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The Use of β-Glucosidase Enzyme in Black Table Olives Fermentation

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Abstract

Edincik-Su variety black olives, grown in Marmara region, were processed by three different debittering methods [fermentation with *Lactobacillus plantarum* (T I), fermentation with β-glucosidase enzyme+*L.plantarum* (T II), modified ripe-olive fermentation (T III)], and the effects of the applied methods on physico-chemical and sensory characteristics of fermentation media were investigated. The average salt concentrations of the brines were determined as 5.718, 5.769 and 4.825%, for T I, T II and T III, respectively. Salt content of T III was found lower than other treatments. TIII olives displayed the lowest acidity (0.270%) with the highest pH (5.96) depending on removal of most of the sugars and nutrients by lye treatment and following washings. The pH presented a sharp decrease, on the contrary to acidity, within the first days of fermentation in all treatments. The average reducing sugar concentrations of treatments were determined as 0.748, 0.692 and 0.441% for T I, T II and T III, respectively. Although the lowest oleuropein values were obtained in T III olives, the overall liking scores revealed that T II olives had higher organoleptic acceptability (as 27.9). T II and T III olives were found to be more typical to consumers. The correlations of the different sensory attributes with overall acceptability indicated that the panel was negatively influenced by bitterness and skin separation. It appears that the panelists preferred olives with a acidic/bitter taste and firm appearance.

Keywords: olive, β-glucosidase, *Lactobacillus plantarum*, lye, sensory properties

Introduction

Table olives, either green or black are the basic components of the Mediterranean diet renowned for its beneficial effect on the health and they are recognised as a valuable functional foods. The nutritional value of olives depends mainly on the lipid, vitamin and mineral contents, and the phenolic antioxidant compounds (Marsilio *et al.*, 2001; Boskou and Visioli, 2003). The main purpose of table olive processing is the removal, at least partially, of the natural bitterness of the fruit, to make it acceptable as food or as appetizer (Soler-Rivas *et al.*, 2000). Oleuropein, the bitter-tasting secoiridoid glucoside, is the most abundant biologically active coumarin-like phenolic compound in olives and it is an ester of 2'-(3',4'-dihydroxyphenyl) ethanol (hydroxytyrosol) (Briante *et al.*, 2002; Bianchi, 2003, Malik and Bradford, 2006), and it undergoes a notable decrease during the course of fruit ripening and processing (Amiot *et al.*, 1989; Ryan *et al.*, 1999; Ongen *et al.*, 2000; Piga *et al.*, 2001; Ferreira *et al.*, 2002).

There are several processing methods to obtain palatable olives (Vaughn, 1975; Brenes *et al.*, 1995; Marsilio *et al.*, 1996; Papoff *et al.*, 1996; Garrido-Fernandez *et al.*, 1997; Sanchez *et al.*, 2001; Leal-Sanchez *et al.*, 2003). The processing requires a brining stage during which the olives are fermented to have the characteristic texture, aroma and flavour. However, black olives can be debittered before brining by successive treatments with dilute NaOH solution (lye) known as Spanish-style, used mainly for green olives. During the intervals between lye treatments the fruits are suspended in water through which air is bubbled and the olives are darkened progressively due to oxidation of orthodiphenols (Kilic, 1986, 1989; Brenes *et al.*, 2004).

The reason for lye treatment is to eliminate the bitterness of olives by hydrolysation of the oleuropein and diffusion into the brine (Brenes and de Castro, 1998). The lye treatment and subsequent washing process comprises a very complex mechanism of removal of some water-soluble compounds, like reducing sugar and organic acids, from the olive fruit as well as oleuropein (Garrido-Fernandez *et al.*, 1997). In addition, the lye could dissolve the epicuticular waxy coating and enhance the diffusion from fruit flesh and result in the softening (Araujo *et al.*, 1994; Jiménez *et al.*, 1995; Coimbra *et al.*, 1996; Marsilio *et al.*, 1996; Sanchez-Romero *et al.*, 1998). The spontaneous lactic acid fermentation of olives is due to yeast, lactic bacteria, or both. The acid pH produced by the metabolism of the microorganisms, could aid in the preservation of the product and is necessary to hydrolyze oleuropein in directly brined olives (Ciafardini *et al.*, 1994).

In search for alternative processing methods to lye and/or brine treatment, enzymatic hydrolysis of the glycosidic linkage of oleuropein by β-glucosidase activity, which results in formation of glucose and oleuropein aglycone,

could be taken into consideration. The glycosidases catalyse the hydrolysis of the glycosidic bonds and are an important group of enzymes for many biochemical, biomedical and industrial applications. β-glucosidases of microbial origin were investigated for substituting NaOH treatment in olive processing (Materassi *et al.*, 1975; Limirolli *et al.*, 1995; Briante *et al.*, 2000; Ciafardini and Zullo, 2000).

The aim of this work was to monitor the fermentation media for changes in physico-chemical and sensory characteristics on Edincik-Su black olive variety, grown in Marmara region and processed by using three different debittering methods; i) fermentation with lactic starter, ii) fermentation with β-glucosidase enzyme + lactic starter, and iii) ripe-olive fermentation.

Materials and methods

The olive variety used in present study was Edincik-Su (*Olea europea* cv. Edincik-Su) black olives, grown in Marmara Region, Turkey. The starter culture was *Lactobacillus plantarum L2-1* and it has being supplied by DANISCO CULTOR Niebüll GmbH, Denmark. β-glucosidase enzyme (EC 3.2.1.105) was obtained by DSM Food Speciality Company, Izmir, Turkey.

Preparation of Starter Culture

Prior to experimental use the cultures of *Lactobacillus plantarum L2-1* were propagated in MRS broth (Man *et al.*, 1960) and incubated overnight at 30°C. 24-hours. Young cultures were harvested by centrifugation at 4000 *g* for 10 min and the precipitate was suspended in sterile physiological water. The culture was diluted so that a 1 mL inoculum would produce a concentration of approximately 10^8 cfu mL^{-1}. This suspension of lactic starter was used to inoculate fermentation containers as 1% (v/v) of total volume.

Olives were transported to the laboratory within 24 h after harvesting. On arrival, they were sorted to remove fruits with colour defects, blemishes, cuts and damages as well as over-ripened ones. After washing with water under pressure to remove any impurities the olives were divided into three batches and processed by three different methods described below. Fermentation experiments were carried out in 5 L total capacity screw-capped glass fermenters filled with freshly prepared pasteurised brine (85°C for 30 min) containing 8% (w/v) NaCl. After brining, the fermenters were stored at room temperature for a period of 90 days. The NaCl level in the brines was kept constant throughout fermentation by adding solid salt at regular time intervals. Fermentation was observed by physico-chemical and sensorial analyses on duplicate samples taken periodically.

Treatment I (T I): Fermentation with *Lactobacillus plantarum*

The olives were placed in fermenters with cover brine and the starter culture (1%, v/v) was added.

Treatment II (T II): Fermentation with β-glucosidase Enzyme + *L. plantarum*

The olives were placed in fermenters with cover brine and the pH value of the brine was set to pH 4.70 for optimum enzyme activity, with lactic acid (90%, v/v). Following pH adjustment starter culture (1%, v/v) and 0.5% β-glucosidase enzyme were added.

Treatment III (T III): Ripe-olive fermentation

After sorting and washing, the olives were placed in 1.5% (w/v) NaOH solution for 7–12 h to remove bitterness. Lye solution was allowed to penetrate ¾ of the olive flesh. Then, olives were washed by replacing lye with tap water, kept in water for 30 min and changed three times at intervals of 10 h. On ensuring total removal of NaOH from olives starter culture (1%, v/v) was added.

Chemical analysis

Changes in the physico-chemical properties of brines were studied by measuring pH using a Nel pH 840 model pH meter, titratable acidity in terms of lactic acid (Uylaser and Basoglu, 2000) and salt content by the titrimetric method of International Olive Oil Council (IOOC, 1990). Reducing sugar values were determined by Luff-Schoorl method (Cemeroglu, 1992). Oleuropein content was realised according to the absorbance values of samples at 345 nm (Tzika *et al.*, 2004; Mastorakis *et al.*, 2004). All analyses were done in triplicate.

Sensory evaluation

Ten experienced panelists chosed from the Department of Food Engineering, Uludag University staff, evaluated the sensory characteristics of the processed olives, after preliminary training sessions. The panelists were asked to rate colour, mechanical properties (firmness, skin separation, freestone strength) and flavour (odour, taste, bitterness, saltiness), by a modified interval sensory rating scale of 1 to 5 (unacceptable/excellent) described by Marsilio (2002) and Di Biase *et al.* (2002). Overall appreciation is evaluated as a sum of colour, mechanical and flavour attributes for each treatment. Colour was evaluated as 1: grey-brown to 5: black.. Flesh/stone attachment represents the easiness/difficulty in separation of stone from the flesh (1: difficult deattachment; 5: easy deattachment). Taste attribute is the combination of acidty, saltiness, bitterness, and aroma balance of the olive and expresses mainly the mouthfeel characteristics. Saltiness is represented as 1: very salty and 5: low salt. Bitterness, due to phenolic compounds, is expressed as bittter to sweet.

Statistical Analysis

The significance of differences among samples was determined by analysis of variance (ANOVA), using 99%, and for some 95%, confidence intervals on random parcel. Trial pattern was run on each of the physico-chemical variables to disclose possible differences among the samples for the two factors "treatment" and "fermentation time". For the sensory properties "treatment" was the only variation factor. All analyses were performed using the Minitab for

Windows (Ver. 14) Statistical Software Package (Minitab Inc., State College, PA).

Results and discussion

Raw Olive Characteristics

Prior to processing the physical and chemical properties of Edincik-Su variety olives were determined (Tab. 1). The properties of olives depend not only on the variety, harvesting time and the cultivation conditions (climate, geographical area, quality of soil, irrigation technique, etc), but also on the differences of maturity at the time of harvest (Garrido-Fernandez *et al.,* 1997; Shibasaki, 2005).

The Edincik-Su variety produces large spherical fruits with an average size of 202 olives kg^{-1}, an average fruit weight of 4.95 g, 16.71% oil content, a round and very small stone, 38.84% dry matter content and a flesh-to-pit ratio higher than 8:1. The fruits have a high moisture content which gives the variety its name. Particular care must be taken during harvesting and processing due to its soft texture with very delicate skin and pulp. They are hand harvested from mid-October to mid-November when they reached a grey-black surface colour. Edincik variety is grown mainly in coasts of Edincik and Erdek, Marmara Region, and is consumed widely as a black table olive variety (Canozer, 1991).

Tab. 1. Physical and chemical properties of Edincik-Su variety raw olives (n = 3)

Number of fruit kg^{-1}	207.00±32.24
Fruit size (mm)	
Length	22.48±2.12
Width	17.96±1.60
Stone size (mm)	
Length	15.61±1.56
Width	7.68±0.57
Flesh : stone ratio	6.02±0.21
Dry matter (%)	39.20±1.97
Ash (%)	1.46±0.02
Reducing sugars (g/L)	3.62±0.19
Protein (%, dry matter basis)	3.29±0.03
Fats (%, dry matter basis)	24.49±0.14
Oleuropein (abs.)	1.583±0.012

Assesment of physico-chemical changes during the fermentation of olives

In order to investigate the effect of treatment on physico-chemical properties of olives during fermentation pH, titratable acidity, salt, reducing sugar and oleuropein contents were determined. Olives undergo rapid physical and chemical changes following brining due to an onset of material exchange between the brine and the olives, reaching an equilibrium after a certain period. Fermentable sugar and other nutrients diffuse in the brine supporting the activation of lactic acid bacteria leading to a significant increase in acidity as fermentation advanced up to 14th day, after which the acidity of the brine displayed a slight increase (Tab. 2). Changes in physico-chemical composition were found to be in strict relation with concomitant microbial flora and pre-fermentation processes.

The effect of treatments and fermentation time were significant on physico-chemical characteristics ($P<0.01$, Tab. 2). As designated, the acidity and reducing sugar increased as fermentation advanced, whereas salt and oleuropein contents and pH, with some fluctuations, declined (Fig.s 1-5). The salt concentration is an important factor in governing the diffusion of soluble constituents as well as the microbial growth, and therefore the rate of the fermentation (Fernandez-Diez *et al.*, 1985).

The salt contents of each treatment, with an initial value of 8%, decreased from the 1st day of fermentation due to osmosis and material exchange, and the average salt concentrations were found as 5.718, 5.769 and 4.825%, for T I, T II and T III, respectively (Tab. 2). The lye solution applied in T III resulted in solubilisation of pectic and hemicellulosic polysaccharides and cellulose, degradation of cell walls and enhanced-permeability due to breakage of ester and hydrogen bonds (Mafra *et al.*, 1996). Enhanced cell wall permeability eventuated in high salt-uptake of flesh, thus salt content of cover brine in T III was found lower than other treatments (Fig. 1).

The lye treated olives (T III) displayed the lowest acidity (0.270%) with highest pH (5.96%) in brine as most of the sugars and nutrients were removed by the effect of the lye treatment and washings applied to completely remove excess alkaline. The pH presented a sharp decrease within the first days of fermentation in all treatments. Higher acidity values observed with other treatments (0.371 and 0.370% for T I and II, respectively) and lower pH were similar to Borcakli et al. (1993) and might reflect the activity of starter culture depending on the availability of fermentable material diffused to the brine for their growth (Fig. 2). Acidity of black olives is reported to range up to 1% during controlled fermentation (IOOC, 1990; Fernandez-Diez, 1984).

With spontaneous fermentation or lye treatment the final pH and acidity values may often not reach desired levels to ensure safe storage of the end-product (Marsilio, 1993) and spoilage may occur due to subsequent contamination by other microorganisms (Fernandez-Diez, 1983; Garrido-Fernandez *et al.*, 1997). Acidification of the initial brine with organic acids (lactic, acetic), addition of fermentable material, inoculation with a pure culture of lactobacilli, and use of brine from a previous fermentation are some ways to minimize the the risk of spoilage (Tassou *et al.*, 2002; Panagou and Katsaboxakis, 2006).

The use of selected strains of Lactic acid bacteria (LAB) as starter culture, and thus controlled fermentation, is emphasised as a food preservation technique. LAB have a progressive acidification activity of the fermenting

Tab. 2. Effects of treatment and fermentation time on the pysico-chemical properties of fermented olives

Treatment Code	N	Salt content (%)	pH	Titratable acidity (%)	Reducing sugars (g/L)	Oleuropein (abs.)
I	22	5.718±0.197^{a}	4.574±0.048^{b}	0.371±0.191^{a}	0.748±0.604^{a}	0.397±0.096^{a}
II	22	5.769±0.181^{a}	4.073±0.087^{c}	0.370±0.201^{a}	0.692±0.558^{a}	0.325±0.098^{b}
III	22	4.825±0.081^{b}	5.962±0.079^{a}	0.270±0.136^{b}	0.441±0.204^{b}	0.074±0.012^{c}
Fermentation time (days)†						
1	6	6.849±0.310^{a}	5.263±0.498^{a}	0.049±0.090^{i}	0.531±0.030^{de}	1.087±0.277^{a}
3	6	6.129±0.334^{b}	5.100±0.487^{b}	0.151±0.012^{h}	0.883±0.191^{bc}	0.632±0.195^{b}
5	6	6.030±0.359^{b}	4.687±0.460^{g}	0.163±0.018^{h}	1.346±0.568^{a}	0.123±0.030^{e}
7	6	5.674±0.305^{c}	4.477±0.389^{h}	0.214±0.054^{g}	1.207±0.460^{a}	0.078±0.031^{fg}
10	6	5.580±0.300^{c}	4.492±0.389^{h}	0.328±0.056^{e}	1.067±0.417^{ab}	0.227±0.049^{d}
14	6	5.217±0.212^{d}	4.893±0.388^{e}	0.298±0.074^{f}	0.724±0.167^{cd}	0.301±0.107^{c}
21	6	5.169±0.111^{d}	4.990±0.328^{c}	0.445±0.055^{d}	0.340±0.035^{ef}	0.064±0.027^{g}
28	6	4.907±0.115^{e}	4.940±0.282^{d}	0.418±0.024^{c}	0.301±0.053^{efg}	0.126±0.021^{e}
45	6	4.907±0.133^{e}	4.831±0.282^{f}	0.510±0.098b	0.229±0.063^{fg}	0.104±0.013^{ef}
60	6	4.663±0.068^{f}	4.982±0.232^{c}	0.560±0.110^{a}	0.122±0.014^{fg}	0.115±0.022^{e}
90	6	4.682±0.050^{f}	4.910±0.263^{c}	0.569±0.111^{a}	0.065±0.033^{g}	0.061±0.013^{g}
ANOVA						
Treatment (T)		**	**	**	**	**
Fermentation time (FT)		**	**	**	**	**
T x FT		**	**	**	**	**

Values are means of all fermentation time ± standard error; different superscript letters on the same column indicate significant differences; Duncan's multiple range test (P<0.01,**).

†The beginning of fermentation was taken as the first day of the fermentation period

brine with a consequent pH decrease and the production of antimicrobial substances and bacteriocins (Durán *et al*., 1994; Nychas *et al*., 2002; Panagou *et al*., 2003; Marsilio *et al*., 2005).

Reducing sugars and other fermentable material were utilised by concomitant bacteria fundamentally for proliferation, and subsequently converted to secondary metabolites, through homo- or heterofermentative metabolism, such as lactic acid, acetic acid, CO_2, ethanol. Changes of reducing sugar contents in brine was given in Fig. 3. As fermentation proceeded the fermentable substances diffused into brine and their concentrations increased to maximum values of 1.607, 1.809 and 0.623% in T I, T II and T III,

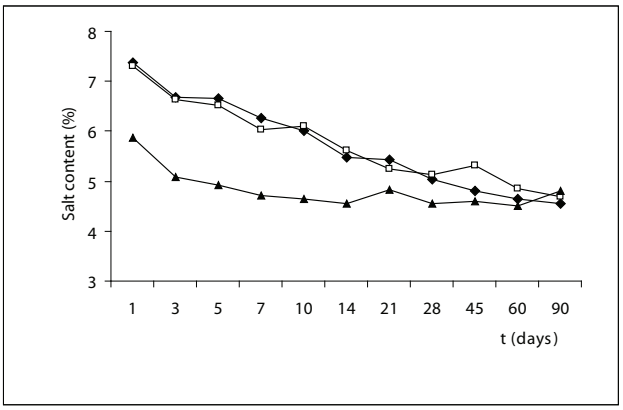
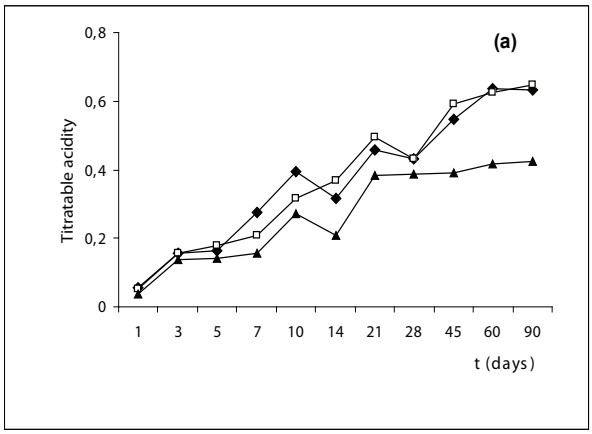


Fig. 1. Changes in salt content during fermentation of T I olives (♦), T II olives (□) and T III olives (▲)



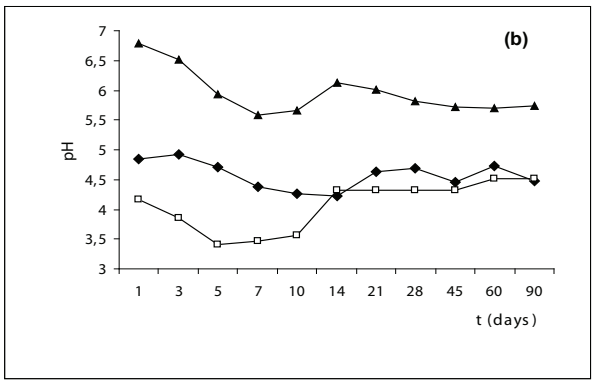


Fig. 2. Changes in (a) titratable acidity and (b) pH content during fermentation of T I (♦), T II olives (□) and T III olives (▲)

respectively after 5 days of brining. The average reducing sugar concentrations of treatments were found as 0.748, 0.692 and 0.441%. Lower concentrations in T III might be related to low initial reducing sugar values due to the removal of fermentable substances by lye treatment and subsequent washings. Lye treatment is an important step in the fermentation process. It results not only in debittering of the fruit but also in softening of olive flesh (Papamichael-Balatsouras and Balatsouras, 1988). Excessive washings leads to elimination of most of the fermentable substance in the context of limiting growth and activity of bacteria.

Changes in oleuropein content, the main phenolic compound in olive flesh and indicator of edibleness, during fermentation for each treatment are given in Fig. 4. The effects of treatment and fermentation time were found significantly important on oleuropein content of Edincik variety olives. The oleuropein values were observed to decrease throughout fermentation and determined as 0.092, 0.067 and 0.024 for T I, T II and T III, respectively after 90 days of brining. With the lye treatment and following washings in T III olives initial oleuropein level was lower than T I and T II olives due to hydrolisation of this bitter-tasting glycoside, and thus final oleuropein content was

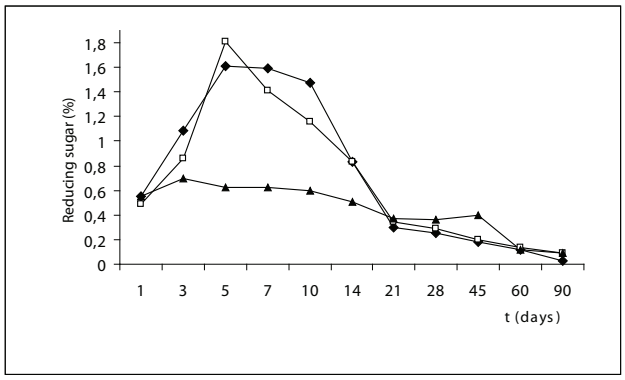


Fig. 3. Changes in reducing sugars during fermentation of T I olives (♦), T II olives (□) and T III olives (▲)

lowest.

The phenolic composition within the olive varieties is very complex and depends on the maturation stage, part of the fruit, variety and season (Brenes *et al.*, 1993; Soler-Rivas *et al.*, 2000). In ripening period of the olive fruit, oleuropein accumulates during the growth phase. Follwing the green maturation phase, where reduction in chlorophyll and oleuropein contents are observed, anthocyanins appear and oleuropein level is reduced at the black maturation phase (Amiot *et al.*, 1989). In the black cultivars the level of oleuropein displays a decrease during maturation (Limiroli *et al.*, 1995). The final oleuropein value and its degradation products in processed olives depends on the processing method. Throughout pre-processing, fermentation and storage phenolic composition simultaneously

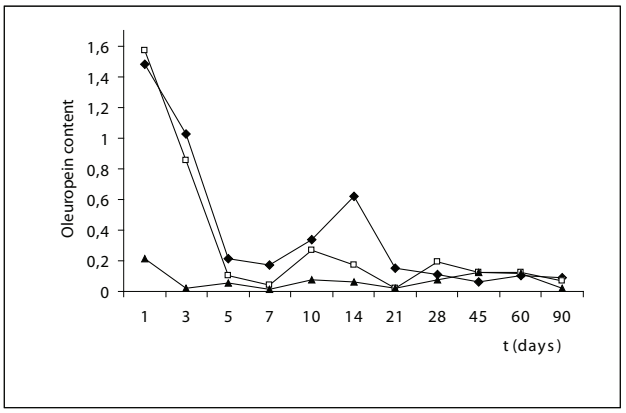


Fig. 4. Changes in oleuropein content during fermentation of T I olives (♦), T II olives (□) and T III olives (▲)

change due to hydrolysis of the glycosides initially present in the flesh, and these variations largely explain the difference in colour and taste within olives.

Sensory Properties of Fermented Olives

Black olives, even the bitter compound oleuropein moiety declines throughout ripening, are not appropriate for consumption without some kind of curing, and in most cases, excluding dry-salting, olives do undergo fermentation in brine solution not only for preservation but also to increase palatability (Panagou *et al.*, 2006). Olive brining depends upon reduction of the activity of the enzymes responsible for chemical changes involved in fermentation and the inhibition of growth of deteriorative microorganisms. Therefore, acid formation is essential in conjunction with the salt to inhibit the softening due to enzymatic activity (Akpinar-Bayizit *et al.*, 2007). Marsilio *et al.* (2005) expressed that addition of suitable starter culture during green olive processing shortened the fermentation time and enhanced the organoleptic quality with controlled conditions.

The scores for analysed sensory attributes and their standard errors in Edincik-Su variety black olive samples at the end of fermentation are given in Tab. 3. One-way ANOVA showed that the olives were significantly different in terms of colour, flesh/stone attachment, odour, taste and bitterness. The effects of treatment for attributes where the differences were significant are summarized in Tab. 3 ($P<0.01$,**; $P<0.05$, *).

The highest scores for colour were achieved in T II and T III olives as black where T I olives were assigned as grey-black. The sensory scores for firmness, skin separation, saltiness and overall appreciation were not significantly different. T I olives had the highest scores for skin seperation whereas the lowest for colour, firmness, flesh/stone attachment and overall appreciation. Eventhough T I olives had higher mean value for bitterness than T II olives, taste scores for both treatments were similar. As with lye treatment bitter tasting compound was hydrolised, the loss in the bitterness, resulting in higher score, which

Tab. 3. Effects of treatment on the sensory properties of fermented olives

Sensory Property		N	Treatment Code		
			I	II	III
Colour**		10	2.8±1.476[b]	4.1±0.568[a]	4.1±0.738[a]
Mechanical properties	Skin separation*	10	1.9±0.316[ns]	1.6±0.517[ns]	1.8±0.422[ns]
	Flesh/stone attachment **	10	4.1±0.995[b]	4.6±0.517[ab]	4.9±0.316[a]
	Firmness*	10	3.6±0.517[ns]	4.2±0.789[ns]	4.2±0.633[ns]
Flavour	Odour*	10	3.5±0.707[b]	3.7±0.483[ab]	4.3±0.824[a]
	Taste**	10	3.7±1.060[ab]	3.6±0.517[b]	4.6± 0.517[a]
	Bitterness**	10	3.3±0.949[b]	2.7±0.675[b]	4.6±0.517[a]
	Saltiness*	10	3.4±0.844[ns]	3.4±0.516[ns]	3.7±0.824[ns]
Overall appreciation*		10	26.3±2.669[ns]	27.9±1.663[ns]	27.3±1.703[ns]

Values are means of all fermentation time ± standard error; different superscript letters on the same column indicate significant differences; Duncan's multiple range test (P<0.01,**; P<0.05, *)

is seeked by the consumer revealed in lower scores of T III olives for odour and taste attributes. Changes in organoleptic characteristics of any product are not only relatively easy to discern and identify by the consumer than physico-chemical and microbiological properties, but also provides an evident description of the product with a starting point to evaluate product abnormalities, defects and spoilage. Despite the fact that the level of lactic acid was found lower than the requirements established by the

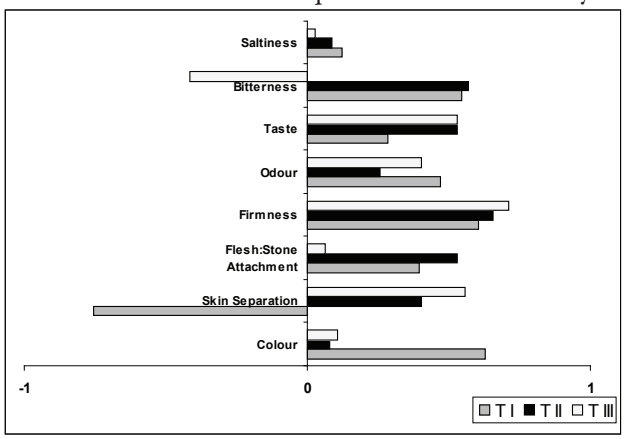


Fig. 5. Effect of treatment on sensorial attributes of black olives compared to overall appreciation

IOOC (1990) for the trade of table olives (Tab. 2), its presence influences the aroma of final olive product. Panagou *et al.* (2006) stated that consumers mainly prefer medium to large-sized olives with sound appearance, crisp and good aroma, which are well defined sensory expectations of "typical" olives. These attributes were observed to be influential on shaping up the judgement of the panelists, with the consideration of variations that occur in the organoleptic profile within individual table olives according to processing method and fermentation time.

T II olives yielded a product with low salt content during fermentation period, with significantly black colour and higher acceptability among the judges compared to T I olives. Scores for T III olives were similar to or for some attributes not significantly higher than T II olives. The overall appreciation perceived T II and T III olives to be more typical to consumers than T I olives (Tab. 3). The results revealed that olives fermented with addition of enzyme + starter culture (T II) had higher overall organoleptic appreciation scores (as 27.9) than olives fermented with starter culture (T I) only (as 26.3), however, the variations between treatments were found insignificant. The correlations of the different sensory attributes with overall acceptability indicated that the panel was positively influenced by saltiness, taste, odour, firmness, flesh/stone attachment and colour and negatively influenced by bitterness and skin separation (Fig. 5). Even though the panelists were not asked to compare the olive samples with commercially fermented 'typical' olives of clearly defined expectations as dominant black colour and acid/salt aroma, it is evident that the panelists preferred olives with a acidic/bitter taste and firm appearance as being similar to 'typical' black table olive features.

Conclusions

The findings of the present work may provide processors with valuable information on how to achieve specific quality olives by targeting oleuropein hydrolysis. At this point, it should be noted that potential use of enzyme in solubilisation of oleuropein instead of traditional lye treatment can result in products of high consumer acceptability. During lye treatment, the lye solution with washing wastewaters cannot be discharged into public sewers, streams or rivers because of their high chemical and biological oxygen demand, mainly due to dissolved organic and inorganic contents. By using β-glucosidases oleuropein can be hydrolysed into glucose and oleuropein aglicone which further biotransforms to other non-bitter tasting metabolites. The results of the study revealed that using β-glucosidase prevents the loss of fermentable material during the washing step of lye treatment, enhances

the initial growth of lactic acid bacteria and results in a favoured end-product with high sensory quality.

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