Food Microbiology 66 (2017) 190–198

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Efficacy of gaseous ozone to counteract postharvest table grape sour rot

L. Pinto, L. Caputo, L. Quintieri, S. de Candia, F. Baruzzi*

Institute of Sciences of Food Production, National Research Council of Italy, Via G. Amendola 122/O, 70126 Bari, Italy

ARTICLE INFO

Article history:
Received 28 December 2016
Received in revised form
28 April 2017
Accepted 1 May 2017
Available online 2 May 2017

Keywords:
Grape decay
Microbial control
Shelf-life
Ethanol
Acetic acid

ABSTRACT

This work aims at studying the efficacy of low doses of gaseous ozone in postharvest control of the table grape sour rot, a disease generally attributed to a consortium of non-*Saccharomyces* yeasts (NSY) and acetic acid bacteria (AAB). Sour rot incidence of wounded berries, inoculated with 8 NSYstrains, or 7 AAB, or 56 yeast-bacterium associations, was monitored at 25 °C up to six days. Sour rot incidence in wounded berries inoculated with yeast-bacterium associations resulted higher than in berries inoculated with one single NSY or AAB strain. Among all NSY-AAB associations, the yeast-bacterium association composed of *Candida zemplinina* CBS 9494 (Cz) and *Acetobacter syzygii* LMG 21419 (As) showed the highest prevalence of sour rot; thus, after preliminary *in vitro* assays, this simplified As-Cz microbial consortium was inoculated in wounded berries that were stored at 4 °C for ten days under ozone (2.14 mg m^{-3}) or in air. At the end of cold storage, no berries showed sour-rot symptoms although ozonation mainly affected As viable cell count. After additional 12 days at 25 °C, the sour rot index of inoculated As-Cz berries previously cold-stored under ozone or in air accounted for 22.6 ± 3.7% and 66.7 ± 4.5%, respectively. Molecular analyses of dominant AAB and NSY populations of both sound and rotten berries during post-refrigeration period revealed the appearance of new strains mainly belonging to *Gluconobacter albidus* and *Hanseniaspora uvarum* species, respectively. Cold ozonation resulted an effective approach to extend the shelf-life of table grapes also after cold storage.

1. Introduction

Italy is one of the leading grape (*Vitis vinifera* L.) producers and consumers, and is also among the most important trade markets for this fruit in the world (USDA-FAS, 2014, 2015). The marketability of this product is strongly affected by postharvest microbial tissue decay resulting in undesirable physiological and chemico-physical changes and shelf-life reduction.

Sour rot is a grape disease (Guerzoni and Marchetti, 1987; Nigro et al., 2006) caused by opportunistic non-*Saccharomyces* yeasts (NSY) and acetic acid bacteria (AAB) (Barata et al., 2008, 2012a, b, c; Loureiro et al., 2012) mainly affecting late ripening cultivars under postharvest conditions (Hashim-Buckey et al., 2008; Puelles Tamsec and Sepulveda Ramirez, 2012). Rotten bunches show a strong and pungent odor of vinegar as the result of the production of microbial metabolites such as acetic acid, glycerol, ethyl acetate, ethanol, galacturonic acid, acetaldehyde and gluconic acid (Marchetti et al., 1984; Zoecklein et al., 2001). In particular, acetic and gluconic acid are usually considered chemical markers of sour rot development (Barata et al., 2012c).

The NSY-AAB consortium can be composed of different microbial species such as *Acetobacter malorum*, *A. cibinongensis*, *Gluconobacter oxydans*, *Pichia terricola*, *Hanseniaspora uvarum*, *Candida zemplinina*, and *Zygoascus hellenicus* as recently reported by Barata et al. (2012c).

The severity of grape sour rot is strongly promoted by the action of *Drosophila* spp. flies, attracted by volatile organic compounds released from sour rotten berries, that contribute to inoculate and disperse sour rot related microorganisms (Barata et al., 2012c).

To date, the control of microbial spoilage of table grapes under postharvest conditions is almost exclusively performed by using sulphur dioxide fumigation or applying SO_2-releasing pads (Lichter et al., 2006). However, excess of sulphur dioxide induces fruit and stem bleaching (Snowdon, 1990; Crisosto and Mitchell, 2002) and may result in sulphite accumulation on table grape; thus, the content of sulphur dioxide residuals is internationally regulated (EPA, 1989; EU directive 2006/52/CE). Therefore, alternative tools

* Corresponding author.
E-mail address: federico.baruzzi@ispa.cnr.it (F. Baruzzi).

http://dx.doi.org/10.1016/j.fm.2017.05.001
0740-0020/

for controlling postharvest decays of table grapes such as natural antimicrobials, decontaminating agents and physical methods have been recently proposed (Romanazzi et al., 2012). Among alternative postharvest decay control means (Feliziani et al., 2016; Pinto et al., 2015, 2016), ozone is increasingly gaining success owing to its broad antimicrobial spectrum and the lack of harmful residues, so as to be a compound generally recognised as safe (GRAS; Graham et al., 1997; FDA, 2001). High ozone concentrations were effective in controlling the germination of *Botrytis cinerea* conidia, reducing grey mould development during grape cold storage (Gabler et al., 2010; Ozkan et al., 2011). Low doses of gaseous ozone (0.21–0.31 mg m^{-3}) were also successfully used to limit the spread of grey mould on grape during postharvest refrigerated period (Feliziani et al., 2014). The antimicrobial action of ozone is generally attributed to its oxidant activity, also sustained by the release of reactive oxygen species, causing microbial cell membrane disruption, enzyme inactivation and nucleic acids damages (Hinze et al., 1987; Khadre et al., 2001).

Despite these results, to the best of our knowledge, there are no studies reporting antimicrobial efficacy of ozone treatments against the yeast-bacterium consortium responsible for grape sour rot. Thus, in this work one single yeast-bacterium consortium responsible for grape sour rot was selected. Then, ozone treatments were carried out to reduce viable load of selected microbial consortium when inoculated in healthy berries to counteract sour rot development during cold storage and post-refrigeration period.

2. Materials and methods

The flow chart, describing the experiments carried out in this work, is depicted in Fig. 1.

2.1. Yeast and bacteria strains and culture conditions

In this work, eight yeast strains (*Candida vanderwaltii* CBS 5524, *C. zemplinina* CBS 9494; *Hanseniaspora guilliermondii* DSM 3432,

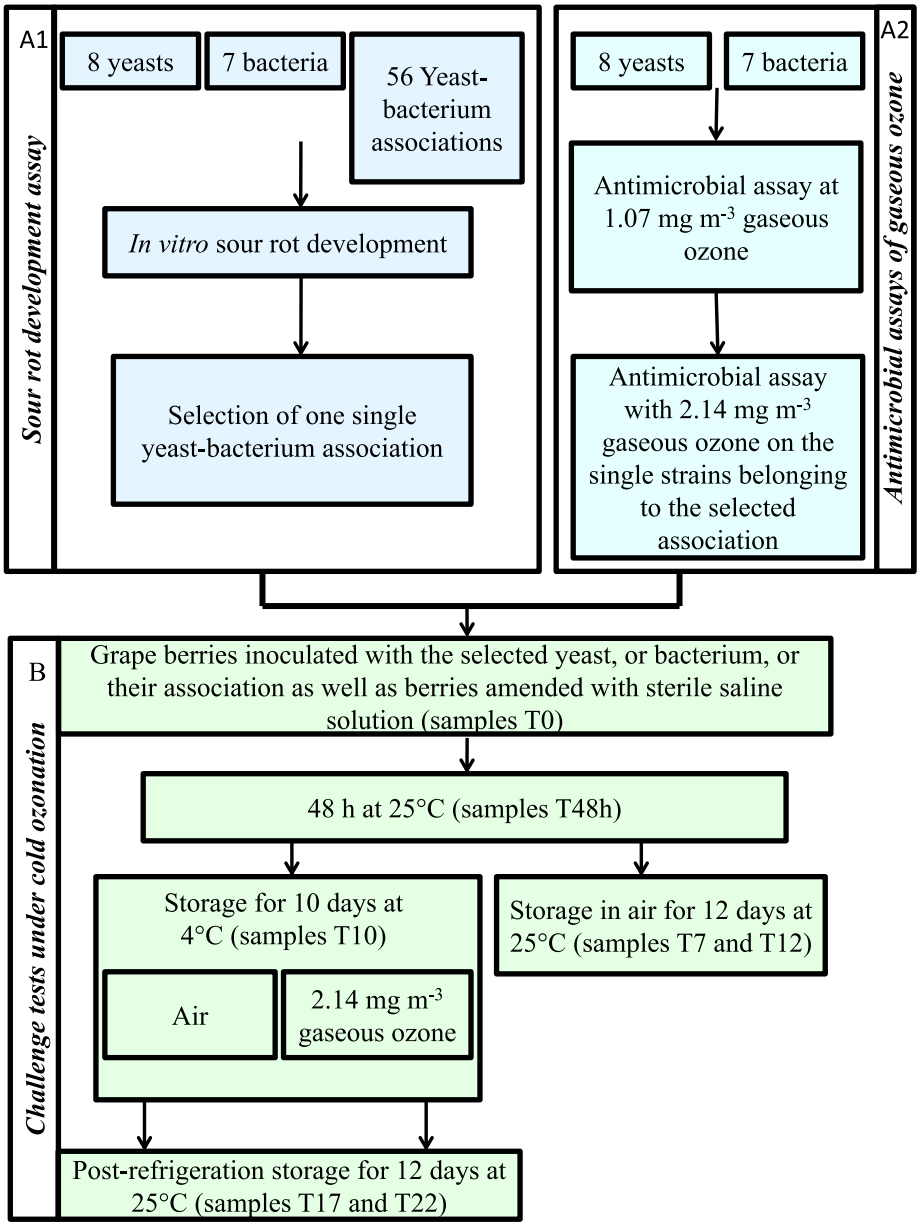

Fig. 1. Flow chart of the experiments carried out in this work.