Thermochimica Acta 630 (2016) 31–36

Contents lists available at ScienceDirect

Thermochimica Acta

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

Microcalorimetric monitoring of grape withering

Ksenia Morozova [a], Andrea Romano [a], Francesco Lonardi [c], Roberto Ferrarini [c], Franco Biasioli [b], Matteo Scampicchio [a,*]

[a] Free University of Bozen-Bolzano, Faculty of Science and Technology, piazza Università 1, 39100 Bolzano, Italy
[b] Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach (FEM), Via E. Mach 1, 38010 San Michele all'Adige, Italy
[c] PerfectWine s.r.l., Via della Pieve 70, 37029 S. Floriano, Italy

ARTICLE INFO

Article history:
Received 26 September 2015
Received in revised form 12 January 2016
Accepted 17 January 2016
Available online 22 February 2016

Keywords:
Microcalorimetry
Proton transfer reaction-mass spectrometry
Grapes
Withering

ABSTRACT

This work aimed at monitoring the metabolic activity of grapes during withering by microcalorimetry. Samples of *Corvina* grapes, a cultivar used in the production of *Amarone* wine, were dehydrated for about 120 days at an industrial scale plants (*fruttaia*). Single berries, sampled in the course of the withering process, were closed in ampoules and maintained at constant temperature. As biochemical events (i.e. berry respiration, microbial growth, etc.) are always accompanied by the production of heat (q), the heat-flow (dq/dt) emitted by berries enclosed in the ampoules was used to monitor their metabolic activity during withering, i.e. respiration. For each sampling time, the heat rate production of the berries at 298 K was monitored till a steady state signal was achieved (within 60 h). Such heat flow value was used as marker during the entire withering process (120 days). Its trend allowed to characterize the changes in the metabolic activity of the grape berries along the withering process. To understand the origin of such changes, the emission of volatile organic compounds (VOCs) were also measured by proton transfer mass spectrometry (PTR-MS). The use of microcalorimetry associated with the analysis of specific VOCs fragments offered a valuable information to describe the withering process.

1. Introduction

Grape withering is a technological process occurring during dehydration of grapes and widely used to produce the so-called *passito* wine [1]. Such dehydration process is a source of stress for the grapes that induces significant modifications on their metabolism [2]. The main effects of dehydration are the loss of water. Up to 40% of the initial weight of the grapes can be loss. As a result, the content of total soluble solids and polyphenols increases. Also, a partial loss of water may enhance the activity of cell wall enzymes, that, in turn, increases berry respiration and volatile emission [2–4]. Along the dehydration process, yeasts and other fungi can colonize the berry surface [5]. Also, the reduced water content decreases the water activity of the berries. This selects the type of microorganisms able to growth. Under favorable condition, this growth can provide a positive infection known as *noble rot* [6]. Noble rot occurs when the fungi *Botrytis cinerea* become one of the predominant species [7]. Its growth may ultimately affect the profile in volatile organic compounds of grapes [8–11].

The monitoring of the withering process is of great interest for wine makers. Recently, a visible near-infrared spectroscopy was used for the analysis of chemical change on grapes during withering [12]. The impact of *Botrytis cinerea* on the aroma components of *Amarone* was evaluated by gas chromatography [13]. The analysis of the molecular events supporting withering was explained by amplified fragment length polymorphism-transcriptional profiling [14]. However, these analytical techniques provide only an indirect information about the changes occurring to grapes. As such, their results may reflect side effects (such as acidification sustained by microbial growth) that are in delay respect to the main degradation process [15].

With respect to the aforementioned techniques, microcalorimetry has a greater potential to directly and continuously monitor the kinetics of complex phenomena such as microbial growth or enzymatic activity. First of all, it is a non-destructive technique. Ampoule of different size and materials can be used to contain whole grape berries. Thus, the metabolic events occurring on a berry can be studied directly when the main degradation process appears and without the need of manipulation of the sample (such as crushing, extraction with solvents, etc.).

* Corresponding author at: Free University of Bozen-Bolzano, Faculty of Science and Technology, piazza Università 5, 39100 Bolzano, Italy.
E-mail address: matteo.scampicchio@unibz.it (M. Scampicchio).

http://dx.doi.org/10.1016/j.tca.2016.01.011
0040-6031/

Microcalorimetry measures the heat transferred between the sample contained in an ampoule and its surrounding. As any metabolic event is accompanied by the production of heat, the resulting heat-flow signal can be used to follow the extent of the reaction [16]. Furthermore, when the main exothermic reaction is the respiration of the fruit, then the value of heat-flow can be used to directly measure the rate of respiration of the sample.

Microcalorimetry was used in the past to quantify the growth of molds [17], yeasts such as *Saccharomyces cerevisiae* [18], enzyme activity [19], wounding respiration of vegetables [20–22]. Here, microcalorimetry was used, for the first time, to measure the metabolism of whole berry occurring during withering process.

In this work, we describe the metabolism of *Vitis vinifera cv. Corvina* grapes, one of the main varieties used to produce *Amarone* wine [23,24], during the withering process. From the monitoring of the rate of heat production, we monitored the extent of different metabolic phases occurring to grape berries during the withering process. This, in turn, allowed to identify precisely the changes in the grape berry respiration. The nature of such changes was confirmed with the analysis of volatile organic compounds (VOCs) by proton transfer reaction mass spectrometry (PTR-MS). The evolution of specific volatile fragments during the withering process was used to explain the complex calorimetric signal.

2. Materials and methods

2.1. Grape samples

Vitis vinifera, cultivar *Corvina* were grown in vineyards from Verona-Valpolicella region (latitude 45°38′ N and longitude 10°87′ E), North-East Italy. Grapes were harvested on the 25th of September 2014 stored in *plateaux* in industrial drying plants and left drying until the 15th of January 2015. The drying plant was designed to control manually the temperature and humidity through a combination of natural and ventilation system as well as the presence of dehumidifiers. Temperature and relative humidity were recorded during the whole period of dehydration. Initial drying conditions (September 2014) were 14–23 °C (min–max) and 40–85% (min–max) relative humidity (the high variability of the humidity values are due to the exceptionally rainy season in 2014). Final conditions (January 2015) were 4–10 °C and 54–77.5% relative humidity (minimum and maximum values are given).

About 1 kg of *Corvina* grapes were received and immediately analyzed. Samples were monitored during the 1st week after harvest and at weeks no. 2nd, 4th, 6th, 8th, 14th and 16th.

At harvest, *Corvina* grapes were placed on trays, in a single layer. The trays were stacked in rows several meters high, in well-ventilated storage areas (*fruttaia*). Initial soluble sugars content of the grapes were 23.8 ± 1.2 °Brix. During about 120 days of withering, the weight loss was about 40%, and soluble solids increased up to 45.1 ± 0.5 °Brix.

Vitis vinfera cv. Corvina berries were sampled from the *fruttaia* in different moment of the withering process. The berry (average masses from 0.98 to 0.46 g to depending on the withering stage) was individually placed inside closed ampoules.

2.2. Microcalorimetry

A microcalorimetry thermostat (Thermal Activity Monitor, Model 395 TAM III, TA Instruments) equipped with 12 stainless steel ampoules was used to measure the heat flow signal. Each channel of the instrument is a twin calorimeter where the two units are positioned above each other. In isothermal mode, the oil in the thermostat was maintained at the set temperature 25 °C with a precision of ±0.00005 °C (manufacturer's data). The microcalorimeters had installed metal reference specimens having a heat capacity approximately equal to that of a measurement ampoule. Ampoules (TA Instruments) were of stainless steel, with height of 50 mm and diameter of 13 mm. Internal volume of the ampoules is about 5 cm^3. The ampoule is resistant to the pressure up to 7 bar. Any heat generated or absorbed was measured continuously over time. Each channel was calibrated prior to measurement using gain calibration procedure with electric impulses supplied by the instrument manufacturer. The operator was wearing vinyl gloves to prevent the wetting of the ampoules. The analysis of a single berry was repeated six times. In details, six berries were randomly picked from different parts of the grape so that the data took into account the variability in weights and sizes. With the samples obtained from the first week of withering, some berries were excluded due to their bigger size. This was necessary to avoid any damage of the surface of the berry during the insertion into the ampoule. Single berries with pedicel were cut with scissors to avoid damages. The berry was placed inside the ampoule and sealed tightly with a cap. Ampoules were sequentially introduced into the instrument previously stabilized at 25 °C. Ampoules were first lowered into the thermal equilibration position (15 min) before being lowered into the measurement position. The heat-flow of each individual berry was continuously monitored for 60 h. The heat flow value used as index of the metabolic activity was arbitrarily taken at 60 h.

2.3. VOCs analysis

Volatile profile measurements were performed with a high-sensitivity proton transfer reaction mass spectrometer – PTR-QMS (Ionicon Analytik GmbH, Innsbruck, Austria). Instrumental parameters were the following: drift voltage 550 V, drift pressure 2.0 mbar and drift temperature 80 °C, leading to an E/N value of 140 Td (1 Td = 10^{-17} cm^2/V s). Sampling was performed at a flow rate of 80 ml/min, by means of a heated (80 °C) PEEK transfer line. The instrument operated in the "scan" mode in the range 20–200 Thompson (Th), with 100-ms dwell time for each mass channel. Each measurement consisted of five spectral scans and each analysis was repeated six times, with the exception of the sampling at week 1, where the measurement was performed in triplicate. For every day of measurement, three blank (empty) ampoules were also analyzed. Clean air was supplied by means of a needle connected to an activated charcoal filter. The signal resulting from each nominal mass channel was blank subtracted. All signals with maximum intensity exceeding 1 ppbV were selected for further analysis. After ruling out signals related to water (m/z 21 and 37) interferences (m/z 30 and 32), or redundant data (e.g. water clusters and minor isotopologues) 24 masses were obtained. VOC data after subtraction of blank (empty ampoule with air of the laboratory) is expressed in concentration as parts per billion in volume (ppbV). To determine the concentrations, the formulas described by Lindinger and Jordan [25] were employed. A relative transmission curve was calculated employing a standard gas mixture (Restek, Bellefonte, PA) and following a previously published procedure [26]. A constant reaction rate coefficient was assumed ($k_R = 2 \times 10^{-9}$ cm^3/s) for H_3O^+ as primary ion. Tentative identification of mass peaks was performed, based on the known fragmentation patterns of pure compounds [27–29].

VOC analyses of *Corvina* grape berries were performed on a single berry placed into a screw-capped, 40-ml, glass ampoule suited for volatile compound analysis. Ampoules were equilibrated at 25 °C for 30 min before each analysis.