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Statistical interpretation of chromatic indicators in correlation to phytochemical profile of a sulfur dioxide-free mulberry (*Morus nigra*) wine submitted to non-thermal maturation processes

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ABSTRACT

The four different methods of color measurement of wine proposed by Boulton, Giusti, Glories and Commission International de l'Eclairage (CIE) were applied to assess the statistical relationship between the phytochemical profile and chromatic characteristics of sulfur dioxide-free mulberry (*Morus nigra*) wine submitted to non-thermal maturation processes. The alteration in chromatic properties and phenolic composition of non-thermal aged mulberry wine were examined, aided by the used of Pearson correlation, cluster and principal component analysis. The results revealed a positive effect of non-thermal processes on phytochemical families of wines. From Pearson correlation analysis relationships between chromatic indexes and flavonols as well as anthocyanins were established. Cluster analysis highlighted similarities between Boulton and Giusti parameters, as well as Glories and CIE parameters in the assessment of chromatic properties of wines. Finally, principal component analysis was able to discriminate wines subjected to different maturation techniques on the basis of their chromatic and phenolics characteristics.

1. Introduction

Mulberry originated from Asian is extensively grown in China. The fruits are highly perishable and is swiftly processing into wine to extend their shelf life. Moreover, mulberry fruits are renowned for its health benefits due to its high content in phytochemicals. Besides, phenolic compounds have been demonstrated to be accountable for wine color (Monagas, Martín-Álvarez, Bartolomé, & Gómez-Cordovés, 2006).

Considering the direct impact of color on the acceptability of wine by consumers, winemaking practices have been established to improve the sensorial attributes of wine like chromaticity. Maturation is an oenological technique which is usually employed to stabilize and enhance the organoleptic attributes of wine (Lago-Vanzela et al., 2014). The traditional maturation of wine in oak barrel requires a long time in order to acquire a sensorial complexity and typical red color (Tao et al., 2012). However, the red color of mulberry wine is chiefly attributable to flavonoids, predominantly anthocyanin pigments which are prone to chromatic alteration during maturation (Lago-Vanzela et al., 2014). Moreover, new trends of berry wines without addition of sulfites nor matured in oak with deep red-purplish color are nowadays demanded by wine consumers' due to the side effect of sulfur dioxide which can induce allergic reactions in some consumers (Santos, Nunes, Saraiva, & Coimbra, 2012).

Novel technologies (pulse electric field, ultraviolet,...) have been introduced in an attempt to substitute the usage of sulfur dioxide and accelerate maturation process by shortening the time (Santos et al., 2012). Moreover, these non-thermal processes (NTP) may impact on reactions (copigmentation, oxidation, condensation, polymerization...) which occur during maturation, thus affecting the chromatic properties of wine (Santos et al., 2012). Recently, we have conducted a thorough study on the effect of non-thermal accelerating aging techniques on sulfur dioxide-free wine from mulberry (*Morus nigra*). The alteration in phenolic profile during pressurization, sonication and manosonication have previously been reported (Tchabo et al., 2017).

According to Monagas et al. (2006), the change in phenolic composition of wine may alter the chromatic profile of wine. Although, studies (El Darra et al., 2016; Gao et al., 2015; Monagas et al., 2006) have been conducted to assess the relationship between the phenolic profile and color of red wine produced under different oenological and aging conditions, there is a paucity of information on fruit wines maturation using NTP. In this context, it is of industrial importance to assess the influence of pressurization, sonication

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and manosonication on chromatic properties of aged fruit wines, specifically that of mulberry wine. Hence, the aim of the present investigation was to assess the statistical relationship between the phytochemical profile and chromatic characteristics of mulberry wine subjected to ultrasound (US), high pressure (HP) and manosonication (MS) maturation techniques. Moreover, this work seeks to identify which phenolic compounds contribute most to the color of aged mulberry wines (AMW).

2. Materials and methods

2.1. Chemicals

The phenolics standards and solvent were provided by Sigma-Aldrich (St. Louis, USA). Pectinex UF was obtained from Novozymes (Beijing, China). The yeast Actiflore F33 was purchased from Laffort (Bordeaux, France). Other chemicals were of analytical grade and were acquired from Sinopharm Chemical Reagent (Shanghai, China).

2.2. Winemaking

Mulberry fruits (*Morus nigra var. Zhen Jiang* No.1) were collected in a farm situated in Jiangsu province (China). The mulberry must was prepared as reported by Tchabo, Ma, Engmann, and Zhang (2015). In brief, the fruits were mashed and subjected to simultaneous treatment of ultrasound (34 kHz) and enzyme (0.010% v/w) during 12 min at 20 °C with a pulsating time of 10 s on and 5 off at constant power of 60 W. Afterwards, the fermentation was carried out as described by Tchabo, Ma, Kwaw, Zhang, and Li (2017). In brief, the °Brix and pH value of the must were adjusted to 26.00 and 4.00, respectively. Then, alcoholic fermentation was performed in darkness at 25 °C by inoculation of 10% (v/v) of yeast. The fermentation process was assumed completed when the weight and °Brix value was stable. Subsequently, the broth was centrifuged (10,000 rpm for 15 min at 4 °C) and filtered using cellulose filters. Before maturation, the filtered wines were stabilized for 1 month at −3 °C in darkness and thereafter packaged in polyethylene bags.

2.3. Maturation techniques

The optimal conditions of the non-thermal maturation techniques were chosen based on preliminary investigation that guarantees microbial safety and better polyphenolic preservation of the AMW (Tchabo et al., 2017).

2.3.1. Ultrasound maturation technique

Packaged wines were ultrasonicated at constant temperature of 10 °C for 30 min with an ultrasonic processor (Wuxi Fanbo Biological Engineering, Wuxi, China). The generator was set to operate at a frequency of 26 kHz with a pulsating time of 10 s (on) and 5 s (off) at fixe power of 60 W. Then, the ultrasonicated wines were stored for 2 months at 15 °C (in dark). Thereafter, the aged ultrasonicated wines (USW) were kept at −20 °C prior to analysis.

2.3.2. High pressure maturation technique

Packaged wines were pressurized at constant temperature of 10 °C for 20 min in the vessel (440 mm in height and 150 mm in diameter) of a hydrostatic press (Intelligent Super High Pressure Food Processing Device, Jiangsu University, China) using di-octyl sebacate as transmission fluid. After treatment, the high pressurized wines were aged under same conditions as described for the USW. Afterward, the aged high pressurized wines (HPW) were kept at −20 °C prior to analysis.

2.3.3. Manosonic maturation technique

Packaged wines were manosonicated at constant temperature of 10 °C for 15 min at frequency of 24 kHz and a pressure of 200 MPa. After treatment, the manosonicated wines were aged under same conditions as described for the USW. Afterward, the aged manosonicated wines (MSW) were kept at −20 °C prior to analysis.

2.3.4. Control

Packaged wines without SO_2 addition and not subjected to any treatment were aged under the same conditions as that of the treated wines and used as a control. Afterward, the aged control wines (CON) were kept at −20 °C prior to analysis.

2.4. Phytochemical analysis

2.4.1. Phytochemical families

The total phenolic index (TPI), total flavonol index (TFI) and total anthocyanin index (TAI) were determined according to Mazza, Fukumoto, Delaquis, Girard, and Ewert (1999). The condensed tannin index (CTI) was measured as described by Broadhurst and Jones (1978). The assays were performed using an UV-1600 spectrophotometer (Beijing Rayleigh Analytical Instrument Corporation, Beijing, China). The phytochemical families were expressed in mg per 100 ml equivalent of gallic acid for TPI, rutin for TFI, cyanidin-3-*O*-glucoside for TAI and catechin for CTI.

2.4.2. Polyphenols determination

The separation, identification and quantification of polyphenols were performed using a Shimadzu HPLC system (Shimadzu Corporation, Kyoto, Japan) (Tchabo et al., 2017). The HPLC system was equipped with Class VP software, a model LC-10AT pump, a model DGU-14A degasser, a model CTO-10AS column oven, a model SPD-M10A diode array detector and a model SCL-10A system controller. The chromatographic separation was carried out with 20RBAX-SB C18 column (4.6 mm × 250 mm, 5 μm particle size, Agilent, Santa Clara, USA). The diode array detector data were recorded over 200 and 600 nm range. The data of individual polyphenols are reported in Tchabo et al. (2017).

2.5. Chromatic analysis

The chromatic analysis of the Boulton, Giusti and Glories methods were performed using an UV-1600 spectrophotometer (Beijing Rayleigh Analytical Instrument Corporation, Beijing, China). The CIE method was performed with a Color Quest XE (Hunter lab, Reston, USA) in the total transmission mode with a light source D65 and a 10° observer angle.

2.5.1. Boulton method

The total color of pigments (TCP) and wine color (WC) were computed from the color fraction attributable to monomeric anthocyanin (MA), polymeric anthocyanin (PA) and copigmented anthocyanin (CA) (Levengood & Boulton, 2004). The color stability (CS) was calculated as described by Martínez-Pinilla, Martínez-Lapuente, Guadalupe, and Ayestarán (2012).

2.5.2. Giusti method

The polymeric color (PC), color density (CD) and percent polymeric color (%PC) were calculated according to Giusti and Wrolstad (2001).

2.5.3. Glories method

The color tonality (CT), color intensity (CI), Percent of pure red produced by flavylium cations (%dA), percent of yellow (%Ye),