

Introduction

Annatto is commonly used as a food colorant in cheddar cheese production to give the signature orange color in the final product. It is added to cheese milk during cheese making to achieve a consistent color to appeal the consumer. As a co-product of cheese making, liquid whey stream contains valuable whey proteins that can be further processed into powder form. However, annatto residues can be found in liquid whey which can lead to undesirable color in the whey protein powders. Therefore, liquid whey is commonly bleached prior to downstream processing. Currently, hydrogen peroxide and benzoyl peroxide (Kang et al., 2010) are two approved bleaching agents in the whey industry. However, recent studies have shown that the bleaching agent impart negative flavor effects on whey protein based ingredients (Kang et al.2010). Ozone, as a renewable and environmental-friendly source of oxidizing agent, may have potential to bleach annatto in liquid whey (Kang et al. 2012) .

Objective

The objective of this study was to examine the effectiveness of treatment time, temperature, and holding time of ozone as a whey bleaching agent.

Material and Methods

Experimental Design

- Bleaching efficiency of ozone was first evaluated in a model aqueous annatto solution at 3 different temperatures (10, 20, and 35°C).
- In the next step, bleaching efficiency of ozone in reconstituted whey was evaluated at different treatment temperatures (10, 20, and 35°C), recirculation time (1 and 2 min), and holding time (0, 5, 10, 20, and 30 min) as independent factors.
- All the experiments were carried out in duplicate.

Model Annatto Solution

- 0.085 g of Annatto (Edger A Webber & Co., IL) was added to 500 g water.
- Incubated at 10, 20, 35 °C and exposed to ozone by pumping through the custom-build ozone generator (CleanCore Technologies, NE) in continuous mode as shown in Figures 1 and 2.



Figure 1. Ozone generation setup used in the present study

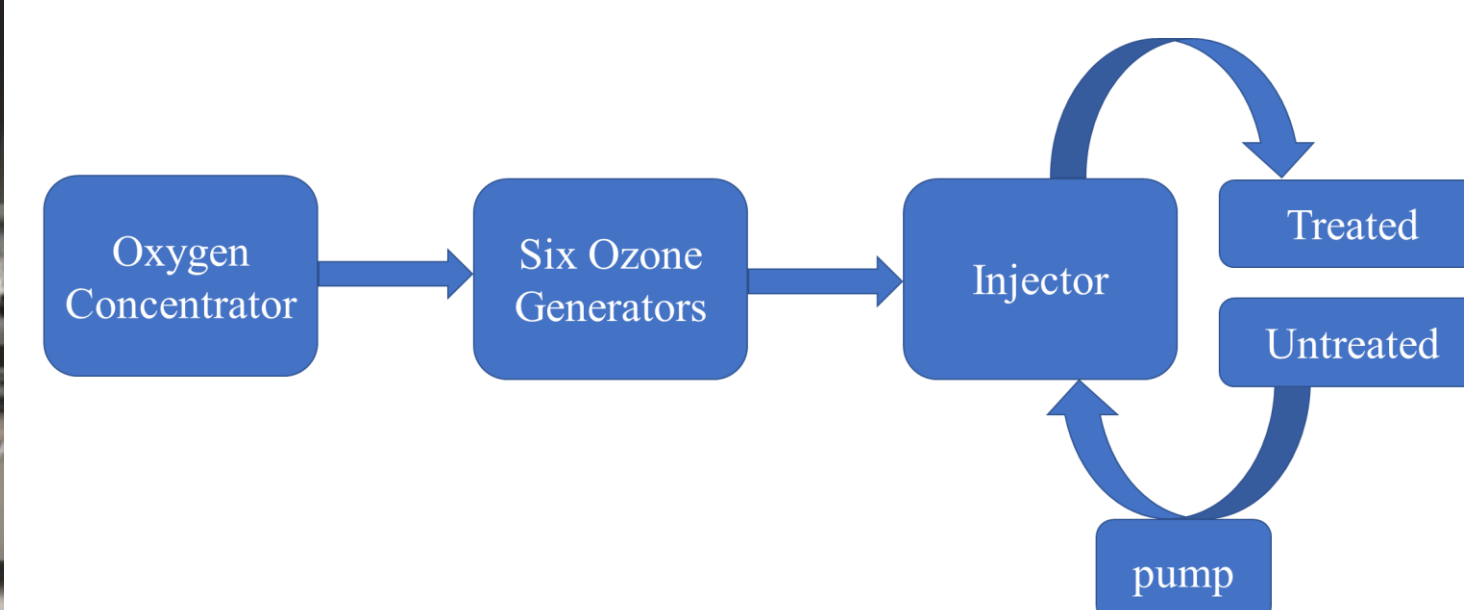


Figure 2. Schematic representation of the ozone treatment set up

Reconstituted Cheese Whey

- Sweet whey powder (Saputo Inc., CA) was dissolved to make 7.6% (w/w) solution under constant stirring at 55°C for 30 min.
- Subsequently, annatto was added (0.017%) to the whey solution to simulate unbleached cheese whey. Whey was incubated at 10, 20, and 35 °C as per the experimental design.
- The temperature-adjusted whey was exposed to ozone by circulating through an ozone generator for 1 and 2 min as per the experimental design.
- Immediately after the ozone treatment, the treated whey was incubated at the same temperature and evaluated for bleaching efficiency at 5, 10, 20, and 30 min. At the end of incubation, the treated whey was heated to 55°C to remove residual ozone.

Measuring Residual Annatto in Water and Whey

- Residual Annatto was measured using
 - Colorimeter (Miniscan XE, HunterLab, Reston, VA).
 - Absorbance at 458 nm using a spectrophotometer (Metash Inc, Shanghai, China).
- Analysis was carried out in duplicate.

Results and Discussion

Model Annatto Solution

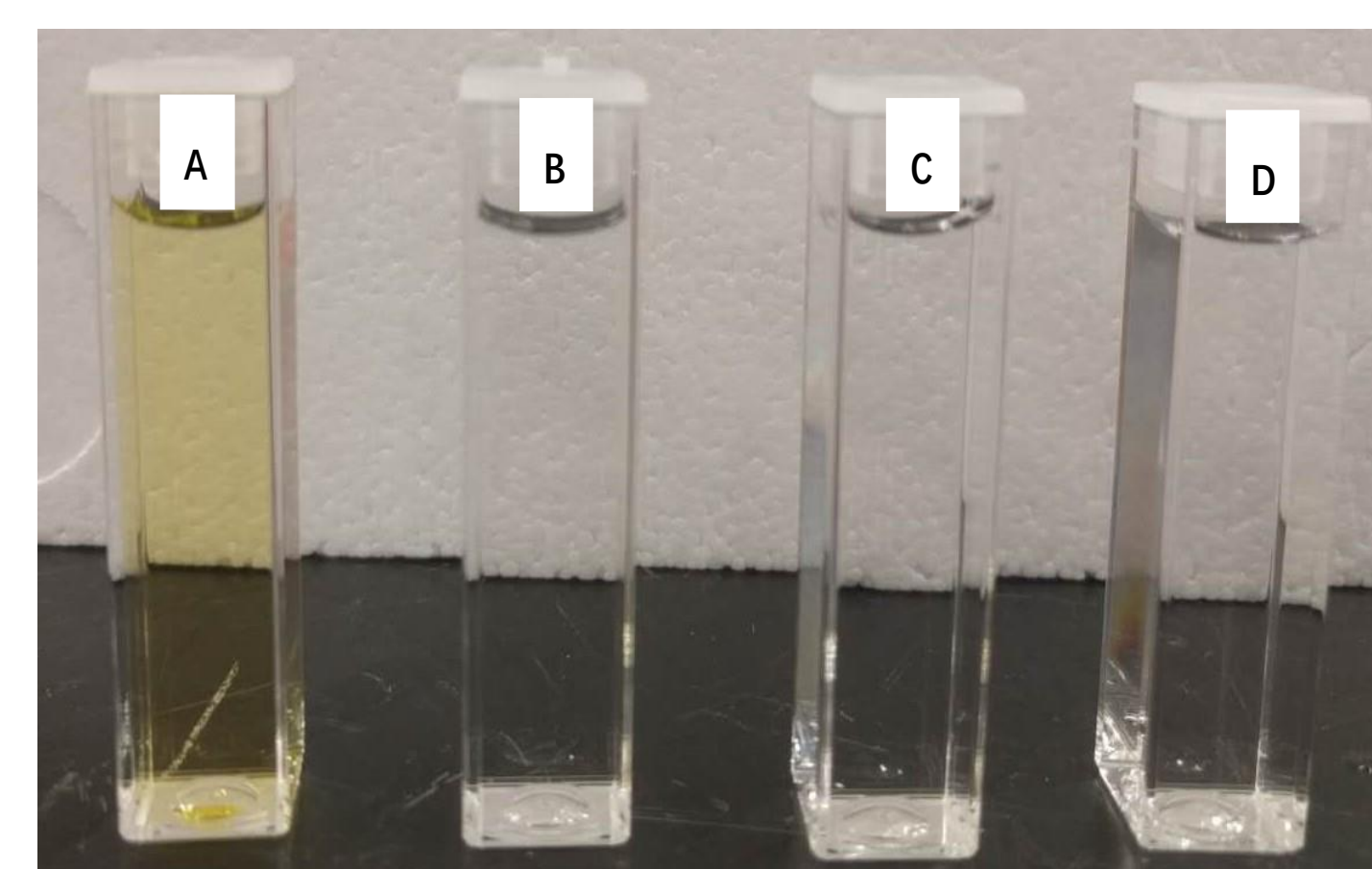


Figure 3. Photograph showing (A) untreated annatto solution, exposure to ozone at (B)10°C, (C) 20°C, and (D) 35 °C.

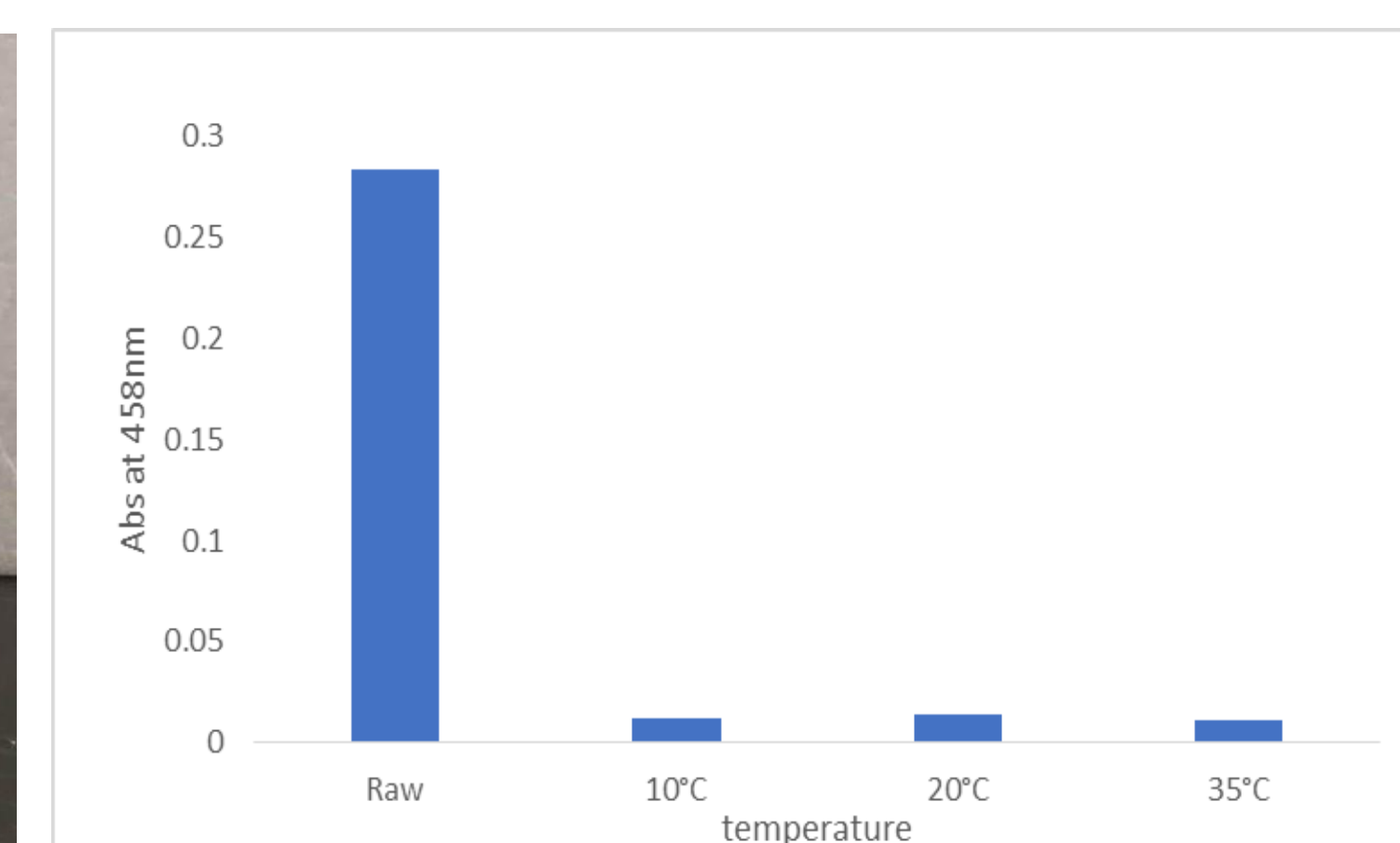


Figure 2. Change in absorbance (458 nm) of annatto model solution before and after ozone exposure at 10, 20, and 35°C.

- Ozone had strong bleaching effect on annatto in model solutions (Figure 3).
- Treatment temperature did not affect the bleaching efficiency.
- Bleaching efficiency was 96, 95, and 96% at 10, 20, and 35°C, respectively.

Reconstituted Cheese Whey

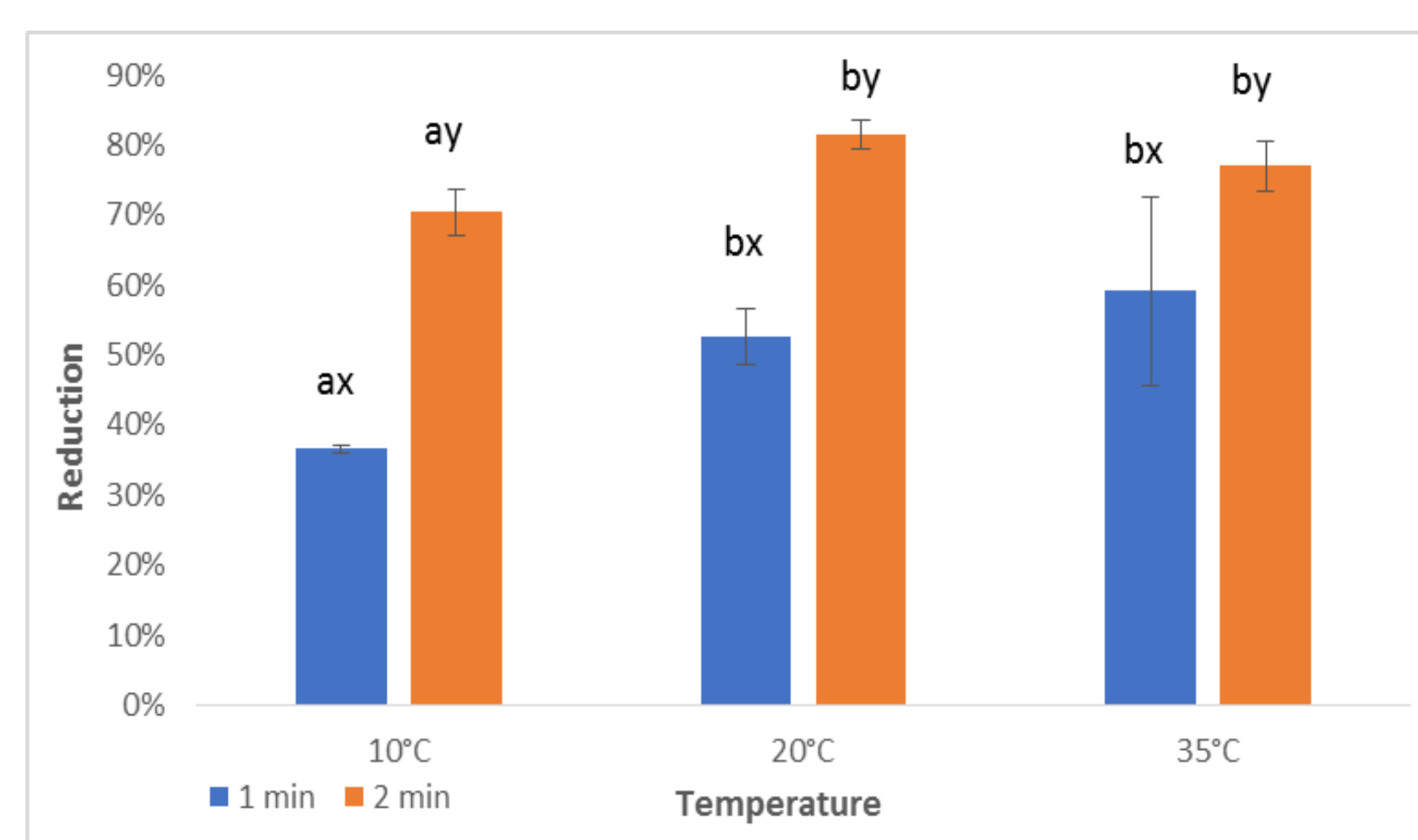


Figure 3. Percent reduction in annatto after 1 and 2 min of ozone exposure at 10, 20, and 35 °C. The error bar represents the standard deviation from duplicate measurements.

- Time and temperature of ozone exposure significantly affected annatto reduction ($P < 0.05$).
- Increase in whey temperature from 10 and 20 °C significantly improved the annatto reduction ($P < 0.05$). However, further increase in temperature to 35°C did not significantly impact on annatto color reduction.
- The optimal ozone bleaching condition to achieve 80% annatto reduction was found at 20°C for 2 min recirculation (Figure 5).

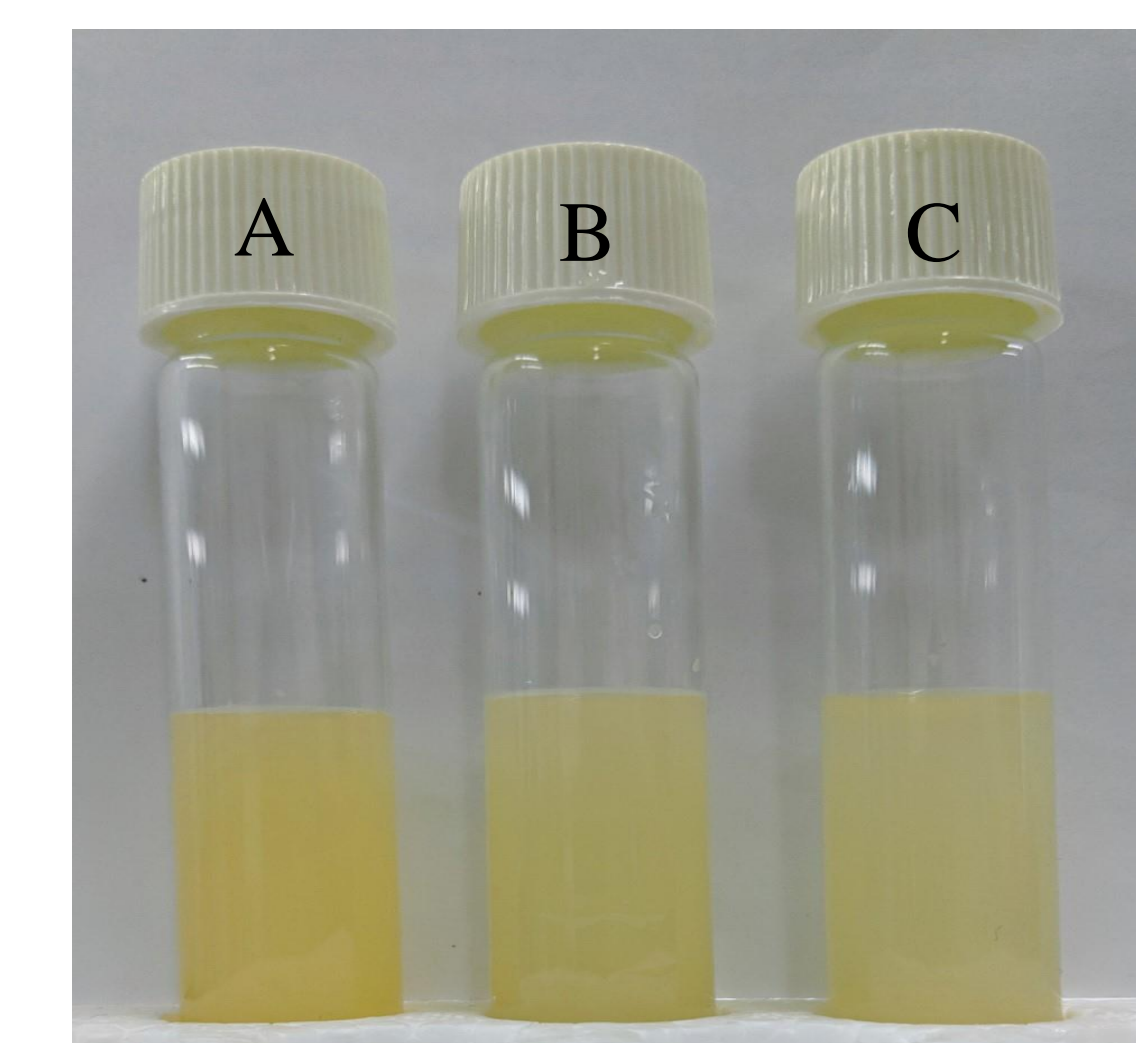


Figure 5. Photograph showing (A) untreated annatto whey solution, exposure to ozone after (B) 1 and (C) 2 min at 20°C.

Table 1. Change in color of whey after 1 and 2 min ozone exposure at 10, 20, and 35°C. L*(white to black),a*(red to green),b*(yellow to blue)

	L*	a*	b*
10°C-1 min	57.7	-0.3	8.1
10°C-2 min	58.3	-0.6	5.8
20°C-1 min	58.3	-0.3	7.7
20°C-2 min	58.2	-0.5	6.2
35°C-1 min	58.4	-0.4	6.6
35°C-2 min	58.2	-0.5	6.7

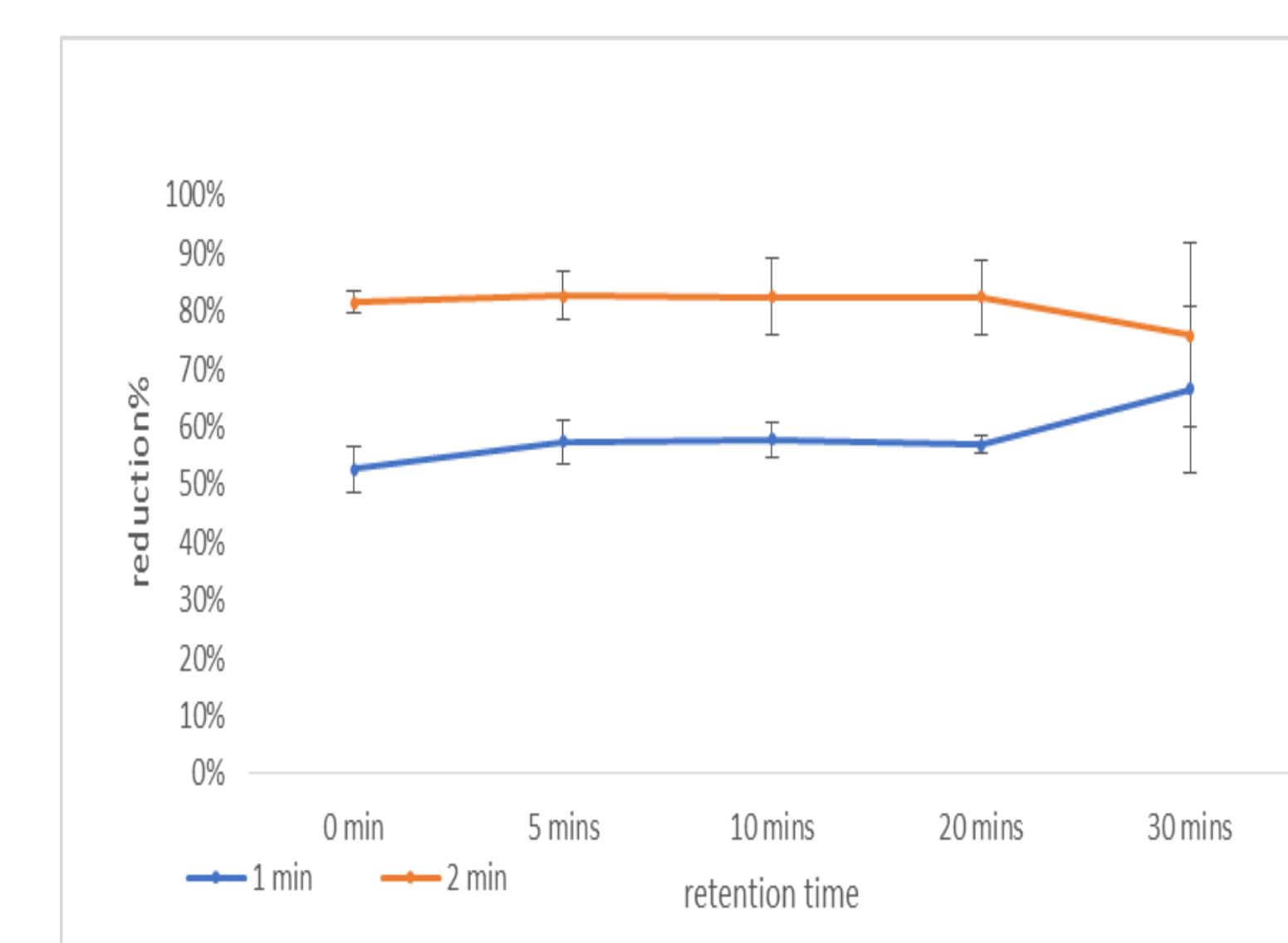


Figure 5. Percent of annatto reduction change after 1 and 2 min of ozone exposure at 20 °C after 0, 5, 10, 20, and 30 min. The error bar represents the standard deviation from duplicate measurements.

- Holding time had no effect on annatto reduction at 10, 20, and 35°C.

Conclusion

- Ozone has shown excellent bleaching effect to remove 95% of annatto in model solution.
- Increase of treatment time and temperature significantly improved annatto reduction in reconstituted cheese whey.
- Holding time had no effect on ozone bleaching efficiency

References

- Kang, E. J., Campbell, R. E., Bastian, E., & Drake, M. A. (2010). Invited review: Annatto usage and bleaching in dairy foods. *Journal of dairy science*, 93(9), 3891-3901.
- Kang, E. J., Smith, T. J., & Drake, M. A. (2012). Alternative bleaching methods for Cheddar cheese whey. *Journal of food science*, 77(7).