Ozone technology for sanitization and product quality in the dairy supply chain

Vanessa Eramo (vanessa.eramo@unitus.it)

Department for Innovation in Biological, Agro-food and Forest systems, University of Tuscia, Viterbo, Italy

Tutor: Prof. Rinaldo Botondi

The first activities of the PhD thesis project are described belove. The application of low levels of gaseous ozone during Toma Piemontese PDO cheese ripening to control microbial and fungal growth on the crust area was tested. After ozone treatments, cheese spoilage was monitored for 1 month. Every 7 days of ripening, microbiological and technological analyses, and consumer tests on cheese with a minimum of 40 days of ripening, were carried out to evaluate the product quality. Results showed that treatments with low levels of gaseous ozone can significantly affect spoilage microflora, without altering the overall cheese quality.

La tecnologia dell’ozono per la sanitizzazione e la qualità di prodotto nella filiera di produzione casearia

Di seguito sono descritte le prime attività del progetto di tesi di dottorato. È stata testata l'applicazione di bassi livelli di ozono gassoso durante la stagionatura del formaggio Toma Piemontese DOP per controllare la crescita microbica e fungina nell'area della crosta. Dopo i trattamenti con ozono, la crescita microbica e fungina sul formaggio è stata monitorata per 1 mese. Ogni 7 giorni di stagionatura, sono state effettuate analisi microbiologiche e tecnologiche e condotti test sui consumatori su formaggi con minimo 40 giorni di maturazione per valutare la qualità del prodotto. I risultati hanno mostrato che i trattamenti con bassi livelli di ozono gassoso possono influenzare significativamente la crescita della microflora alterante, senza compromettere la qualità globale del formaggio.

**Key words**: Cheese ripening, ozone technology, product quality, cheese storage, food spoilage, sensory analysis.

# **1. Introduction**

In accordance with the PhD thesis project previously described (Rolle *et al.*, 2022), this poster reports the main results of the first activities concerning:

(A1) the determination of the optimal doses of ozone, contact times and other treatment variables;

(A3) the analysis of the product quality;

(A4) the study of shelf life.

# **2. Materials and Methods**

The batch test involved a control (CTL) and two different samples: gaseous ozone at 400 ppb (0.856 mg m-3), called OZ 400 A, with treatment every other day (8h – overnight) for the entire period of cheese ripening and gaseous ozone at 300 ppb (0.642 mg m-3), called OZ 300 C, all nights (8 h per day) until the end of ripening. Eight cheeses per type of sample were placed in three different rooms (8.0 m3 each), one with normal atmospheric air for the CTL sample, and the other two rooms connected to the ozone generator (C32-AG; Industrie De Nora Spa, Milan, Italy) via silicone tubes. Rooms were set at 8°C, with 85% relative humidity (RH) (same parameters used by the producer). After 60 days of aging (of which 25 days of cheese ripening by the manufacturer), all samples were stored in the same room (8.0 m3) set at 8°C for a month to monitor the spoilage microflora growth of cheeses not sold immediately. For weight loss, three cheese samples per treatment were weighed at each sampling time with a technical balance (Adam Equipment Co., Ltd., Milton Keynes). Cheese firmness was evaluated with cylindrical samples by compression test with the Instron Universal Testing Machine (mod. 4301; Instron Inc., Canton, MS, USA) and expressed in N mm-1. The Instron machine was also used to evaluate the cheese chewiness (N) with cube-shaped samples by double compression cycle (Texture Profile Analysis–TPA) (AGRIS). Color evaluation was carried out using a Minolta colorimeter (Minolta C2500; Konica Minolta, Ramsey, NY, USA): chromaticity values L\* (lightness), a\* (green to red), and b\* (blue to yellow) were determined, and color difference CIE 1976 - ΔE\* = (ΔL\*2 + Δa\*2 + Δb\*2) (1/2) - was calculated. For microbiological analysis, three sub-samples were taken from each cheese wheel per type of sample (2.35 cm2) with a 1 cm diameter round, selecting only the rind. The preparation of samples was made according to ISO 6611 (ISO 6611:2004). For the determination of Total Microbial Count (TMC) and Total Fungal Count (TFC), YEA (Yeast Extract Agar) and PDA (Potato Dextrose Agar) were used, respectively. Plates were incubated at 25 °C for 48h. Data were expressed as the number of colony-forming units (log CFU cm−2 of cheese rind). Liking tests were conducted according to ISO 4121 (ISO 4121:2003). Product acceptability for "appearance", "smell", "taste", "aroma", "texture" and "overall acceptability" on a scale from 1 (extremely bad) to 9 (extremely good) was tested by ten people for each session, who was asked to also indicate flavors perceived on the palate. Data were expressed as the means ± standard error (SE). Analysis of variance (ANOVA) and Tukey's test at the 5% level were carried out, using DSAASTAT tool in EXCEL®.

# **3. Results and Discussion**

## **3.1 Determination of the ideal parameters for batch test**

## Optimal doses of ozone, contact times, and other treatment variables were found after carrying out a preliminary test on Toma Piemontese PDO cheese.

## **3.2 Determination of the effects of gaseous ozone treatments on spoilage microflora and evaluation of qualitative parameters during cheese ripening**

All samples gradually lose weight. At the end of ripening, 400 A samples show a lower water loss than CTL (significant difference). For color variation, no significant differences were recorded at any single time between samples. At the end of ripening, all samples appear to have a perceptible color variation compared to the beginning of the batch test (0 days). Firmness data show that treated samples appear to be less consistent than the control (significant difference) at the end of the batch test, probably due to less water loss. However, chewiness analysis shows no significant differences between samples at each sampling time. For TMC, in the long term, ozone-treated samples managed to keep the microbial load at the beginning of the ripening almost stable (no significant difference), compared to that of the CTL sample which, also, significantly grows at the end of ripening by about 0.8 log and 0.9 log compared to the OZ 400 A and OZ 300 C samples. For TFC, treated samples show a significant reduction in the microbial load at the end of ripening of about 1.5 log and 1.4 log for the OZ 400 A and OZ 300 C samples than CTL, respectively. Our results are in agreement with the previous study on pecorino cheese (Grasso *et al.*, 2022). Sensory evaluation shows no significant difference at each session time, except for the appearance score at the end of ripening between CTL and OZ 300 C samples (7,4 vs 6) and for aroma, texture and overall acceptability between the two treated samples which appear to be no different from CTL sample (Table 1), with characteristic nuances of milk and fresh butter perceived more frequently in the treated ones.

***Table 1*** *Consumer test (mean values ± SE) for the control and the ozone-treated samples (400 and 300 ppb) at 35 days of ripening for Toma Piemontese PDO cheese samples.*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Days** | **Thesis** | **Appearance** | **Smell** | **Taste** | **Aroma** | **Texture** | **Overall acceptability** |
| 35 | CTL | 7.4 | ± | 0.3 a | 6.4 | ± | 0.2 ns | 6.5 | ± | 0.3 ns | 6.4 | ± | 0.3 ab | 7.2 | ± | 0.3 ab | 7.1 | ± | 0.2 ab |
| OZ 400 A | 6.9 | ± | 0.2 ab | 6.9 | ± | 0.5 ns | 7.3 | ± | 0.4 ns | 7.4 | ± | 0.3 a | 7.4 | ± | 0.4 a | 7.5 | ± | 0.3 a |
| OZ 300 C | 6 | ± | 0.5 b | 6 | ± | 0.4 ns | 6.4 | ± | 0.3 ns | 6.3 | ± | 0.3 b | 5.6 | ± | 0.6 b | 6.4 | ± | 0.3 b |

*Note: Different lowercase letters for the same column indicate significant differences between cheeses with different treatments (P < 0.05).*

## **3.3 Evaluation of microbiological and overall quality during cheese storage after ozone treatments**

After the batch test, untreated and treated samples were stored in a room (normal air) for 1 month. Weight loss significantly increases over time for all samples. Also for color variation and firmness no differences were found between samples at the end of storage. For chewiness data, comparing the start and the end of storage for each type of sample, only the CTL sample significantly reduces its chewiness value. TMC decreases over time for all samples, with a significantly lower value for ozone-treated samples. Instead, TFC increases over time for all samples, but data show a significant difference in value from the CTL sample of about 1 log lower for OZ 400 A and 300 C samples. Cheese samples were considered non-different (no significance) by tasters. Typical nuances of milk and fresh butter were confirmed at the end of the storage period. The evaluation of the possible formation of peroxides and other impacts on the nutritional quality of the cheese will be carried out. The first experimental results are promising, so the original PhD thesis project can proceed without any substantial change.

# **4. References**

AGRIS Sardegna – Sardegna Ricerche, Progetto Contaminazioni, Relazione intermedia – Linea 3. Available at: http://www.sardegnaricerche.it/index.php?xsl=370&s=311546&v=2&c=15068&nc=1&sc= (Accessed: 06 June 2023).

Grasso C, Eramo V, Lembo M, Forniti R, Carboni C, & Botondi R (2022) Effects of gaseous ozone treatment on the mite pest control and qualitative properties during ripening storage of pecorino cheese. *Journal of the Science of Food and Agriculture* **103(4)**: 2124-2133.

ISO 4121:2003 Sensory analysis — Guidelines for the use of quantitative response scales. Available at: https://www.iso.org/standard/33817.html (Accessed: 06 June 2023).

ISO 6611:2004 (IDF 94). Milk and Milk Products – Enumeration of Colony-Forming Units of Yeasts and/or Moulds – Colony-Count Technique at 25 Degrees C. Available at: https://www.iso.org/standard/40473.html (Accessed: 06 June 2023).

Rolle L G C, Alessandria V, Bertolino M, Botta C, Cardenia V, Cocolin L, ... & Zuccolo E (2022) Ozone technology for sanitization and product quality in the dairy supply chain. In Proceedings of the 26th Workshop on the *Developments in the Italian PhD Research on Food Science, Technology and Biotechnology*, Asti (Italy), 19-21 September, 2022, pp. 81-82.