Food Microbiology 36 (2013) 70–78

Contents lists available at SciVerse ScienceDirect

Food Microbiology

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

Effects of some oil-born yeasts on the sensory characteristics of Italian virgin olive oil during its storage

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ARTICLE INFO

Article history:
Received 25 September 2012
Received in revised form
28 January 2013
Accepted 12 April 2013
Available online 25 April 2013

Keywords:
Microbiota
Olive oil
Olive oil quality
Sensory profile
Yeasts

ABSTRACT

The olive oil microbiota, mainly composed of yeasts, is associated with the suspended fraction of freshly produced olive oils. Some olive oil yeasts are considered useful as they are able to hydrolyse the bitter tasting secoiridoid compound of the oil, whereas others are considered harmful as they can damage the quality of the oil. Present research demonstrated the influence of some yeast strains belonging to *Candida adriatica*, *Candida diddensiae* and *Candida wickerhamii* species on the olive oil sensory characteristics during its storage. All the tested yeasts survived in the inoculated extra virgin olive oil and, after four months of storage, the suspended yeast cells recovered from the olive oil varied between 50% and 80% of the initial total yeasts, according to their sedimentation capacity. The mean of five analytical indices (free fatty acids, peroxide value, K_{232}, K_{270} and ΔK) were quite similar and about 60% of the treated samples analysed after four months of storage, on the basis of these indices, were still classed as extra virgin. Completely different results were obtained from the analyses of volatile and non volatile carbonyl compounds according to the yeast used. In the samples of oil treated with *C. adriatica* and *C. wickerhamii*, instead of some strains of *C. diddensiae*, a lower concentration of C6 volatile carbonyl compounds and polyphenols, responsible for positive oil attributes, were found. The sensory attributes of the treated olive oils varied according to the composition of the volatile and non volatile carbonyl compounds produced with the treatments. "Muddy-sediment", "rancid" or both defects were found in olive oil samples treated with *C. adriatica* DAPES 1933, *C. wickerhamii* DAPES 1885 and *C. diddensiae* DAPES 1912 and 1913 strains, whereas olive oil samples treated with *C. diddensiae* DAPES 1918 and 1922 after four months of storage were defect-free, and still categorized as extra virgin, according to the requirements of both chemical and sensory quality indices of European Community Regulations.

1. Introduction

Olive oil is a basic component of the Mediterranean diet and is well known for its high nutritional value and sensory characteristics. Extra virgin olive oil is produced by mechanical means directly from olives without any further refining processes. The International Olive Council (IOC) and the European Community (EC) have defined the quality of olive oil, based on chemical parameters that include free fatty acids, peroxide values, UV specific extinction coefficient (K_{232} and K_{270}) and sensory score. The classification as "extra virgin" is granted if the chemical analysis and, even more importantly, the sensory evaluation conducted by a panel of accredited and trained olive oil tasters (EN ISO/IEC, 2005), confirm conformity with the requirements of the European Community's 640/2008 (EC, 2008). The classification of olive oil from a sensory point of view is based on the detection of certain negative attributes (fusty, musty-humid, muddy-sediment, winey-vinegary, metallic and rancid), as well as the measurement of the intensity of three positive attributes (fruitiness, bitterness and pungency). Samples that show a medium of defect not above zero (=0) and a medium of fruitiness above zero (>0) are categorized as extra virgin which represents the highest classification level an olive oil can achieve (IOC, 2007). The sensory attributes are directly ascribable to the stimulation of the human sensory receptors by both volatile and some non-volatile carbonyl compounds present in virgin olive oil (Angerosa, 2002). Non volatile compounds such as phenolic compounds stimulate the tasting receptors and also the free endings of trigeminal nerves, the former eliciting the perception of bitterness, the latter pungency, astringency and metallic attributes (Kalua et al., 2007). On the other hand, volatile carbonyl compounds that are mainly produced by the oxidation of fatty acids, stimulating the olfactory receptors, are responsible for the whole

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0740-0020/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fm.2013.04.006

aroma of the virgin olive oil (Morales and Tsimidou, 2000). It is generally agreed that endogenous plant enzymes, through the lipoxygenase pathway, are responsible for the positive aroma perception in olive oil, whereas chemical oxidation and exogenous enzymes, usually from microbial activity, are associated with sensory defects. The activity of healthy olive microbiota contributes greatly to the flavour and overall quality of olive oil during the olive storage, fruit crushing and malaxation process as well as oil storage. Storage of olives in unsuitable conditions has heavy negative repercussions on the sensory quality of the resulting oils. In fact, an important growth of yeasts, bacteria and moulds responsible of the "winey", "vinegary" or "fusty" defects can occur, especially if the temperature is relatively high (Agar et al., 1998; Garcia et al., 1996). During the olive oil extraction phase Vichi et al. (2011) reports that in some cases the effect of olive microbiota on oil characteristics was greater than the effect exerted by malaxation time and temperature, and could be a critical point for the quality. The olive oil profile changes during storage because of the simultaneous drastic reduction of compounds from lipoxygenase pathways and the neo formation of some volatile compounds responsible for some defects known as "rancid" and "muddy sediment" attributes (Frankel, 1985). In the olive oil habitat, the microbiological profile is quite different compared to the healthy olive fruit microbiota. In fact, during processing, a large part of the olive microbiota migrates in the oil where, although following the selective action exerted by the new habitat, only a few microbic forms survived, whereas the others succumbed in a short time (Ciafardini et al., 2004). Among the microbic forms found most frequently in the extra virgin olive oil we have detected some yeasts belonging to the *Candida diddensiae*, *Candida boidinii*, *Candida wickerhamii*, *Williopsis californica*, *Candida guilliermondii*, *Candida parapsilosis* and *Saccharomyces cerevisiae* species (Ciafardini et al., 2006a). However, olive oil is a heterogeneous habitat where yeasts with biochemical and genetic characteristics not identical to known yeast species also live (Zullo et al., 2010). Cadez et al. (2012) recently described two new yeast species namely *Candida adriatica* and *Candida molendinolei*, isolated from olive oil and its by-products. Some yeast species are responsible for the appearance of the "heating" or "winey" defect in the oil extracted from olives which underwent a fermentation process before being processed (Angerosa, 2002). Studies have shown that some yeast species found in olive oil produce enzymes that may affect the oil's quality in both positive and negative ways (Ciafardini and Zullo, 2002; Ciafardini et al., 2006b). Nevertheless, microbiological research carried out up till now on this topic has not yet considered the direct relationship between the olive oil microbiota and the sensory characteristics of the product, and therefore at present it is not known if the above-reported yeasts are able to influence the sensory characteristics of the extra virgin olive oil during its storage. The aim of the present study is to investigate the influence of some dimorphic oil-born yeasts on the sensory profile of an Italian extra virgin olive oil during its storage.

2. Material and methods

2.1. Enzymatic activity and yeasts growth behaviour

The dimorphic yeasts used in the trials reported as follows, were previously isolated from extra virgin olive oil and belonged to the *C. adriatica* (Cadez et al., 2012), *C. diddensiae* (Zullo et al., 2010) and *C. wickerhamii* (Ciafardini et al., 2006b) species. The *C. adriatica* DAPES 1933 strain, *C. diddensiae* DAPES 1912, 1913, 1918 and 1922 strains and the DAPES 1885 strain of *C. wickerhamii* were grown for 3 days at 30 °C in Petri dishes with MYGP agar medium containing 3 g yeast extract (Biolife, Milan, Italy), 3 g malt extract (BBL, Cockeysville, MD, USA) 5 g beef extract powder (BBL), 10 g D-glucose (Merck, Darmstadt, Germany), 1000 mL distilled water, pH 7 as described by Kurtzman and Fell (1998). All the yeast cultures were further characterized considering some important enzymatic activity involved in the oxidation and hydrolysis of the olive oil phenolic compounds and the dimorphic growth behaviour related to the presence or not of olive oil. Among the enzymes responsible for the oxidation process, the phenoloxidase, peroxidase and tyrosinase activity have been studied, while as hydrolytic enzymes the production of β-glucosidase and esterase active respectively on the bitter tasting olive oil oleuropein glucoside and the derivative oleuropein-aglycon have been evaluated.

2.1.1. Phenoloxidase activity

The phenoloxidase activity was assessed according to an enzyme test that involves the oxidation of pyrocatechol to *o*-benzoquinone. 30 mg of biomass from each yeast culture grown 3 days at 30 °C in Petri dish with MYGP agar medium were transferred in sterile test tubes with screw caps containing 3 mL potassium phosphate buffer 0.1 M pH 7 with 0.5% w/v pyrocatechol (Sigma Chemical, St. Louis, Missouri). The yeast cells were suspended in the reaction mixture with a vortex (mod. Maxi mixer, FAVS, Bologna, Italy), then incubate 180 min in agitation (50 rpm) at 30 °C. Two negative controls for each yeast strain containing the same described components without respectively the yeast biomass or pyrocathecol were monitored over the same time period and were subtracted as background. The trials were accomplished using four repetitions. After incubation the yeast suspensions were centrifuged at 10,000 × *g* for 10 min and the supernatants were analysed with a spectrophotometer (Jenway mod. 6300, Essex, United Kingdom) at 398 nm. The enzyme activity was determined on the base of the initial rate of increase in absorbance. An enzyme unit is defined as an optical density change of 0.01/min/g biomass under the above conditions.

2.1.2. Peroxidase activity

The peroxidase activity was performed spectrophotometrically at 470 nm using pyrogallol as a phenolic substrate with hydrogen peroxide (Dìaz et al., 2001). Briefly the reaction mixture contained 0.15 mL of 4% w/v pyrogallol (Sigma Chemical, St. Louis, Missouri), 0.15 mL of 1% v/v H_2O_2, 2.66 mL of 0.1 M potassium phosphate buffer pH 7 and 30 mg of the above yeast biomass. Four repetitions and two negative controls for each yeast culture containing the same components except respectively the yeast biomass or pyrogallol, were accomplished and were subtracted as background. After 180 min of incubation at 30 °C, the yeast suspensions were centrifuged at 10,000 × *g* and the supernatants were analysed as described before. An enzyme unit is defined as an optical density change of 0.01/min/g biomass under the above conditions.

2.1.3. Tyrosinase activity

The assay of tyrosinase enzyme was performed using tyrosine as substrate. The reaction mixture containing 3 mL of 0.1 M phosphate buffer pH 7, 25 mg L-tyrosine (Sigma) and 30 mg of yeast biomass. Four replications and two negative controls without tyrosine or yeast biomass were accomplished as before. After 180 min of incubation at 30 °C in agitation (50 rpm), the reaction mixtures were analysed as before with the spectrophotometer at 398 nm. The optical density of the controls was subtracted as background. One enzyme unit is defined as an optical density change of 0.01/min/g biomass.

2.1.4. β-glucosidase activity

The β-glucosidase test was carried out by transferring 30 mg of each yeast culture in the sterile test tubes with 3 mL of 0.1 M phosphate buffer pH 7, containing 0.4% w/v of the synthetic