Food Chemistry 244 (2018) 266–274

Contents lists available at ScienceDirect

Food Chemistry

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

Balsamic type varietal vinegar from cv. Xinomavro (Northen Greece). Optimization and scale-up of the alcoholic fermentation step using indigenous multistarters

Sofia Lalou, Maria Ferentidou, Fani Th. Mantzouridou, Maria Z. Tsimidou*

Laboratory of Food Chemistry and Technology, School of Chemistry, Aristotle University of Thessaloniki, 54 124 Thessaloniki, Greece

ARTICLE INFO

Keywords:
Balsamic vinegar
Concentrated grape must
cv Xinomavro
Saccharomyces cerevisiae
Hanseniaspora uvarum
Multistarter alcoholic fermentation
Taguchi design
Scale-up

ABSTRACT

Taguchi design was used to examine the effect of parameters that should be optimized in order to control the alcoholic fermentation of the concentrated grape must (CGM) from cv. Xinomavro using the best-performing indigenous *Hanseniaspora uvarum* and *Saccharomyces cerevisiae* strains as multistarters. The "optimum" combination of conditions (cell ratio of *H. uvarum/S. cerevisiae*; inoculum size and inoculation time of *S. cerevisiae*; fermentation time and temperature) resulted in an alcoholic product that meets ethanol (79 g/kg) and residual sugar (164 g/kg) content requirements for further use in the production of balsamic type vinegar. Multistarter fermentation affected positively the varietal organoleptic traits of the fermented CGM. 5-(Hydroxymethyl)-furfural content emerged as a critical factor for the standardization of this process. Scaling up experiments in 12 L barrels verified findings from small scale in 100 mL flasks. The results of this work can be used as a prototype in further similar efforts.

1. Introduction

Traditionally fermented foods and beverages are the result of spontaneous fermentation that takes place with the aid of the best adapted, indigenous, multi-species microflora. The result of their inherent properties is reflected onto the distinctive organoleptic traits of the end products (Speranza, Bevilacqua, Corbo, & Sinigaglia, 2017). However, the variability in the composition of the raw materials and the environmental conditions affect the type, quantity and biological activities of the indigenous strains from batch to batch. Consequently, traditional bioprocesses are difficult to standardize and often lack the required efficacy when scaled up. Given that, there is a challenge for adoption of modern production operations that ensure standardized quality, safety and line-speed but deliver products bearing distinct organoleptic features. To this direction academic and industrial sectors collaborate to develop starter cultures of defined strains with known properties (Speranza et al., 2017)that can be commercially available. This practice is common in the production of fermented products like table olives (e.g. Benincasa, Muccilli, Amenta, Perri, & Romeo, 2015), sausages (e.g. Montanari et al., 2016) and wine (e.g. Manfroi, Silva, Rizzon, Sabaini, & Glória, 2009). Still, evidence from the wine sector suggests that because these commercial starters consist of specific domesticated microbial strains, may exert low dominance in the fermentation process resulting in failure (Ciani et al., 2016; Steensels et al., 2014; Tristezza et al., 2016). Another drawback of the application of commercial starter inoculum is the limited sensorial complexity of the end product (Ciani et al., 2016; Speranza et al., 2017). To overcome these constrains, indigenous strains selected from the predominant microflora can be used after their phenotypic, genotypic and safe use characterization and examination of technological performance (Speranza et al., 2017). For example, to further improve the quality of wines, the best-performing indigenous *Saccharomyces* and non-*Saccharomyces* strains have to be combined to achieve a controlled multistarter fermentation process (Ciani et al., 2016; Steensels et al., 2014).

The experience from the winemaking can be transferred to similar production lines such as the alcoholic fermentation of the concentrated grape must (CGM), the first step in the production of traditional balsamic vinegars. As known, alcoholic fermentation and acetification steps take place in the same barrel by indigenous yeasts and bacteria before ageing of balsamic vinegar in a battery for many years (Giudici, Lemmeti, & Mazza, 2015). Nowadays, the interest in the isolation and characterization of the yeast microflora and their succession during the alcoholic fermentation is increasing, rendering the first step of the double fermentation line an independent one that needs optimization (Giudici et al., 2015; Solieri, Landi, De Vero, & Giudici, 2006). Such a need stands also true in the development of new balsamic type products

* Corresponding author.
E-mail address: tsimidou@chem.auth.gr (M.Z. Tsimidou).

http://dx.doi.org/10.1016/j.foodchem.2017.10.058
Received 20 July 2017; Received in revised form 9 October 2017; Accepted 9 October 2017
Available online 12 October 2017

beyond the traditional Italian ones from double fermentation of CGM or raisin extracts (Lalou et al., 2015).

In the course of our efforts to develop a new generation of balsamic type vinegars from the double fermentation of CGM from cv. Xinomavro, the present work focuses on the simultaneous exploitation of the two wild yeast strains, a *S. cerevisiae* and a *Hanseniaspora uvarum*, which were recently isolated and fully characterized (Lalou, Capece, Mantzouridou, Romano, & Tsimidou, 2016). Xinomavro is a typical indigenous red grape cultivar of Northern Greece known for producing supreme quality varietal wines with high aging potential (Kyraleou et al., 2015).

Regarding the selection of Xinomavro grape must as a starting material for balsamic vinegar production, its high acidity was considered a desirable characteristic in order to increase the acidity of the final product (Giudici et al., 2015). The latter has been considered as positive for this type of products by trained panelists (Hatzidimitriou, Papadopoulou, Lalou, & Tsimidou, 2015). Moreover, the two wild yeasts seemed to be promising for the development of a multistarter inoculum due to their strong osmophilic and glucophilic behaviour, high tolerance to CGM stress factors (high sugar content, low pH value, presence of furanic derivatives) and other desirable characteristics (Lalou et al., 2016).

The use of multistarters requires optimization of parameters such as the total cell population, the relative ratio between cell strains, the inoculum strategy (simultaneous *vs.* sequential) and the fermentation time and temperature. To address this complexity, a systematic optimization approach using the Taguchi method was employed to find out what type of parameters combination ensures the production of the intermediate alcoholic product with varietal traits that then can serve as an appropriate substrate for acetification. Scaling up experiments in barrels were also carried out to test the outcomes from flask fermentation in real conditions. To the best of our knowledge, the present investigation is the first report about the development of a new generation balsamic type vinegars with varietal characteristics based on fermentation processes and not on CGM-vinegar blending practices. This work can be used as a prototype as it gives evidence on the multiple challenges encountered in the first step of the double fermentation production line of balsamic type vinegar.

2. Materials and methods

2.1. Samples and CGM preparation

Fresh, non-sulfited grape must from cv. Xinomavro (Naoussa, Northern Greece, 408420 N, 218470 E, 300 m altitude) from three successive harvest years (2013–2015) was used. Each year, fresh must was boiled down to about 50% of the initial volume, within 24 h after its production, using an open flame at laboratory scale and kept at −18 oC until further use. Three similar set-ups consisting of an aluminum pot (12 L) with slightly round bottom and a burner connected to its gas tank were employed.

2.2. Yeast strains and inoculum preparation

The *S. cerevisiae* 121 and the *H. uvarum* 143 strains used had been previously isolated from CGM (cv. Xinomavro) and fully characterized (Lalou et al., 2016). The yeast strains were periodically sub-cultured and maintained on YEPD agar plates containing 10 g/L yeast extract (Panreac-Quimica S.A., Barcelona, Spain), 10 g/L peptone, 20 g/L glucose, 20 g/L agar (Biolab, Budapest, Hungary) at 4 °C till application. Inoculum of both strains was prepared by transferring a loopful of cells from the YEPD agar plates to 30 mL CGM in 50-mL Erlenmeyer flasks. Cultures were grown aerobically in a shaking incubator at 25 °C/180 rpm to a final OD_{660} value of ~2.

2.3. Evaluation of yeast growth

Enumeration of *H. uvarum* cells was performed using YEPD agar medium supplemented with 2 μg/mL of cycloheximide (YEPD + CYH agar) according to Pérez-Nevado, Albergaria, Hogg, and Girio (2006). YEPD and YEPD + CYH agar plates were incubated at 25 °C for 2–4 days and enumeration was performed when no further increase in colony forming unit (cfu) values was observed. The number of *S. cerevisiae* viable cells in the multistarter culture was calculated as the difference between the total number of colonies on YEPD agar plates and the total number of colonies on YEPD + CYH agar plates according to Eq. (1).

$$cfu_{S.cerevisiae} = cfu_{Total} - cfu_{H.uvarum} \quad (1)$$

As a prerequisite test, enumeration of single culture of *H. uvarum* was also conducted on both agar media and no statistical difference in the populations was observed (data not shown).

2.4. Optimization of multistarter fermentation at laboratory scale

2.4.1. Taguchi experimental design

Optimization studies were based on a Taguchi experimental design (Roy, 1990). The input parameters were the cell ratio of *H. uvarum/S. cerevisiae* (*H/S*), the inoculum size of *S. cerevisiae* (Is) (log_{10}cfu/mL), the inoculation time of *S. cerevisiae* (t_I) (days), the fermentation time (t_F) (days) and the temperature (T) (°C). Each parameter was assessed at three different levels resulting in a L_{27} (3^5) Taguchi orthogonal array (Table 1). The experimental design was built up using R language and environment for statistical computing (version 2.15.2). The responses examined were cell viability of *H. uvarum* (log_{10}cfu/mL), ethanol concentration (g/kg) and those of selected volatile compounds (alcohols, esters, acetoin) (g/kg). The concentration of alcohols refers to the sum of the concentrations of isobutanol, 3-methyl-1-butanol (isoamyl alcohol), 2,3-butanediol and 2-phenylethanol whilst the term concentration of esters is used for the sum of the hexanoic, octanoic and decanoic ethyl ester concentrations. All 27 experiments were carried out in duplicate in 100 mL Erlenmeyer flasks containing 60 mL of CGM (harvest year 2014). The flasks were withdrawn at the defined time points and their content was analyzed for cell viability of *H. uvarum* and the content of ethanol and major volatile compounds.

2.4.2. Data analysis

Experimental results were analyzed in terms of mean values (i.e. the average response values for each combination of input parameter levels) with the aim to determine the main effect for each parameter and the level that either minimizes or maximizes the corresponding mean value. Analysis in terms of signal-to noise (S/N) ratio was also performed in order to recognize the significant factors that influence the response variability (Roy, 1990). In this study, maximum output (the larger the better) is desired for cell viability of *H. uvarum*, ethanol, alcohols and esters concentration and the respective S/N ratios were calculated from Eq. (2):

$$\frac{S}{N} = -10\log\left(\frac{1}{n}\sum_{i=1}^{n}\frac{1}{y_i^2}\right) \quad (2)$$

On the contrary, minimization of acetoin concentration is intended so the “smaller the better” criterion is employed and the S/N ratio was calculated from Eq. (3):

$$\frac{S}{N} = -10\log\left(\frac{1}{n}\sum_{i=1}^{n}y_i^2\right) \quad (3)$$

where n is the number of experiments and y_i is the response value for a given experiment. In both cases, the response signals are maximized and the effect of random noise factors is minimized resulting in the improvement of the process performance (Roy, 1990). Analysis of