Annals of Microbiology, **54 (2)**, 161-168 (2004)

Presence of microorganisms in flavoured extra-virgin olive oil

G. CIAFARDINI*, B.A. ZULLO, G. PECA

Department of Animal, Plant and Environmental Sciences, Agriculture Faculty, University of Molise, Via De Sanctis, 86100, Campobasso, Italy

Abstract - The paper reports a study on the presence of microorganisms in the flavoured extra-virgin olive oils. The microbiological analysis of the commercial extra-virgin olive oils flavoured with lemon, oregano, garlic and red chilli pepper indicated the presence of a different type of microflora according to the ingredient. Moulds were present in all of the types of commercial flavoured olive oils, yeasts were only found in the oil enriched with oregano, while bacteria were only occasionally observed. The trials performed in laboratory using experimental extra-virgin olive oil, flavoured with the same ingredients, at zero concentration (control), 1, 5 and 10% (w/v), confirmed the survival of the microorganisms in the flavoured olive oil in a different way according to the type and concentration of the ingredients. In fact, after 40 days of storage, yeasts were present in the olive oil flavoured with 1% of oregano; moulds were widespread in all of the samples of oil flavoured with lemon, oregano and red chilli pepper, while bacteria were only observed in the oil enriched with 5 and 10% of red chilli pepper. The results obtained from both of the trials demonstrated that microorganisms are capable of surviving in the flavoured extra-virgin olive oils, but the ingredients condition the life of the microbes in the oil in a selective way according to their chemical characteristics.

Key words: flavoured olive oil, ingredients, oil microorganisms, survival of microorganisms.

INTRODUCTION

The extra-virgin olive oil is obtained through the grinding and pressing of the olives gathered directly from the plant at the beginning of the ripening period. The newly produced olive oil has a turbid appearance caused by the presence of numerous solid and liquid particles suspended in the oily mass. During storage, the physico-chemical characteristics of the oil are improved due to the sedimentation of the suspended material and to the hydrolytic processes, which involve complex forms of phenolic compounds (Cortesi *et al.,* 1995). The chemical modifications of the oil during storage were attributed to the activity of some

*Corresponding Author. Phone: +39 0874404689; Fax: +39 0874404855; E-mail: ciafardi@unimol.it

enzymes that, together with the solid particles, migrate to the oily mass during the extraction process (Montedoro *et al.,* 1993). Nevertheless, recently microbiological research has also demonstrated that the micro-organisms present on the fruits are capable of migrating in the oil where they join on to the suspended material remaining vital for many months (Ciafardini and Zullo, 2002a, 2002b). Such microorganisms, formed principally from yeasts, are able to modify the chemical characteristics of the oil through the production of several hydrolytic enzymes. In fact, among the microorganisms present in the oil various types of yeasts belonging to the *Saccharomyces cerevisiae*, *Candida wickerhamii*, *Williopsis californica* e *Candida boidinii* (Ciafardini, 2003) species were isolated and distinguished. Some of these yeasts allowed the sweetening of an extra-virgin olive oil through the production of a β-glucosidase active on the bitter glucoside of the oil known as oleuropein, hydrolyzing it into simpler compounds which are no longer bitter in taste (Ciafardini and Zullo, 2002a). From the research reported above, it results that extra-virgin olive oil represents a very selective habitat, where only some types of microorganisms can multiply according to the chemical composition of the medium and of the storage conditions. Nevertheless, other than the extra-virgin olive oil whose habitat is fairly defined, recently there has also been a rapid expansion in the market of olive oils enriched with aromatic products, commercialized as flavoured olive oil, whose habitat is modified differently according to the ingredient used. From the scientific information available, it is not possible to establish if microorganisms exist in the flavoured olive oils and if the aromatic ingredients used are able to influence the survival of the microorganisms in the oily mass. The aim of the present research was to investigate the presence of microorganisms in flavoured extra virgin olive oil.

MATERIALS AND METHODS

Microbiological analysis of commercial flavoured olive oils. The commercial flavoured olive oils were exhibited in 2003 at the "Mostra Internazionale dell'Alimentazione (MIA)" (International Food Exhibition) in Rimini (Italy), by various oil producers from the south of Italy. In particular, analyses were carried out on samples of oils flavoured respectively with lemon, oregano, garlic, and red chilli pepper, produced by three companies situated in Sicily between the provinces of Enna and Catania. A representative part of each sample was first diluted with sterile distilled water and then used to inoculate the Petri dishes containing the specific substrate. During the preparation of dilutes with factor 10 and the inoculation of the Petri dishes, the samples were emulsified and kept in agitation using a vortex mod. Maxi Mixer (FAVS, Bologna-Italy). Total bacteria were evaluated on Standard Plate Count Agar (Oxoid, Basingstoke, Hampshire, England) after 5 days incubation at 32 °C. The moulds were evaluated after 7 days incubation at 28 °C using Potato Dextrose Agar (Becton Dickinson and Company Cockeysville, MD 21030, USA), enriched with 100 μg mL^{-1} of tetracycline. The tetracycline hydrochloride (Sigma Chemical Co., St. Louis, MO) dissolved in distilled water was sterilized using a nitrocellulose filter membrane with a porosity of 0.2 μm (Minisart NML-Sartorius, Göttingen, Germany) and then was added to the PDA just before the solidification of the medium. The

total yeasts were evaluated after 7 days of incubation at 28 °C using the Malt Yeast Glucose Peptone Agar reported by Kurtzman and Fell (1998).

Microbiological analysis of experimental flavoured olive oils. In laboratory conditions four types of extra-virgin olive oil were prepared using respectively lemon, oregano, garlic and red chilli pepper. The experimental flavoured olive oils were obtained in the laboratory according to the traditional methodology used by the Italian commercial firms.

After being washed under tap water and dried by air, the lemons were peeled by hand separating the peel from the rest of the fruit. Next the peel was homogenized for 10 min with a Tefal Mixer (FAVS, Bologna, Italy) and finally added to the oil in the proportions reported below. The oregano flowers were air-dried for about a month, then finely triturated with a mortar and finally added to the oil. The garlic was first divided into cloves, then peeled by hand, homogenized for 10 min with the mixer and finally added to the oil. The red chilli peppers were air-dried for about a month, then finely triturated with a mortar and added to the oil.

Each type of flavoured olive oil was prepared using an extra-virgin olive oil of the “Leccino” variety enriched respectively with zero (control), 1, 5 and 10% (w/v) of lemon, oregano, garlic and red chilli pepper. The oil enriched with the various ingredients in different proportions was mixed by hand for about a minute and then divided into portions of 750 mL in dark bottles with metal tops, previously sterilized in autoclaves at 121 °C for 20 min. For each type and concentration of the ingredient other than the untreated control, four repetitions were set up and the experiment was repeated twice using the same type of oil and the same ingredients. The samples prepared as described were preserved at room temperature in a dark room and analysed after forty days.

At the moment of the analyses the samples were shaken by hand for about a minute and then 10 mL of flavoured olive oil were sampled under sterile conditions and transferred into sterile Test tubes with screw caps. At the end, the samples taken were analysed from a microbiological aspect using the same procedure and the same mediums used in the microbiological analyses of the commercial flavoured olive oils reported above.

RESULTS AND DISCUSSION

The microbiological analyses carried out on the commercial flavoured olive oil indicated the presence of yeasts and moulds in the analysed samples. In particular, bacteria were only found sporadically (data not shown), yeasts were found in the oil flavoured with oregano, while moulds were present in all of the samples of the commercial flavoured olive oils (Fig. 1). Nevertheless, the number of colony formant units (CFU) of moulds was respectively higher in the oil flavoured with red chilli pepper and lower in that containing garlic (Fig. 1). The presence or not of yeasts and the differing content of moulds found in the commercial flavoured olive oils, confirm the hypothesis according to which the different types of ingredients added to the oil are able to affect the survival of the microorganisms by modifying the habitat. In fact, the results as a whole obtained from the subsequent microbiological analyses carried out on the

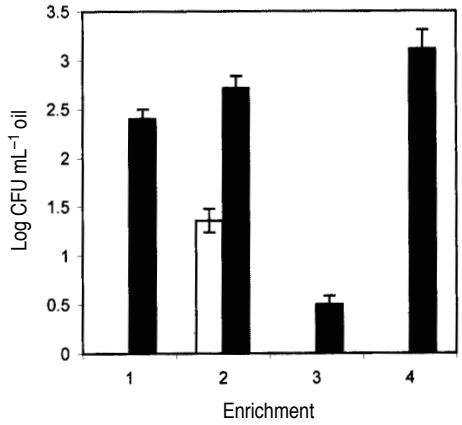

FIG. 1 – Yeasts (□), moulds (■) and bacteria (sporadic), evaluated in commercial flavoured olive oil after one year of storage: (1) flavoured olive oil with lemon; (2) flavoured olive oil with oregano; (3) flavoured olive oil with garlic; (4) flavoured olive oil with red chilli pepper. Data are means ± standard deviation; n = 4.

experimental flavoured olive oil accomplished in the laboratory, strongly confirm this hypothesis. The analyses carried out directly on the ingredients used to prepare the experimental flavoured olive oils in the laboratory, indicated that apart from the garlic, the other ingredients were contaminated by various microorganisms according to the ingredient (Table 1). These findings are in accordance with other authors who observed that spices and herbs may be contaminated due to the conditions in which they were grown and harvested and that fewer microorganisms are present in the spices with higher antimicrobial activity (Pafumi, 1986; Giese, 1994; Kneifel and Berger, 1994). The absence of microorganisms found in the samples of garlic may in part be due to the methods of preparation, where some contaminating microbes present on the cloves were removed through peeling, and partly due to the antimicrobial activity of its chemical components acting on the growth of the microbes present in the samples (Feldberg *et al.,* 1988; Ankri and Mirelman, 1999) (Table 1).

Nevertheless the results obtained from the microbiological analyses, carried out on the experimental flavoured olive oils accomplished in the laboratory using the above ingredients, demonstrated that both the microflora present at

TABLE 1 – Microbiological analyses of the ingredients used to prepare the experimental flavoured extra-virgin olive oils

Ingredient	Total yeasts (Log CFU g^{-1})[a]	Total moulds (Log CFU g^{-1})[a]	Total bacteria (Log CFU g^{-1})[a]
Red chilli pepper	0	2.82±0.09	5.78±0.20
Garlic	0	0	0
Oregano	0	4.00±0.76	5.21±0.15
Lemon	2.12±0.01	2.91±0.50	0

[a] Mean ± standard deviation; n=4.

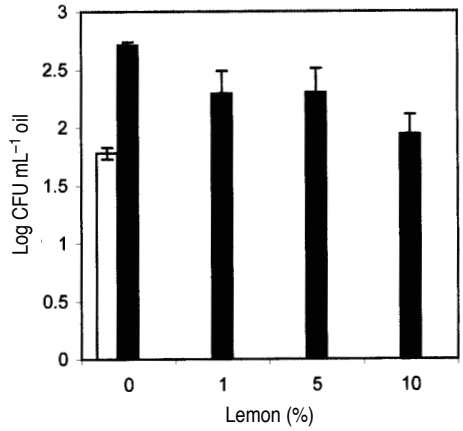

FIG. 2 – Yeasts (□), moulds (■) and bacteria (absent), evaluated in the experimental flavoured olive oil with lemon. Data are means ± standard deviation; n = 4.

the origin in the untreated extra-virgin olive oil (control), and the contaminating microflora introduced to the oil with the ingredients, survive in a different way in the flavoured oils according to the type and concentration of the ingredients used. In fact, in the experimental lemon flavoured olive oil only the presence of moulds was found in concentrations slightly lower in respect to the control, especially in the trial containing 10% lemon (Fig. 2). From the comparison of this data with that shown in Fig. 1 and with the microbiological analyses reported in Table 1, it can be seen that the habitat of the olive oil flavoured with lemon inhibits the survival of yeasts (Fig. 2).

The results of the microbiological analyses carried out on the oregano flavoured olive oil indicated the presence of yeasts and moulds in the samples

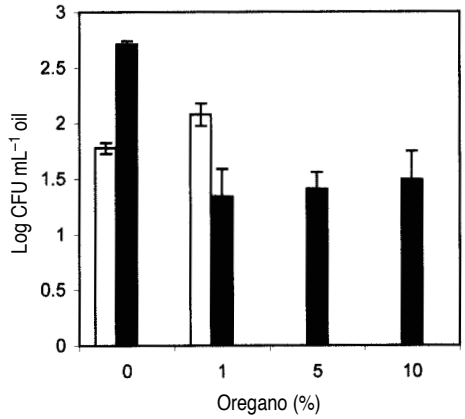

FIG. 3 – Yeasts (□), moulds (■) and bacteria (absent), evaluated in the experimental flavoured olive oil with oregano. Data are means ± standard deviation; n = 4.

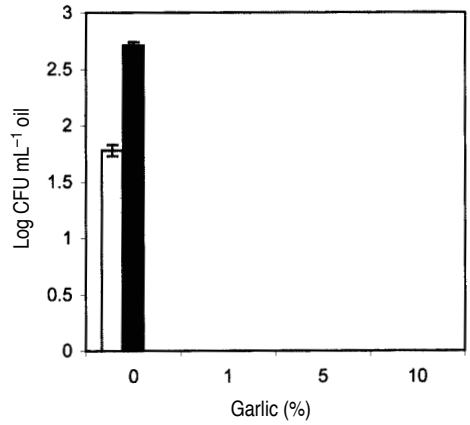

FIG. 4 – Yeasts (□), moulds (■) and bacteria (absent), evaluated in the experimental flavoured olive oil with garlic. Data are means ± standard deviation; $n = 4$.

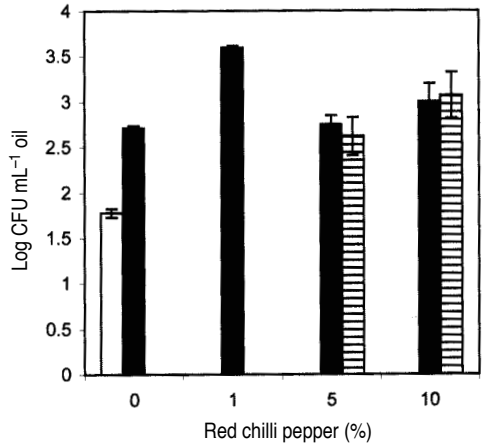

FIG. 5 – Yeasts (□), moulds (■) and bacteria (▣), evaluated in the experimental flavoured olive oil with red chilli pepper. Data are means ± standard deviation; $n = 4$.

containing 1% of the ingredient, while in the samples containing a higher concentration of oregano only the presence of moulds was found (Fig. 3). Nevertheless, in this type of flavoured olive oil the oregano was initially contaminated not by yeasts but by moulds and bacteria (Table 1), therefore it can be assumed that the yeasts observed in the trial with 1% of oregano originate solely from the extra-virgin olive oil used in the experiment (Fig. 3). In this type of flavoured olive oil the survival of the yeasts seems to depend on the concentration of oregano used as an ingredient, while regarding the bacteria, even if their presence was abundant on the oregano (Table 1), they were never found in the flavoured olive oil. The data reported in Fig. 3 confirms the results of the micro-

biological analyses carried out on the commercial olive oil flavoured with 1% of oregano where both the presence of yeasts and moulds can be observed (Fig. 1).

The oil flavoured with garlic clearly differentiated itself from all the other types. In fact, the recognised antimicrobial action of some components of garlic, reported by various authors (Uchida *et al*., 1975; Yoshida *et al*., 1987, 1999), was confirmed both in the experimental flavoured olive oil, where the complete absence of any microorganism was observed (Fig. 4), and in the commercial flavoured olive oil characterized by a low content of moulds (Fig. 1).

The oil flavoured with red chilli pepper prevented the survival of yeasts in all of the samples, while moulds were always found present confirming what had already been observed in the commercial flavoured olive oils (Fig. 5). In this type of flavoured olive oil, when the concentration of the ingredient was above 5%, the presence of some types of bacteria was also observed which originally contaminated the red chilli peppers (Table 1).

In conclusion, the results of the microbiological analyses carried out on the commercial flavoured olive oil and on the experimental flavoured olive oil demonstrate that microorganisms can survive in the extra-virgin flavoured olive oils. The type of ingredient and its concentration in the oil strongly influence the survival of the microorganisms, independently of the type and amount of initial contaminant. Such conditioning can be clearly observed during the first 40 days of storage, especially when ingredients characterized by high levels of antimicrobial activity such as garlic are used.

REFERENCES

Ankri S., Mirelman D. (1999). Antimicrobial properties of allicin from garlic. Microbes and Infection, 1(2): 125-129.

Ciafardini G., Zullo B.A. (2002a). Microbiological activity in stored olive oil. International Journal of Food Microbiology, 75: 111-118.

Ciafardini G., Zullo B.A. (2002b). Survival of microorganisms in extra virgin olive oil. Food Microbiology, 19: 105-109.

Ciafardini G. (2003). Presenza di microrganismi nell'olio extra vergine d'oliva. L'Informatore Agrario, 44: 39-42.

Cortesi N., Azzolini M., Novellini P., Fedeli E. (1995). The minor, polar components of the virgin olive oils: hypothesis of structure through LC-MS. Rivista Italiana delle Sostanze Grasse, 72: 241-251.

Feldberg R.S., Chang S.C., Kotik A.N., Nadler M., Neuwirth Z., Sundstrom D.C., Thompson N.H. (1988). *In vitro* mechanism of inhibition of bacterial cell growth by allicin. Antimicrobial Agents and Chemotherapy, 32(12): 1763-1768.

Giese J. (1994). Spices and seasoning blends: A taste for all seasons. Food Technology, 48(4): 87-98.

Kneifel W., Berger E. (1994). Microbial criteria of random samples of spices and herbs retailed on the Austrian market. Journal of Food Protection, 57: 893-901.

Kurtzman C.P., Fell J.W. (1998). The yeasts. A Taxonomic Study. Elsevier, Amsterdam, pp. 320-351.

Montedoro G.F., Servili M., Baldioli M., Selvaggini R., Miniati E., Macchioni A. (1993). Simple and hydrolyzable compounds in virgin olive oil. 3. Spectroscopic characterizations of the secoiridoid derivatives. Journal of Agricultural Food Chemistry, 41: 2228-2234.

Pafumi J. (1986). Assessment of microbiological quality of spices and herbs. Journal of Food Protection, 49: 958-963.

Uchida Y., Takahashi T., Sato N. (1975). The characteristics of the antibacterial activity of garlic. The Japanese Journal of Antibiotics, 28(4): 638-642.

Yoshida S., Kasuga S., Hayashi N., Ushiroguchi T., Matsuura H., Nakagawa S. (1987). Antifungal activity of ajoene derived from garlic. Applied and Environmental Microbiology, 53(3): 615-617.

Yoshida H., Katsuzaki H., Ohta R., Ishikawa K., Fukuda H., Fujino T., Suzuki A. (1999). Antimicrobial activity of the thiosulfinates isolated from oil-macerated garlic extract. Bioscience Biotechnology and Biochemistry, 63(3): 591-594.