POTENTIAL USE OF KOMBUCHA CRUDE EXTRACT IN POSTHARVEST GRAPE MOULDS CONTROL

Bogdan MATEI, Florentina MATEI, Camelia DIGUȚĂ, Ovidiu POPA

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
Faculty of Biotechnologies, 59 Mărăștiti Blvd., District 1, Bucharest, Romania

Corresponding author email: florentina.matei@biotehnologii.usamv.ro

Abstract

Postharvest diseases of fruits and grapes are caused by fungi and bacteria and the losses in this step increase several folds than in the field. In the case of the grapes, most damages are due to the presence of filamentous fungi belonging to species like Botrytis sp., Penicillium sp. or Aspergillus sp. Several non-chemical treatments have been proposed for fungal decay control, including acetic acid. Our experiments have targeted the potential inhibitory effect of different tea sourced Kombucha crude extracts on the most common moulds of the grapes in pre and postharvest steps. Kombucha is known mainly as a consortium (SCOBY - symbiotic acetic/lactic bacteria and yeast). Because of the presence of acetic bacteria the final content in acetic acid of Kombucha suspensions vary between 8.5 and 17 g/l. Kombucha tea suspension has been prepared starting from three different source of tea plants, respectively green tea, green tea with Melissa officinalis L. and oolong tea. The most significant inhibition has been registered in the case of Botrytis cinerea (38 -55%), less significant on Penicillium expansum (4-8%) and not significant on Aspergillus flavus and Aspergillus carbonarius. It is proposed further to investigate the inhibition of Kombucha extracts, in vivo, on artificially infected grape berries with Botrytis cinerea and to validate the in vitro results.

Key words: grape, postharvest, mould, Kombucha crude extract.

INTRODUCTION

Postharvest diseases of fruits and grapes are caused by fungi and bacteria and the losses in this step increase several folds than in the field (Coates et al., 1997; Scholberg, 2009; Meneses et al., 2014). In the case of the grapes, most damages are done by the presence of filamentous fungi belonging to species like *Botrytis* sp*., Penicillium* sp., *Aspergillus* sp. or *Rhizopus* sp. (Scholberg, 2009). Postharvest diseases of the grapes are controlled by the application of sulphur dioxide, either by weekly fumigation in storage rooms or by packing grapes in polyethylene-lined boxes with sulphur dioxide generator pads (Hafez, 2008).

Several non-chemical treatments have been proposed for fungal decay control. Although these approaches have been shown to reduce postharvest rots of fruit or grapes each has limitations that can affect their commercial applicability (Tripathi et al., 2004). The exploitation of natural products to control fruits postharvest diseases and to prolong their storage life has received special attention in the last decade. Biologically active natural products such as flavour compounds, acetic acid, propolis, chitosan or plant extracts, have the potential to replace synthetic fungicides in postharvest (Tripathi et al., 2004; Xu et al., 2007; Scholberg, 2009; Meneses et al., 2014).

Acetic acid can be employed with no restriction as preservative and represents a possible substitute to sulphur dioxide. The use of acetic acid in postharvest treatments have been reported for citrus fruits (Venditti et al., 2009), stone or berry fruits (Scholberg, 2009; Radi et al, 2010), as well as for table grapes (HAfez, 2008; Vendetti et al., 2012).

Kombucha is known mainly as a consortium (SCOBY - symbiotic acetic/lactic bacteria and yeast) used to prepare from green tea a slightly acidulous beverage with several reported positive health effects, but these effects still have to be scientifically demonstrated. In the recent years, the interest in cultivating Kombucha consortium is linked to the production of bacterial cellulose of very diverse use (medical, textile, industrial, etc) because of its versatile structure; in this process are

involved acetic bacteria from the SCOBY, like by *Gluconacetobacter* sp. or *Acetobacter* sp. (Dutta et al, 2007; Nguyen et al. 2008). Acetic acid concentration in Kombucha range from 8.5 to 17-18 g/L if the tea is allowed to ferment more than 21 days to 30 days (Jayabalan et al., 2014; Chakravorty et al., 2016). In the case of cellulose production, the suspension, otherwise used as beverage, is a residue and its possible uses are under screening. Because of the acetic acid levels, may be used also as treatment in postharvest fruits or grapes.

In this context, the main aim of our research it was to test the effects of different tea sourced Kombucha extracts on the growth of the most common moulds which lead to the decay of the grapes in the pre and postharvest steps.

MATERIALS AND METHODS

Kombucha filtrate preparation. The Kombucha SCOBY consortium has been procured from Medica Farmimpex S.R.L., Otopeni, Romania through the kindly help of Dr. Ionut Moraru.

Kombucha tea suspension has been prepared starting from three different source of tea plants, respectively green tea *Camellia sinensis*, green tea with *Melissa officinalis* L. and oolong (dark green *Camellia sinensis*) tea. The sweetened tea (90 g sucrose/L and 10 g/L plant) has been fermented with Kombucha SCOBY during 18 days at 28°C. After the fermentation, the suspension has been centrifuged at 1000 rpm during 10 minutes to separate the debris, followed by sterile filtration through Millipore membrane of 0.2 µm pores size.

Measurement of pH and acetic acid content. After sterilisation, the pH has been determined with an electronically pH meter and the crude extract (100%) has been used in the antagonistic tests. The acetic acid was determined in Kombucha crude extract by high performance liquid chromatography (HPLC). The mobile phase was 20 mM potassium dihydrogen phosphate, pH 2.4 with a flow rate of 1.0 mL/min and running time of 40 min. The column temperature was maintained at 28°C and the detection was carried out at 220 nm by comparing the retention time of the standard compounds. The concentration of acetic acid was quantified from standard curves.

Fungal pathogens. Four different moulds have been taken into account in the experiments, being known as most common contaminants of the grapes, respectively *B. cinerea* MI-Aligote H, *P. expansum* MI-BB H, *A. flavus* MI-35 and *A. carbonarius* MI-32. All moulds strains have been isolated from grapes and identified in the laboratory of Applied Microbiology from UASMV Bucharest Faculty of Biotechnologies, Romania. Cultures are maintained on PDA (potato-dextrose-agar) medium at 4°C.

Inoculum preparation. Targeted moulds have been cultivated on PDA plates during 14 days at 20°C in the case of *B. cinerea* and at 28°C for the other moulds. Spore suspension has been prepared by flooding the 2-weeks plates with a small volume of sterile distilled water containing 0.05% (v/v) Tween-80, and spores were removed by gently scraping with a glass spatulum. By the aid of a hemacytometer has been determined the spore content in the suspension. Further dilutions with sterile water were performed to obtain a spore concentration of 10^6 CFU/mL (Karabulut *et al*., 2005).

Antifungal assay has been performed on synthetic medium on plate. For the *in-vitro* tests MEA (Malt-Extract-Agar) has been employed in Petri dishes. The medium has been flooded with 1 mL of different Kombucha crude extracts (prepared as described above) than, in the centre have been placed 10µl of fungal spore suspension. The cultures were incubated during 7 days at 20°C for *B. cinerea* and 28°C for the other three fungi (*P. expansum*, *A. flavus* and *A. carbonarius*). As control has been used MEA plates inoculated with same fungal spores suspension without Kombucha crude extract. The radial mycelial growth was measured daily, and the percentage of inhibition was calculated on the basis of growth in control plates as follows:

$$\frac{\text{mycelial growth in control} - \text{mycelial growth in Kombucha}}{\text{mycelial growth in control}}$$

The experiment design has taken into account three replicates for each sample.

Statistical analysis

Analysis of variance was performed. To determine differences in radial growth between samples and controls, Duncan's and Tukey's multiple pairwise comparisons tests were applied to the results (*p*-levels at 0.01 and below were considered significant).

RESULTS

The Kombucha crude extract has been prepared as described above; for all Kombucha samples the final pH after 18 days of cultivation has reached values of 2 ± 0.15 which is in agreement with other reports (Jayabalan et al., 2014; Chakravorty et al., 2016). Regarding the acetic acid content, the results were close to the one obtained by Jayabalan et al. (2014), respectively 10 ± 0.5 g/L acetic acid in the crude extract.

In the *in vitro* tests have been taken into account four of the most common moulds which affect the grapes in pre- and postharvest steps, respectively *B. cinerea, P. expansum*, *A. flavus* and *A. carbonarius*.

The radial growth of the moulds have been measured after 7 incubation days at optimal temperature for each mould (table 1).

Table 1 - Growth and inhibition of moulds on synthetic medium in the presence of different Kombucha crude extracts

	Radial growth (mm)			Inhibition %		
	Control	**K1**	**K3**	**K4**	**K1**	**K3**	**K4**
B. cinerea MI-Aligote H	47.2	20.9*	21.6*	29.0*	55.6	54.3	38.6
P. expansum MI-BB H	50.0	46.0*	46.0*	48.0	8.0	8.0	4.0
A. flavus MI-35	48.3	47.0	47.2	48.0	2.7	2.3	0.6
A. carbonarius MI-32	49.1	48.5	48.7	49.0	1.2	0.8	0.2

Values represent means of measurements made on three independent plates per treatment; * $p \leq 0.01$ vs. respective control.
K1 - Kombucha on green tea; K3- Kombucha on green tea with *Melissa officinalis;* K4 Kombucha on oolong tea
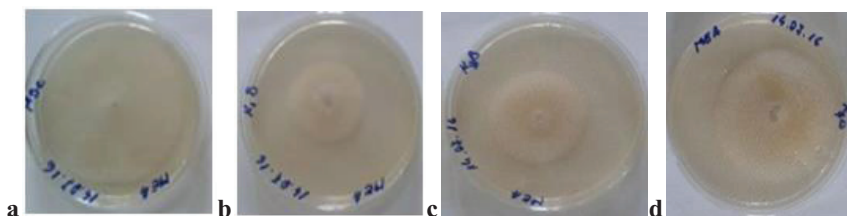

a   **b**   **c**   **d**

Figure 1. On-plate aspects of *Botrytis cinerea* growth's inhibition by different Kombucha crude extracts: **a -** control on MEA; **b -** K1 (Kombucha made of green tea); **c -** K3 (Kombucha made of green tea with *Melissa officinalis);* **d -** K4 (Kombucha made of oolong tea)

The most significant inhibition, statistically assured, has been registered in the case of *B. cinerea*, respectively 54-55% inhibition in the case of green tea Kombucha and 38.6% in the case of oolong Kombucha. Aspects of the mycelium growth inhibition of *B. cinerea* are presented in figure 1.

Positive reports on *B. cinerea* inhibition by the use of acetic acid, mainly as vapours, have been described for different fruits, like strawberries (Hassenberg et al., 2010), kiwifruit (Lagopodi et al., 2008) or grape (Venditti et al., 2008) and our results are in accordance. The reports on using Kombucha extracts for the inhibition of *B. cinerea* are rather limited (Hafez, 2008) and reveal the high inhibitory effect on the spore germination on table grapes (80% to 100%). In this context it has been suggested that Kombucha treatments may be used as an alternative natural solution to replace the pre and postharvest chemical treatments.

Meanwhile, in our tests, less significant has been the inhibition of all the extracts on *P. expansum* growth (4-8%), while in the case of *Aspergillus* sp. the inhibition was not significant. Radi et al. (2010) have reported the inhibition of *P. expansum* by acetic acid on red apples, but only as heated solutions as 50°C. The reports on the effect of *Aspergillus* sp. are rather limited and are linked mainly to food safety and micotoxin production in food commodities; Hassan et al. (2015) reported the inhibition of *A. flavus* of 45.21% for a

concentration of 10 g/L in acetic acid; this data are not in range with our results and further analysis should be performed in this respect.

CONCLUSIONS

The exploitation of natural products to control fruits postharvest diseases and to prolong their storage life has received special attention in the last decade. The use of acetic acid in postharvest treatments have been reported for citrus fruits, stone or berry fruits, as well as for table grapes.

Kombucha suspensions, in crude extracts, as residue when preparing bacterial cellulose, contain 8.5 to 17 g/L acetic acid, depending on the cultivation time.

Our experiments have targeted the potential inhibitory effect of different tea sourced Kombucha crude extracts on the most common moulds of the grapes in pre and postharvest steps. The most significant inhibition has been registered in the case of *B. cinerea* (38 -55%), less significant on *P. expansum* (4-8%) and not significant on *A. flavus* and *A. carbonarius*. While the results regarding the inhibition on *B. cinerea* are in total agreement with other reports, the data on the other moulds are far to be similar. It is proposed further to investigate the inhibition of Kombucha extracts *in vivo* on artificially infected grape berries with *B. cinerea* and to validate the *in vitro* results.

ACKNOWLEDGMENTS

REFERENCES

Chakravorty S., Bhattacharya S., Chatzinotas A., Chakraborty W., Bhattacharya D., Gachhui R. (2016). Kombucha tea fermentation: Microbial and biochemical dynamics. Int. J. of Food Microbiol. 220:63–72.

Coates L, Johnson G. (1997). Chp.33 Postharvest disease of fruit and vegetables, in Plant pathogens and plant diseases, 533-547.

Dutta, D., Gachhui, R., (2007). Nitrogen-fixing and cellulose-producing *Gluconacetobacter kombuchae* sp. nov., isolated from Kombucha tea. Int. J. Syst. Evol. Microbiol. 57:353–357.

Hafez M.A. (2008), Pre-harvest application of Kombucha filtrate to control postharvest bunch rot of table grapes. Annals Of Agric. Sc., Moshtohor,46(2): 33-44.

Hassan R., El-Kadi S., Sand M. (2015). Effect of some organic acids on some fungal growth and their toxins production. Int. J.of Advances in Biology, 2 (1):1-11.

Hassenberg K., Geyer M., Herppich W. B. (2010). Effect of Acetic Acid Vapour on the Natural Microflora and *Botrytis cinerea* of Strawberries. European Journal of Horticultural Science, 75 (4): 141-146.

Jayabalan R., Malbasa R. V., Loncar E.S, Vitas J.S., Sathishkumar M. (2014). A Review on Kombucha Tea—Microbiology, Composition, Fermentation, Beneficial Effects, Toxicity, and Tea Fungus. Comprehensive Reviews in Food Science and Food Safety, 13: 538 -550.

Karabulut, O.A.; Romanazzi, G.; Smilanick, J.L. and Lichter, A. (2005): Postharvest ethanol and potassium sorbate treatments of table grapes to control gray mold. Postharvest Biol.and Technol., 37: 129–134.

Lagopodi, A. L., Cetiz, K., Koukounaras, A. and Sfakiotakis, E. M. (2009), Acetic Acid, Ethanol and Steam Effects on the Growth of *Botrytis cinerea in vitro* and Combination of Steam and Modified Atmosphere Packaging to Control Decay in Kiwifruit. Journal of Phytopathology, 157: 79–84.

Meneses E.A., Durango D.L., , Diego L., García C.M. (2014). Antifungal activity against postharvest fungi by extracts from colombian propolis. *Quim. Nova,* Vol. 32, No. 8, 2011-2017.

Nguyen V.Y., B. Flanagan, M.J. Gidley, G.A. Dykes (2008), Characterizationof cellulose production by a *Gluconacetobacterxylinus s*train from kombucha, Curr. Microbiol. 57:449–453.

Radi M., Jouybari H.A., Mesbahi G., Farahnaky A., Amiri S. (2010). Effect of hot acetic acid solutions on postharvest decay caused by *Penicillium expansum* on Red Delicious apples. Scientia Horticulturae, 126 (4): 421-425.

Sholberg, P. (2009). Control of postharvest decay by fumigation with acetic acid or plant volatile compounds. Fresh Produce 3: 80-86

Tripathi P., Dubey N.K. (2004). Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables. Postharvest Biology and Technology, 32 (3), 235–245.

Venditti T., Angiolino C, Dore A, Molinu MG, Petretto A, D'Hallewin G. (2009). Treatments with acetic acid followed by curing reduce postharvest decay on Citrus fruit. Commun. Agric. Appl. Biol. Sci. 74(3):723-8.

Venditti T, Dore A, Molinu MG, D'Hallewin G. (2012). Effect of acetic acid repeated treatments on post-harvest quality of "Taloppo" table grape. Commun. Agric. Appl. Biol. Sci. 77(3):219-24.

Xu W.T., Huang K.L, Guo F., Qu W., Yang J.J., Liang Z.H., Luo Y.B. (2007). Postharvest grapefruit seed extract and chitosan treatments of table grapes to control *Botrytis cinerea*. Postharvest Biology and Technology, 46 (1): 86–94.