Food Research International 100 (2017) 161–167

Contents lists available at ScienceDirect

Food Research International

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

Influence of *Brettanomyces* ethylphenols on red wine aroma evaluated by consumers in the United States and Portugal

Megan R. Schumaker[a], Mahesh Chandra[b], Manuel Malfeito-Ferreira[b], Carolyn F. Ross[a,*]

[a] School of Food Science, Washington State University, Pullman, WA 99163-6376, United States
[b] Linking Landscape, Environment, Agriculture and Food Research Center (LEAF), Instituto Superior de Agronomia, University of Lisbon, Lisbon 1349-017, Portugal

ARTICLE INFO

Keywords:
Brettanomyces
Sensory evaluation
Volatile phenols
Wine knowledge
Cross-culture

ABSTRACT

Brettanomyces may add complexity to wine at low concentrations but at high concentrations, can result in objectionable wines. The objective of this study was to determine the concentrations at which consumers from two different locations were able to detect *Brettanomyces* volatile compounds present in a red wine. A red wine blend, used in both countries, was spiked to create five treatments containing different concentrations of 4-ethylphenol (4-EP), 4-ethylguiacol (4-EG), and 4-ethylcatechol (4-EC) in a 5:1:1 ratio, respectively. These treatments were evaluated by consumers in the United States and Portugal (n = 121) using a difference from control test. Consumers were also classified as having low, medium, or high wine knowledge. Among the spiked samples, the greatest degree of difference was found between the second and third treatments, corresponding to reported detection and recognition threshold ranges of 4-EP and 4-EG. For some treatments, consumers from Portugal classified in the medium or high knowledge level reported significantly higher mean differences from the control than those in the low knowledge group ($p < 0.05$). Results demonstrated consumers' ability to detect differences in red wines due to *Brettanomyces* volatile compounds. Results provide useful context on how wine knowledge and cultural variants may affect the detection of *Brettanomyces*.

1. Introduction

Wine faults may be caused by the presence of numerous organisms, with one common spoilage organism being *Brettanomyces bruxellensis*. The growth of *Brettanomyces* in a wine may lead to the production of many aroma and flavor compounds, creating what is commonly referred to as a "Bretty wine." While many wine faults may be the result of a number of spoilage organisms, *Brettanomyces* is currently the only microbial species known to synthesize the volatile compounds resulting in the distinct "Bretty" aroma profile (Loureiro & Malfeito-Ferreira, 2006; Romano, Perello, Lonvaud-Funel, Sicard, & de Revel, 2009). Of the many compounds contributing to the complex aroma profile, three frequently reviewed volatile phenols are 4-ethyphenol (4-EP), 4-ethylguaiacol (4-EG), and 4-ethycatechol (4-EC). These compounds are formed through the actions of a decarboxylase enzyme, acting on hydroxycinnamic acids that are part of the non-flavonoid phenol fraction of the phenolic compounds in grapes, followed by a reduction reaction (Fugelsang & Edwards, 2006; Malfeito-Ferreira, Barata, & Loureiro, 2009; Suárez, Suárez-Lepe, Morata, & Calderón, 2007).

Aroma descriptors associated with *Brettanomyces* include smoky, sweaty, and barnyard. These unpleasant descriptors have the potential to severely alter wine quality (Curtin et al., 2008; Malfeito-Ferreira, 2011; Suárez et al., 2007). First studies reported preference thresholds of 4-EP (620 μg/L) and ratios of 4-EP:4-EG (10:1, 426 μg/L) (Chatonnet, Dubourdieu, Boidron, & Pons, 1992). With continued studies, threshold values ranges have been reported from 230–650 μg/L for 4-EP, and from 33 to 135 μg/L for 4-EG (Lattey, Bramley, & Francis, 2010; Nikfardjam, May, & Tschiersch, 2009; Petrozziello et al., 2014; Wedral, Shewfelt, & Frank, 2010). Threshold values of 4-EC have been reported at 60 μg/L, 100–400 μg/L, and as high as 775 μg/L in Cabernet Sauvignon specifically (Curtin et al., 2008; Hesford, Schneider, Porret, & Gafner, 2004; Larcher, Nicolini, Bertoldi, & Nardin, 2008). While the relatively low sensory perception threshold values for these volatile phenols are suggested to highly contribute to wine aroma, many parameters influence both the determination of these threshold values and the overall liking of wines exhibiting "Brett"-character (Curtin et al., 2008; Petrozziello et al., 2014).

Within the wine industry, the aroma profile incurred by *Brettanomyces* is a topic of reoccurring debate. At low concentrations, *Brettanomyces* may positively contribute a leathery aroma to wine, while at high concentrations, the aroma profile is generally considered to negatively impact overall wine quality. To add to the complexity,

* Corresponding author at: School of Food Science, Washington State University, P.O. Box 646376, Pullman, WA 99164-6376, United States.
E-mail address: cfross@wsu.edu (C.F. Ross).

http://dx.doi.org/10.1016/j.foodres.2017.06.057
Received 25 May 2017; Received in revised form 21 June 2017; Accepted 25 June 2017
Available online 27 June 2017
0963-9969/

ratios of 4-EP and 4-EG present in wines vary across wine varietals. Specifically, the 4-EP:EG ratio is dependent upon the wine varietal, with ratios varying from 10:1 for Cabernet Sauvignon, 9:1 for Bordeaux style red wines, and 3.5:1 for Pinot Noir (Curtin et al., 2008; Wedral et al., 2010).

Not only due to differences among wines, the presentation of the complexities of "Brett"-related compounds vary due to the tasters themselves, including their wine expertise and knowledge (Tempère et al., 2014). Although closely related, the terms wine expert and wine knowledge differ when considering types of consumers. While the term "wine expert" generally refers to someone with experience working in the wine industry, wine knowledge is more indicative of a theoretical-based understanding of wine concepts (Parr, Heatherbel, & White, 2002; Schiefer & Fischer, 2008; Tempère et al., 2014). Specifically, winemakers and those holding academic degrees in wine tasting displayed significantly lower detection thresholds of 4-EP and 4-EG compared to winegrowers or those without tasting degrees, indicating higher sensitivity (Tempère et al., 2014). Other studies have defined experience in terms of consumption patterns or wine knowledge (Hopfer & Heymann, 2014; Schiefer & Fischer, 2008). Segmenting consumers on wine expertise, wine knowledge, or both represents an important marketing area as wine experts often spend and purchase larger amounts of wine than novices (D'Alessandro & Pecotich, 2013). In the present study, wine knowledge was assessed using a questionnaire, with the assignment of a low, medium, or high knowledge level made in accordance to the number of correct responses collected from the questionnaire.

The presence of *Brettanomyces* in a wine is a world-wide issue, and is therefore a great concern to the international wine industry. Specifically, in Portugal of 88 samples of Pinot Noir wines, 57% contained *Brettanomyces* (Deavila & Ayub, 2013). Furthermore, while generally considered undesirable in the United States, consumer preferences and perception may have a cultural underpinning (Wedral et al., 2010). Previous cross-cultural studies have provided insight into consumer variations in preferences on food products including apples, sugar, and caffeine levels (Jaeger, Andani, Wakeling, & MacFie, 1998; Prescott, 1998). Cross-cultural studies examining wine have primarily focused on variations in wine quality, and determined country-specific factors which may influence the overall judgement of wine quality (Sáenz-Navajas, Ballester, Peyron, & Valentin, 2014). Furthermore, the globalization of the wine market has resulted in changing consumer trends. New world wines are becoming increasingly popular, with the United States wines expected to have continued international success (Campbell, Campbell, & Guibert, 2006). Therefore, this study investigating the perceived difference of *Brettanomyces* volatile phenols on red wine aroma was conducted in two major wine-producing areas, Washington State within the United States, a New World producer, and Portugal, an Old-World producer.

In the present study, a difference from control test was used to identify the magnitude of difference that consumers could detect across five treatment levels of 4-EP, 4-EG, and 4-EC prepared in a commercial red wine. In a difference from control test the size of any existing differences between samples may be assessed. The difference from control method is advantageous in situations in which a difference may be detectable, but the size of the difference affects the decisions and conclusions concerning the research objectives (Meilgaard, Carr, & Civille, 2006). The wine was the same in both locations so as to minimize the influence of matrix on volatile phenol perception. Treatment levels were selected based upon previously reported threshold values. Furthermore, this study also used the classification of consumers, through demographic responses and wine knowledge to further add to the existing information of the many factors influencing the perception of *Brettanomyces* aromas.

Table 1
Concentrations of volatile compounds spiked into tested wines.

	4-Ethylphenol (μg/L)	4-Ethguiacol (μg/L)	4-Ethylcatechol (μg/L)	Ratio of added 4-EP:4-EG:4:EC
Base wine	0	0	0	0:0:0
Treatment 1	250	50	50	5:1:1
Treatment 2	500	100	100	5:1:1
Treatment 3	1000	200	200	5:1:1
Treatment 4	1500	300	300	5:1:1
Treatment 5	2500	500	500	5:1:1

2. Materials and methods

2.1. Methods

4-Ethyphenol (> 97%) was purchased from Fisher Scientific (Hampton, NH) while 4-ethyguiacol (> 98%) and 4-ethylcatechol (> 98%) were purchased from Sigma Aldrich (St. Louis, MO, USA). Reagent water used was purified by Milli-Q (Millipore, Bedford, MA, USA). The filtration unit for purification of deionized water that was used for palate cleansing during the sensory test was purchased from EcoLab (Spokane, WA). In Portugal, 4-ethyphenol (> 97%) was purchased from Fluka Chemie AG (Buchs, Switzerland), while 4-ethyguiacol (> 95%) and 4-ethylcatechol (> 98%) were purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan) and Sigma Aldrich (St. Louis, MO, USA), respectively.

2.2. Wine treatments

Concentrations of 4-EP, 4-EG, and 4-EC were spiked into wines at a 5:1:1 ratio, respectively (Table 1). These concentrations were selected based on previously published studies as cited in the introduction. Stock solutions of 4-EP (3 mg/mL), 4-EG (1 mg/mL), and 4-EC (1 mg/mL) were prepared separately in 10% ethanol, and added directly to 3 L of base wine to prepare each treatment. Wine treatments were created 1 day prior to the sensory consumer panel, and were flushed with nitrogen before capping. Wine was stored at 23 °C until use.

2.3. Wine analysis

The base wine was a Piteira 2011 DOC Reserve red wine blend imported from Alentejo, Portugal, with a manufacturer reporting of 14% v/v ethanol. This base wine was characterized using standard wine chemistry measurements (Iland, Bruer, Edwards, Weeks, & Wilkes, 2004). Specifically, ethanol content was determined using an ebulliometer (Alla France, France). Titratable acidity was measured using a TitroLine Easy Autotitration calibrated with pH 4.0 and 7.0 standards (Schott Instruments, Germany). Wine pH was measured using a Fischer Scientific Accumet basic AB15 Plus pH meter (Hampton, NH, USA).

To determine the baseline concentration of 4-ethyphenol (4-EP) and 4-ethyguiacol (4-EG) in the base wine, headspace analysis was performed using headspace solid phase microextraction coupled with gas chromatography and mass spectrometry (HS-SPME/GC–MS). This analysis was also repeated on the wine treatments to verify the concentrations of 4-EP and 4-EG present in the wine prior to sensory evaluation. For the determination of 4-EP and 4-EG, a 65 μm SPME fiber coated with polydimethylsiloxane-divinylbenzene (PDMS-DVB) was used (Supelco, Bellefonte, PA). Prior to use, the fiber was conditioned at 250 °C for 30 min. For each analysis, 4 mL of wine sample and 1.28 g NaCl were placed into a 20 mL vial that was capped with a crimp seal with a Naturkautschuk PTFE magnetic cap (Gerstel INC., Linthicum, MD). Samples were analyzed using a GC 6890N chromatograph coupled with a mass spectrometer (MS 5975) (Agilent