OZONIZATION ON SUNFLOWER SPROUT CULTIVATION

Jordana K. Montagua[1], Verlaine M. Zan[2], Éverton Hansen[1], Janice da Silva[1], Bethania Brochier[1], Suse Botelho da Silva[1]*

[1] *Polytechnic School, Unisinos University, São Leopoldo, RS, Brazil.*
[2] *Health School, Unisinos University, São Leopoldo, RS, Brazil.*
* *Corresponding author's email address: susebs@unisinos.br*

ABSTRACT

Raw sprout consumption is increasing due to the demand for nutritious, healthy foods. Germinating sprouts in certain conditions poses a risk of food outbreaks, which ozonation can help prevent. Ozone effectively sanitizes agricultural products by inactivating fungi, bacteria, and viruses. Thus, the present work evaluated the use of ozone during the cultivation of sprouts from a physiological, physical-chemical, and microbiological perspective. Hydrated sunflower seeds were sown at 20 ± 1 ºC for 7 days, without or with ozone gas flow at 2.5 L/min for 120 minutes daily for 4 days. At the end of cultivation, the sprouts were exposed to an average lighting of 130 lux with a white LED lamp (806 Lumens, 60 W). Microbial counts, pH, moisture, phenolic compounds, chlorophyll content, germination index, height, and root performance were analyzed. As a result, there was no significant difference in the parameters of pH, height, and root performance between the conditions with and without exposure to ozone. For the germination index, both chambers reached the maximum percentage of 62.96%. The moisture content did not show a significant difference in the first three days, reducing the percentage on the last day of cultivation in the ozone chamber. The phenolic compounds and the chlorophyll content were higher for the ozonized sprouts. Ozonation reduced aerobic mesophilic bacteria by 1 cycle log on day three but could not prevent contamination throughout cultivation. Ozone has the potential to be used as a sanitizer for sunflower sprouts, considering its effect over physical-chemical and physiological properties. However, further experimentation under more rigorous conditions is necessary to achieve effective sanitization.

Keywords: sunflower sprouts, ozonation chamber, cultivation, ozone.

1 INTRODUCTION

Sprouts show high nutritional value, and they are good sources of minerals, proteins, vitamins and antioxidant compounds, still having low energy value (MARQUES et al., 2017). However, the consumption of raw sprouts has been associated with the occurrence of food outbreaks due to the conditions in which the sprouts are germinated (soil or agro-industrial residues as substrate) which may thus favor the growth of bacterial pathogens. Pathogens can be transmitted through sunflower sprouts via seeds and the sprout substrate. Therefore, some strategies need to be adopted to prevent microbial growth. Chemical interventions like ozone and chlorine can prevent infections (SANTOS et al., 2022). Ozone is a powerful disinfectant without chemical residues (CABRAL et al., 2024). Ozone is used as a sanitizer in its gaseous form or dissolved in water. In gaseous form, its effectiveness is affected by the amount of ozone generated and the rate at which the gas is decomposed to oxygen (SCHROER et al. 2023). Ozone is widely considered for treating seeds due to its effectiveness against fungi, bacteria, and viruses (RODRIGUES et al., 2015). However, it is unclear if ozonation has any influence on the cultivation of sprouts, either on their physical-chemical characteristics or on the growth and development of the plant. Thus, the present work aims to evaluate the use of ozone during the cultivation of sprouts, from the microbiological, physical-chemical, and physiological point of view of the plant.

2 MATERIAL & METHODS

2.1 Sample preparation and cultivation conditions

The sunflower seeds were donated by Familia Hattori (Viamão, RS). 200 grams of seeds (previously hydrated for 4 hours) were sown per tray, in 6 plastic trays (40 x 20 cm), with burnt rice husk and black soil (3:1 ratio) as substrate. The sunflower sprout trials used two chambers: Chamber 1 (control) and Chamber 2 (ozonation system with compressed air and ozone at 2.5 L/min flow rate - AquaOz ozone, Brazil). Both chambers have an internal volume of 5.4 m³. Chambers were lit by LED lamps for the last 27 hours of cultivation. The chamber was illuminated with a white LED lamp (806 Lumens and 60 W), resulting in an average illumination of 130 lux (Smart Luxmeter application version 1.1.0, Smart Tools.)

2.2 Physicochemical and physiological analyzes

Each 2 days, analyzes of pH, moisture, and total phenolic content were carried out to characterize sprouts during the cultivation. On the last day of the experiment, analyzes of respiration rate and chlorophyll content were carried out. The germination index, height, and root performance of sunflower sprouts were analyzed in 9 samples from each seed tray during the seven days of cultivation in the two chambers. Root performance was evaluated by measuring of each sprout grown in 3 cells of the seed tray.

2.3. Microbiological Analysis

Aerobic mesophiles, and molds and yeasts counts were performed for irrigation water, initial substrate, seeds and sprouts by standard methods as mentioned in APHA, 2001.

3 RESULTS & DISCUSSION

3.1. pH and moisture

The pH values ranged from 6.46 ± 0.01 to 7.13 ± 0.14, with no significant difference between the two chambers over 7 days. This suggests that ozone concentration and temperature did not affect sprout pH. The dry seed's initial moisture was 10.50 ± 0.13%, while the hydrated seed's moisture was 42.17 ± 0.76. The sprouts humidity did not significantly differ between the two chambers for the first 3 days (61.33 ± 1.44 and 61.03 ± 0.90). Non-ozone treated sprouts had higher humidity at the end (92.41 ± 0.60 and 87.76 ± 1.09) possibly due to ozone entering Chamber 2. These findings align with Mercali's (2011) of 94.05% humidity in fresh sunflower sprouts.

3.2 Total phenolic and chlorophyll content

Phenolic compounds values varied significantly over time. From days one to six, values ranged from 16.3 ± 0.1 to 8.8 ± 0.3 mg/100 g of sprout in the control chamber while in the ozone chamber, values ranged from 15.9 ± 0.1 to 10.0 ± 0.1 mg/100 g of sprout. Kang, Park, and Lee (2001) suggest that physical damage during harvest can trigger the biosynthesis of phenolic compounds, potentially explaining the observed value on the last day of Chamber 2 cultivation.

Chlorophylls, the pigments that make vegetables green, can degrade during processing, altering the color of foods. Sprouts' color is an important attribute that affects consumer choice and food acceptability, indicating its quality (WOJDYLO et al., 2020). Figure 1 shows the sprouts before lighting in Chamber 1 (when the green color had not yet developed) and after lighting for 27 h in both chambers. The sunflower sprout samples varied significantly between the two chambers, presenting values of 223.56 ± 0.08 and 262.39 ± 0.08 mg of chlorophyll /g of sprout, with the highest chlorophyll content obtained for the ozonized sample.

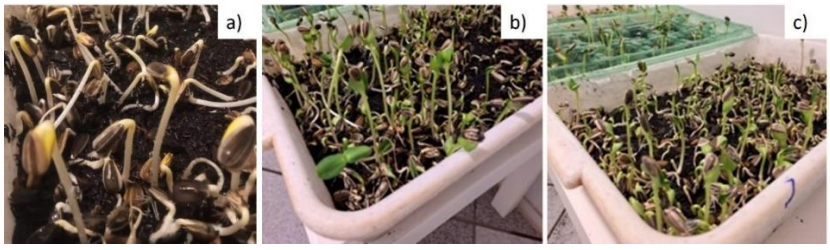

Figure 1 – Sunflower sprouts (a) before lighting, after lighting in Chambers (b) 1 and (c) 2.

3.3 Germination index

Ozone-treated chamber germinated in 1 day, control chamber took 2 days. The ozonated chamber had higher and quicker germination in the first two days, indicating its advantage. The highest seed germination rate, 62.96%, occurred on day 3 without ozone gas treatment, while the best condition for both chambers was on day 6.

3.4 Root height and performance

Figure 2 shows the average growth of sunflower sprouts under two conditions. The height of the sprout stems increased over time, as expected. Sprouts measurements did not significantly differ ($p > 0.05$) when changing the atmosphere, averaging 67 ± 28 mm on the sixth day. The maximum length obtained for Chamber 1 (without ozone) was 117 mm, while for Chamber 2 (with ozone) it was 112 mm. The sprout's height determines production yield, as it is sold by weight in plastic packages. Root performance was only measured on the last day of cultivation. Untreated and ozonized sprouts had root lengths of 67 ± 22 mm and 58 ± 14 mm, respectively, without significant difference in measurements.

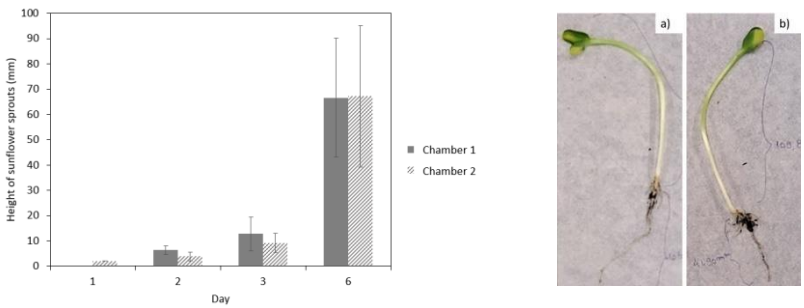

Figure 2 – Average height of sunflower sprouts for the two growing conditions and sunflower sprouts demonstrating the stalk and root. (a) Chamber 1 (control) and (b) Chamber 2 (ozonated).

3.5 Microbial Counts

Table 1 shows the initial counts of aerobic mesophiles, and molds and yeasts in irrigation water, substrate, and seeds. The data indicate that molds and yeasts exhibit higher counts compared to mesophilic bacteria. Prior disinfection is crucial to prevent contamination, particularly for the substrate and seeds, which can be a potential source of initial contamination. Table 2 shows the counts of both treatments were consistently high throughout the experiment, peaking on the last day. This can be attributed to the favorable temperature of 20 ºC, which is characteristic of the crop and encourages the proliferation of microorganisms. While ozonation showed some effectiveness against aerobic mesophilic bacteria in the first 3 days (reduction of 1 cycle log compared to the control), it failed to prevent contamination throughout the entire cultivation process. A stronger set of conditions is needed to address the issue.

Table 1 - Initial microbial counts in the substrate, seeds, and irrigation water.

	Aerobic Mesophilic cfu/g	Molds and Yeasts cfu/g
Irrigation water	1.0 ± 0.0 UFC/mL	3.9 ± 2,0 10^2 UFC/mL
Substrate	3.5 ± 0.6 10^6 UFC/g	1.3 ± 0.8 10^7 UFC/g
Seeds	2.6 ± 0.8 10^4 UFC/g	2.6 ± 0.1 10^5 UFC/g

Table 2 – Molds and Yeasts and Aerobic Mesophilic counts in control and ozonated sprouts

	Control		Ozonation	
Day	Molds and Yeasts cfu/g	Aerobic Mesophilic cfu/g	Molds and Yeasts cfu/g	Aerobic Mesophilic cfu/g
1	1.4 ± 0.1 10^7	2.5 ± 0.2 10^7	2.3 ± 0.8 10^8	3.8 ± 0.5 10^7
3	1.0 ± 0.2 10^8	2.0 ± 0.7 10^8	1.1 ± 0.1 10^8	1.0 ± 0.3 10^7
6	2.9 ± 0.3 10^8	4.5 ± 0.8 10^7	5.5 ± 0.7 10^8	4.5 ± 0.2 10^8

4 CONCLUSION

There was no significant difference in most physical-chemical parameters evaluated, however, the application of gas caused improvements in the content of phenolic compounds and chlorophyll in the sprouts. Regarding physiological parameters, there was no change when using ozone. Ozonation showed effectiveness against aerobic mesophilic bacteria, reducing 1 cycle log compared to the control in the third day, but it failed to prevent contamination throughout the entire cultivation process. A stronger set of conditions is needed to address the issue.

REFERENCES

APHA (AMERICAN PUBLIC HEALTH ASSOCIATION). **Compendium of methods for microbiological examination of foods**. 4.ed. Washington, D.C., 2001. 676p.

CABRAL, K. C. *et al*. Kinetic modeling of *Escherichia coli* inactivation by ozone mist. **Ozone: Science & Engineering**. V. 46, n. 1, p. 64-77.2024. DOI: 10.1080/01919512.2023.2210608.

KANG, J. S.; PARK, W. P.; LEE, D. S. Quality of enoki mushrooms as affected by packaging conditions. **Journal of the Science of Food and Agriculture**, [Londres], v. 81, n. 1, p. 109–114, Jan. 2001.

MARQUES, R. O. *et al.* **Brotos de alfafa para a alimentação humana.** [São Carlos] EMBRAPA-CNPMS, 2017. 7p. (EMBRAPA-CNPNS. Circular Técnica, 76).

MERCALI, C. A. **Estudo do perfil fitoquímico, nutricional e atividades biológicas do broto de girassol (*Helianthus annuus L.*).** Dissertação (Mestrado em Ciências Farmacêuticas) – Universidade Federal do Paraná. Curitiba, PR, 2011.

RODRIGUES, V. O. *et al*.. **Journal of Seed Science**, v.37, n.3, p. 202-210, 2015

SANTOS, G. A. F. *et al.* Edible sprouts: Nutritional quality, microbiological safety and potential application in new products. **Research, Society and Development**, *[s.l.]*, v.11, n.9, p. 2525-3409, 2022.

SCHROER, I. A., J. SILVA, B. BROCHIER, P. R. S. SILVA, S. B. SILVA, AND E. HANSEN. Study of Ozone Misting for Sanitization of Hospital Facilities: A CFD Approach. **Ozone: Science & Engineering** v. 45, n. 3, p. 305-319. 2023. doi:10.1080/01919512.2022.2091512.

WOJDYLO, A. *et al*. Sprouts vs. Microgreens as Novel Functional Foods: Variation of Nutritional and Phytochemical Profiles and Their In Vitro Bioactive Properties. **Molecules**, [Poland], v. 25, p. 4648, 2020.

ACKNOWLEDGEMENTS

The authors would like to thank FAPERGS for the financial support.