated with SAEW 70, NaOCl 35, and tap water than when treated with SAEW 35, and NaOCl 70. Compared with fresh weight and sprout length during the same treatment, the dry weight was probably affected by its weight and length. The edible rate is an important evaluation indicator for edible plants, and may affect the edibility and economic efficiency for consumers and the food industry. The edible rate of sprouts treated with tap water, NaOCl 35, NaOCl 70, SAEW 35, and SAEW 70 were 62.04%, 62.46%, 65.49%, 65.39%, and 65.51%, respectively. Table 2 indicates that SAEW treatment could improve the edibility of the sprouts. Several studies have reported that SAEW can accelerate the growth of mung bean seedlings and germinated brown rice but did not affect the growth of germinated buckwheat and inhibited the growth of broccoli sprouts (Hao, Wu, Li, Wang, & Liu, 2016; Li, et al., 2018; Liu, et al., 2011; Zhang, Xia, Li, & Hung, 2018).

Sodium chloride exerted stress on quinoa sprouts and reduced sprout growth (Fischer, et al., 2017). Therefore, the variation in species and water conditions was possibly the primary reason for differences in the effects on plant growth.

Seed germination is a critical stage in the plant life cycle, which starts with the uptake of water by imbibition of dry seeds and results in the expansion of the seed embryo (Hermann, et al., 2007). Near neutral pH could be more appropriate in the water uptake for seed germination and sprout growth, while a pH range of 5.5 to 6.5 was reported as optimal for seed germination (Janusz Deska & Jankowska, 2011). Therefore, a pH of 5.46~5.57 in SAEW might be more appropriate for seed germination and enhanced sprout growth. The process involved in seed germination and further sprout development depended on the environment and might be affected by natural microbiota counts, the pH levels of the water supply, and ACC in the soaking and germination solution.

3.3. The nutritive compound content

The soluble sugar, vitamin C, total protein, and flavonoid content were determined by assessing the effect of SAEW application on the nutritive indicators of the sprouts. A conventional colorimetric method was used to measure these nutritional properties with commercially available diagnostic kits, and the results are presented in Figs. 4-7.

3.3.1. Soluble sugar content

Soluble sugar is a vital nutritional ingredient for seed germination and is essential for consumption. The soluble sugar content in the sprouts were 9.63, 12.65, 14.90,

15.08, and 14.42 mg/g for tap water, NaOCl 35, NaOCl 70, SAEW 35, and SAEW 70 treatments, respectively, and the results are shown in Fig. 4. Significant differences were evident between the soluble sugar content in the SAEW and the control groups ($p < 0.05$). The results indicated that SAEW application in pea sprout production could increase the soluble sugar levels in the sprouts.

3.3.2. Total protein content

The total protein levels were 44.56, 40.81, 42.42, 44.24, and 48.43 mg/g in pea sprouts treated with tap water, NaOCl 35, NaOCl 70, SAEW 35, and SAEW 70, respectively. As can be seen in Fig. 5, the total protein content significantly increased with SAEW treatment containing ACC of 70 mg/L. When the seeds were washed, the protein content decreased. It is possible that the washing process affected or damaged the outer endosperm, resulting in the loss of protein in water (Fischer, et al., 2017). Different water treatments caused various degrees of damage and loss, resulting in protein content diversity in mature the sprouts.

3.3.3. Vitamin C content

The vitamin C content was 83.29, 76.92, 74.40, 83.33, and 78.66 $\mu\text{g/g}$ in the pea sprouts treated with tap water, NaOCl 35, NaOCl 70, SAEW 35 and SAEW 70, respectively (Fig. 6). Vitamin C, known as ascorbic acid, is associated with the disease, scurvy. Vegetables and fruits are the primary natural sources of vitamin C (Ma, et al., 2017, 2019), and studies that germination could significantly increase its content in some edible seeds and sprouts (Gan, et al., 2017; Gan, Wang, Lui, Wu, & Corke, 2016;

Zhou, et al., 2015). Previous research indicated that environmental conditions such as light and temperature modulated the biotin content of pea sprout tissues by regulating the expression of biotin synthase (Kamiyama, et al., 2016). Therefore, further research is required regarding the mechanism clarification of vitamin C synthesis under the current production conditions.

3.3.4. Flavonoid content

The flavonoid content in the pea sprouts was also investigated and found to be 6.39, 6.56, 5.74, 6.50, and 6.58 mg/g, respectively when treated with tap water, NaOCl 35, NaOCl 70, SAEW 35, and SAEW 70 (Fig. 7). There was no significant difference between the individual treatments ($p>0.05$). However, a previous study indicated that SAEW with different ACC exhibited a different effect on the flavonoid content of broccoli sprouts. Moreover, compared to tap water, the flavonoid levels were higher with SAEW 20 treatment, while they decreased when treated with SAEW 40 and 50 (Li, et al., 2018). Flavonoids are common in plants, and the body requires relatively little of this substance to function normally. Germinated edible seeds can enrich their flavonoid level, due to the activation of endogenous enzymes that participate in the phenylpropanoid pathway, initiated by phenylalanine ammonia-lyase (PAL) (Pajak, et al., 2014). The ACC of SAEW might regulate the activity of PAL or other critical enzymes in the phenylpropanoid metabolism such as chalcone synthase, and chalcone flavanone isomerase, which then modulates the flavonoid content (Li, et al., 2018).

Therefore, several factors should be considered during the seed sprouting process, such as the temperature, humidity, watering, light, and time. The total flavonoid content

of the pea sprouts exposed to yellow, blue, and red LED lighting, as well as white fluorescent lamp light displayed levels of 12.31, 24.55, 21.01 and 24.29 times that of the control group that was kept in the dark. These findings indicated that blue LED lighting could enhance the total flavonoid content (Liu, et al., 2016). In this study, all the applied factors were the same, except watering. Therefore, the differences that were obtained from some measured parameters were due to the various watering techniques that were used in this study. The bioactive compounds, such as vitamins, gamma-aminobutyric acid, and polyphenols could be synthesized, transformed, or influenced by the conditions during the germination process for sprout production (Gan, et al., 2017). Previous research showed that EO water significantly enriched the gamma-aminobutyric acid levels in germinated brown rice, and the content was affected when the grains were treated, rinsed or soaked (Lu, et al., 2010). However, the influence of environmental conditions on the nutritional properties of germinated sprouts remains mostly unclear, and further research is required in this area. In this study, SAEW was proven to possess high antimicrobial qualities, and had a beneficial effect on the nutritive content of the samples. Therefore, SAEW treatment can be applied in seed sprout production to maintain food safety and health.

3.4. Nitrite content

The nitrite content in the pea sprouts that were stored for 7 d was determined, and the results are presented in Fig. 8. The greatest quantity of nitrite was found in the sprouts treated with NaOCl 70 (18.57 $\mu\text{mol/kg}$), followed by the control group (16.89 $\mu\text{mol/kg}$), NaOCl 35 (14.18 $\mu\text{mol/kg}$), SAEW 35 (14.00 $\mu\text{mol/kg}$), and SAEW 70

(13.40 $\mu\text{mol/kg}$). Vegetables are a vital part of the human diet, being a source of minerals, vitamins, and fiber. However, these food sources can also contain nitrates and nitrites, which adversely affect human health. Dietary nitrite is considered to be harmful to human health due to its association with fatal cases of methemoglobinemia following consumption of foods with high concentrations of nitrite (>0.3 percent by weight), and possible links to some gastrointestinal cancers (Gorenjak & Cencic, 2013; Weitzberg & Lundberg, 2013). The nitrite content of the pea sprouts exposed to SAEW was found to have significantly decreased, in comparison with the control sample.

4. Conclusion

SAEW with ACC of 35 and 70 mg/L were applied during the seed sprouting process to eliminate the natural microbiota on the pea seeds and sprouts to improve its safety. The results showed that the population of natural microbiota was up to 1.6 log CFU/g reduced by SAEW treatments. Moreover, SAEW exhibited no significant adverse effect on the measured parameters such as fresh weight, length, soluble sugar, total protein, vitamin C, and flavonoids, in fact, SAEW treatment even improved some of these properties, and the nitrite content was slightly lower than in other treatments after a 7 d storage period. Therefore, SAEW combined with additional appropriate method could be a more promising approach for seed sprout production in consideration of microbial safety, sprout properties, chemical residue, and nutritional safety.

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Competing interest statement

The authors have no conflicts of interest to declare.

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Fig. 1. The counts of total bacteria on pea sprouts by different treatments during germination.

Fig. 2. The counts of coliform on pea sprouts by different treatments during germination.

Fig. 3. The counts of yeast and mold on pea sprouts by different treatments during germination.

Fig. 4. The soluble sugar content of the pea sprouts treated with different solutions. Data labeled with no common letters on the bar are significantly different ($p < 0.05$).

Fig. 5. The total protein content of the pea sprouts treated with different solutions. Data labeled with no common letters on the bar are significantly different ($p < 0.05$).

Fig. 6. The vitamin C content of the pea sprouts treated with different solutions. Data labeled with no common letters on the bar are significantly different ($p < 0.05$).

Fig. 7. The flavonoid content of the pea sprouts treated with different solutions. Data labeled with no common letters on the bar are significantly different ($p < 0.05$).

Fig. 8. The nitrite content of the pea sprouts treated with different solutions after stored for 7 days. Data labeled with no common letters on the bar are significantly different ($p < 0.05$).

Highlights

- SAEW effectively reduced the natural microbiota on pea sprouts
- The biological parameters of the sprouts were slightly enhanced by SAEW
- SAEW showed no adverse effect on nutrients of the sprouts, and even improved
- SAEW could be used in pea sprout production instead of tap water

Table 1. The physicochemical parameters of the treatment solutions.

Treatment solution	ACC (mg/L)	ORP (mV)	pH
SAEW 35	35	912 ± 2.16	5.57 ± 0.02
SAEW 70	70	927 ± 3.56	5.46 ± 0.01
NaOCl 35	35	724 ± 4.03	8.88 ± 0.09
NaOCl 70	70	695 ± 3.09	9.31 ± 0.03
Tap water (control)	< 1 mg/L	531 ± 5.25	7.33 ± 0.01

Table 2. The biological properties of pea sprouts.

Treatments	Fresh weight1 (g/100 strains)	Fresh weight2 (g/100 strains)	Length of sprouts (cm)	Dry weight (g/10g)	Edible rate (%)
Control	95.95 ± 2.59a	59.53 ± 1.33b	21.03 ± 1.01b	0.74 ± 0.02a	62.04%
NaOCl 35	98.38 ± 4.58a	61.45 ± 2.88b	21.81 ± 1.04ab	0.74 ± 0.01a	62.46%
NaOCl 70	99.40 ± 1.22a	65.10 ± 0.09a	22.65 ± 1.15ab	0.70 ± 0.01b	65.49%
SAEW 35	101.62 ± 1.65a	66.45 ± 2.05a	23.69 ± 1.14a	0.71 ± 0.01b	65.39%
SAEW 70	99.47 ± 4.79a	65.16 ± 3.49a	22.35 ± 1.22ab	0.73 ± 0.03a	65.51%

Fresh weight 1: the weighed sprouts included the root, stem, and leaf. Fresh weight 2: the weighed sprouts included only the stem and leaf. Data labeled with no common letters in the same column are significantly different ($p < 0.05$).

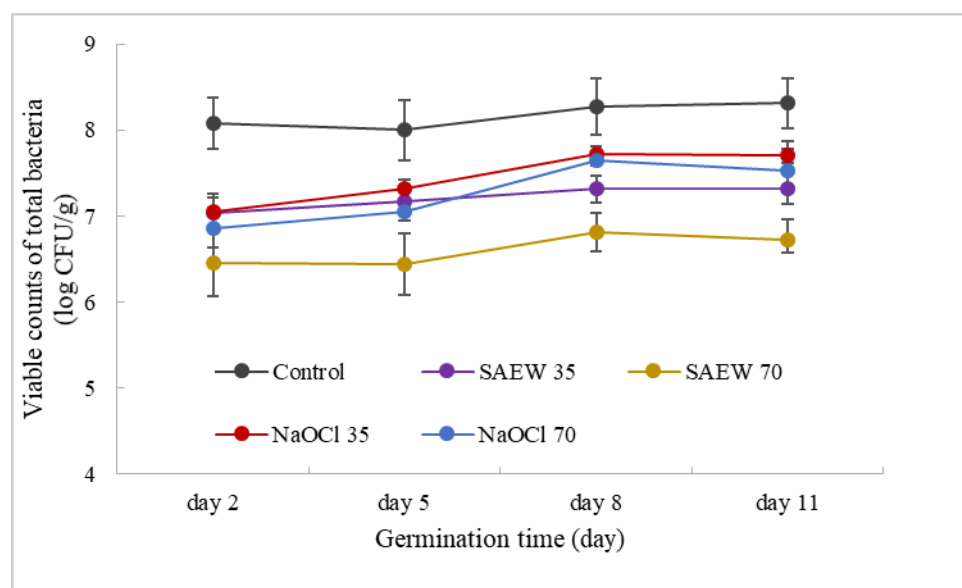


Fig. 1. The counts of total bacteria on pea sprouts by different treatments during germination.

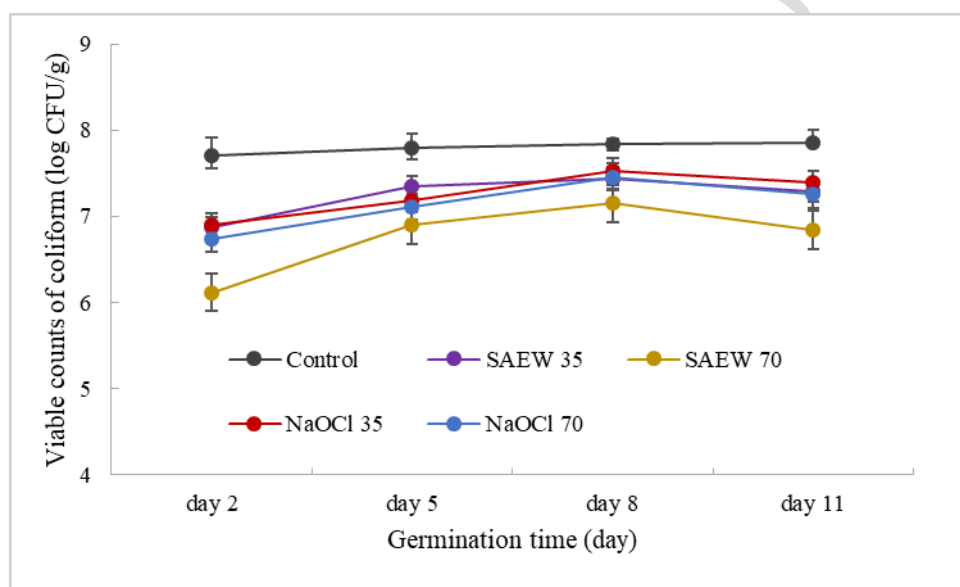


Fig. 2. The counts of coliform on pea sprouts by different treatments during germination.

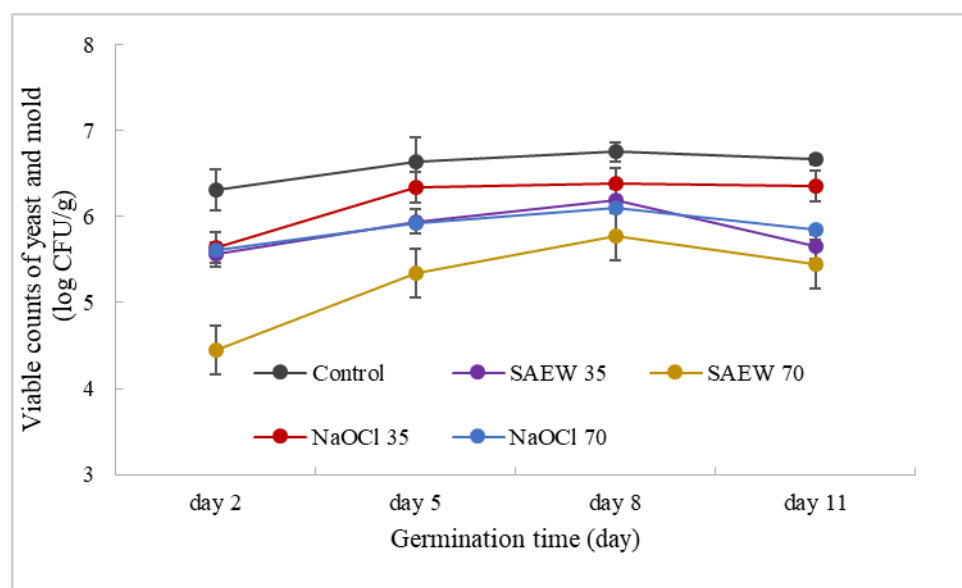


Fig. 3. The counts of yeast and mold on pea sprouts by different treatments during germination.

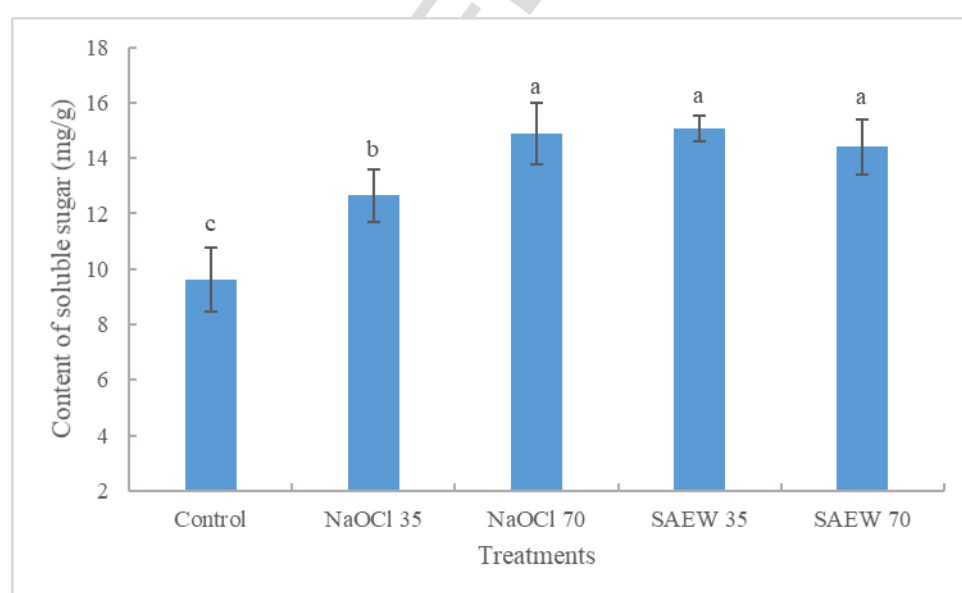


Fig. 4. The soluble sugar content of the pea sprouts treated with different solutions. Data labeled with no common letters on the bar are significantly different ($p < 0.05$).

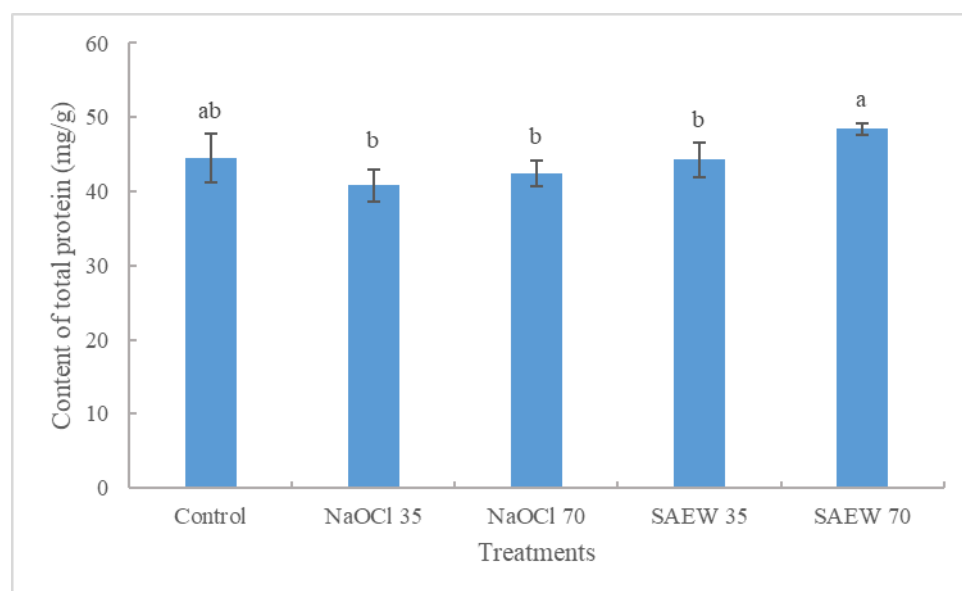


Fig. 5. The total protein content of the pea sprouts treated with different solutions. Data labeled with no common letters on the bar are significantly different ($p < 0.05$).

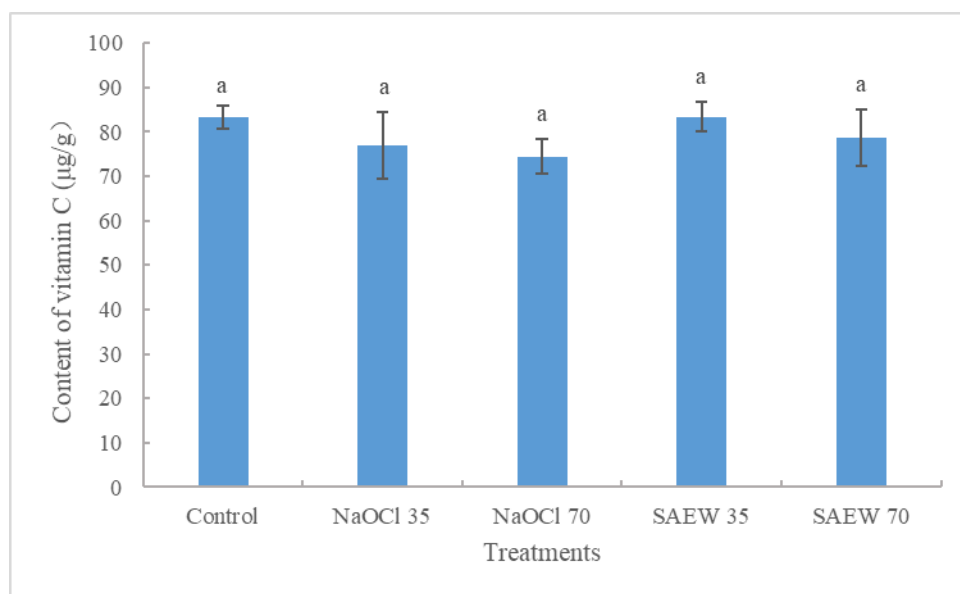


Fig. 6. The vitamin C content of the pea sprouts treated with different solutions. Data labeled with no common letters on the bar are significantly different ($p < 0.05$).

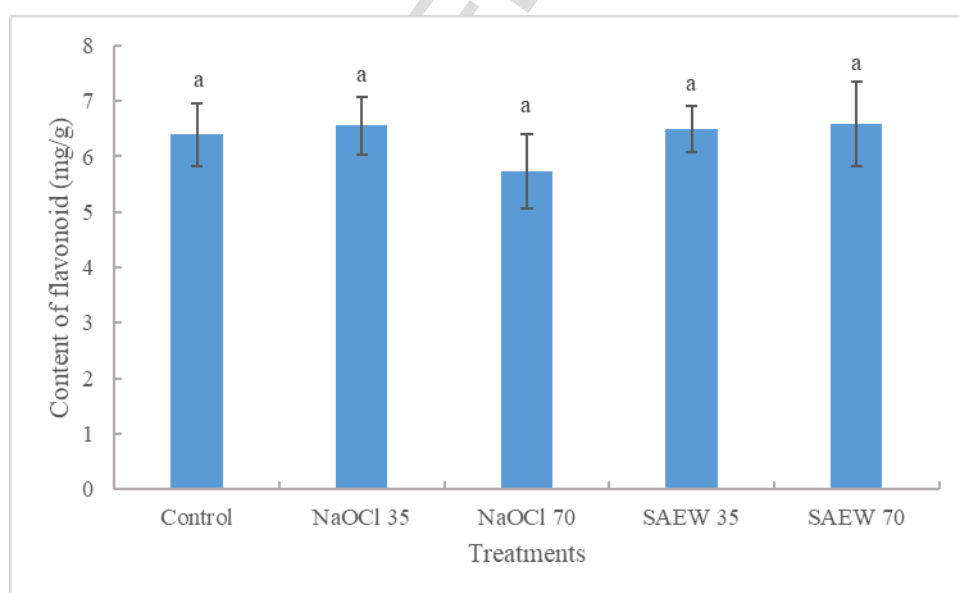


Fig. 7. The flavonoid content of the pea sprouts treated with different solutions. Data labeled with no common letters on the bar are significantly different ($p < 0.05$).

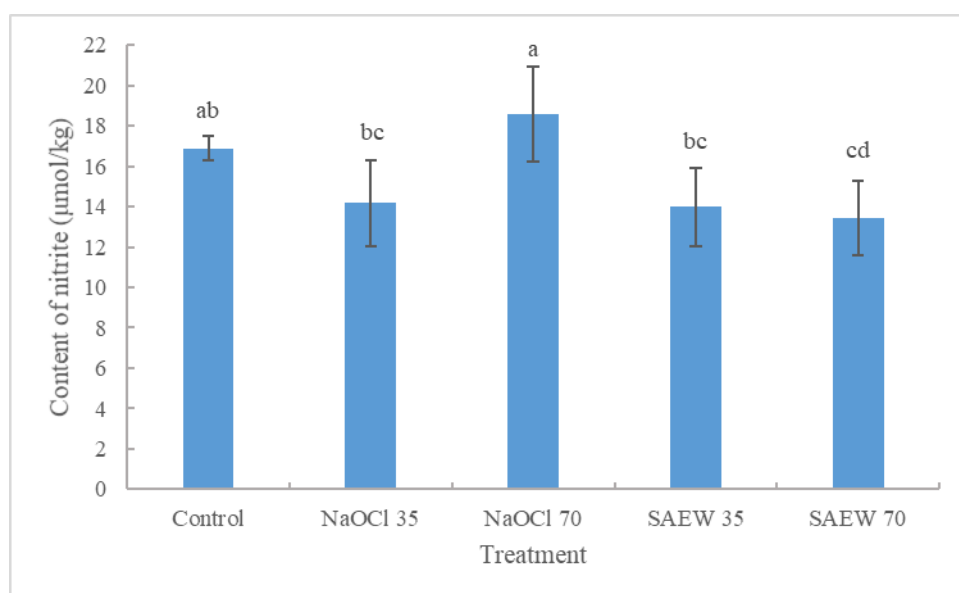


Fig. 8. The nitrite content of the pea sprouts treated with different solutions after stored for 7 days. Data labeled with no common letters on the bar are significantly different ($p < 0.05$).