Report

Microbiological Food Safety of Olive Oil: A Review of the Literature

Mary Palumbo and Linda J. Harris

December 2011

UC DAVIS OLIVE CENTER
at the Robert Mondavi Institute

olivecenter.ucdavis.edu

Copyright © 2011 UC Regents, Davis campus. All rights reserved.

AUTHORS

Dr. Mary Palumbo Dr. Palumbo is a food scientist with the Western Institute for Food Safety and Security at UC Davis. She works on various research and outreach projects, with an emphasis in tree nuts. She also coordinates with UC Food Safety and the Center for Produce Safety at UC Davis, including maintaining information on publications, outbreaks and recalls on the UC Food Safety website.

Dr. Linda J. Harris Dr. Harris is a specialist in UC Cooperative Extension and the Associate Director of the Western Institute for Food Safety and Security at UC Davis. Dr. Harris's research focuses on microbial food safety,emphasizing the microbiology of fresh fruits and vegetables and tree nuts. She develops and validates standard microbiological methods and uses these methods to evaluate the behavior of food borne pathogens on fruits, vegetables and tree nuts under different storage and processing conditions. She uses these methods to evaluate antimicrobial treatments including various sanitizers and thermal processes for their efficacy in reducing microbial populations on various cut and intact produce and tree nut surfaces. She is the co-host of the UC Food Safety website.

UCDAVIS OLIVE CENTER at the Robert Mondavi Institute

The UC Davis Olive Center is the only academic center of its kind in North America, a leader in education and research on olive growing and processing. Learn more at www.olivecenter.ucdavis.edu and on Facebook.

CONTENTS

Microbiological food safety of olive oil: A review of the literature

Summary

Very little data are available on the microbiological safety of edible oils including olive oil. Most of the food safety literature regarding olive oils has been published within the past few years. No outbreaks of foodborne illness linked to olive oil have been reported. There have been no surveys that have assessed the presence of foodborne pathogens in olive oils; surveys that included a determination of general bacterial populations found levels from below the limit of detection (1 to 2 log CFU/ml) to 3 log CFU/ml. Olive oils (virgin and extra virgin) are unique among the edible oils in that they contain small amounts of water in the form of tiny droplets; the pH of the water phase has been reported as generally less than pH 5. Microorganisms have been shown by microscopic evaluation to be present in this water phase but the physical size of the droplets generally constrains microbial numbers. Antimicrobials effective against a broad range of microorganisms including some foodborne pathogens have been shown to be present in extra virgin olive oils. The published studies to date are limited in the number of strains of pathogens assessed, and, in some cases, details in the methods and specifics of the olive oils were lacking. The antimicrobial components, generally ascribed to the soluble phenolic components, can vary in composition and amount for a wide range of reasons including olive variety, production practices, maturity, extraction methods, storage conditions and time. Based on the currently available literature, foodborne pathogens are not likely to occur in extra virgin or virgin olive oils. However, good manufacturing practices and other prerequisite programs that keep all microorganisms at low levels should be followed in the production of olive oil.

Types of olive oil

Trade standards for olive oils are somewhat different in the United States, where retail standards are promulgated by the U.S. Department of Agriculture (USDA, 2010), compared to other olive-producing countries, which follow standards of the International Olive Council (IOC). Much of the pertinent scientific literature comes from countries that use the IOC standards, and therefore, also use the IOC definitions for olive oil types (IOC, 2010). Research studies that have contributed information useful in evaluating the safety of olive oil have been conducted with all types of olive oil as well as olive oil from different geographic regions and products extracted using different production methods.

Dimorphic yeasts include some species considered opportunistic pathogens, such as *Candida guilliermondii* and *Candida parapsilosis*, both of which were identified among the isolates from these oils.[1] Consumption of olive oil is not likely to cause these infections.

Olive oil production methods

To extract oil the olives are first ground or milled to a paste; traditionally this was done with mill stones, but most processors now use a hammer mill. The paste may be slowly stirred or kneaded (malaxation) to allow water and oil droplets to unite into larger droplets, making it easier to separate the water and oil phases. The paste may be pressed by spreading onto fiber plates, stacking the plates, and applying pressure to separate the liquid from the solid material. The resulting cloudy olive oil may then be allowed to separate by gravity to give a clear product. Alternatively, the paste may be centrifuged in a horizontal centrifugal separator, or decanter, to separate the oil from the solid and aqueous phases. In California, two-phase separation systems yielding an oil phase and wet solid phase are most common (Flynn, 2011). Sometimes the oil is filtered to eliminate solid particles remaining in the oil. These variations in production methods produce oils with different characteristics, which may affect chemical and microbial characteristics. For example, Artajo et al. (2007) found that malaxation time had an important effect on the content of alcohols and secoiridoids. They also observed that the concentration of the different phenolic groups in the solid and liquid phases decreased significantly in the mid- and late-season samplings compared to the first-season samplings, perhaps as a result of the advanced ripening stage of the olive fruit. Cerretani et al. (2005) compared the concentration of total phenols and phenolic profiles obtained by high performance liquid chromotography - diode array detector/mass spectrometry detector (HPLC-DAD/MSD) of two monovarietal oils obtained from a continuous industrial plant and a low-scale mill. Di Giovacchino, Sestili, and Di Vincenzo (2002) review the influence of olive oil processing on virgin olive oil quality, including phenolic content.

Microorganisms present in olive oil

Studies of the naturally occurring microorganisms in olive oil have been limited and do not give a complete picture for olive oils produced in all of the olive-producing regions of the world. Ciafardini and Zullo (2002a and 2002b) examined olive oils produced in central Italy, and conducted microbiological

---

[1] Definition of opportunistic pathogen
An opportunistic infection is an infection caused by pathogens (bacterial, viral, fungal or protozoan) that usually do not cause disease in a healthy host, i.e. one with a healthy immune system. A compromised immune system, however, presents an "opportunity" for the pathogen to infect. Candida guilliermondii has been isolated from numerous human infections, mostly of cutaneous origin. One systemic infection has been reported in a patient with aplastic anemia. C. guilliermondii has been isolated from normal skin and in sea water, feces of animals, fig wasps, buttermilk, leather, fish, and beer. Candida parapsilosis is an opportunistic human pathogen which may cause both superficial cutaneous infections, especially of the nail, and systemic disease, especially endocarditis. Environmental isolates have been obtained from intertidal and oceanic waters, pickle brine, cured meats, olives, and normal skin and feces.
Consumption of olive oil is not likely to cause these infections.

From http://en.wikipedia.org/wiki/Opportunistic_pathogen
Mycology Online accessed on March 18, 2011, at http://www.mycology.adelaide.edu.au/Fungal_Descriptions/Yeasts/Candida/Candida_guilliermondii.html
Mycology Online accessed on March 18, 2011, at http://www.mycology.adelaide.edu.au/Fungal_Descriptions/Yeasts/Candida/Candida_parapsilosis.html

analysis for aerobic (Standard Plate Count agar) and lactic acid bacteria (MRS agar), yeasts (Sabouraud medium), and molds (glucose yeast extract agar with gentamicin and chlorampenicol). They reported that yeasts were consistently present both initially and during storage, molds were occasionally found, and bacteria were never found. The molds belonged primarily to the genus *Aspergillus*. The 50 yeast isolates examined were classified as *Saccharomyces cerevisiae* and *Candida wickerhamii* at a ratio of 3:1. Ciafardini and Zullo (2002b) determined the biochemical activities of these species. They also showed, by light microscopy, that the microorganisms and the solid particles were entrapped in micro drops of vegetation water that were suspended in the olive oil. Ciafardini et al. (2004) found *Williopsis californica* and *Candida boidinii* in addition to *S. cerevisiae* and *C. wickerhamii* in olive oil produced in central Italy. Zullo and Ciafardini (2008) found *Candida parapsilosis* in commercial olive oil produced in Italy. Zullo, Cioccia, and Ciafardini (2010) studied the distribution of dimorphic yeast forms in commercial extra virgin olive oil from 23 producers in north and central Italy. Of the 23 olive oil samples analyzed, six contained dimorphic yeasts. Populations of total yeasts in the samples ranged from 23 to 15,000 CFU/ml of oil; in the six samples that contained dimorphic yeasts, their populations ranged from 3 to 5300 CFU/ml. Dimorphic yeasts include some species considered opportunistic pathogens, such as *Candida guilliermondii* and *Candida parapsilosis*, both of which were identified among the isolates from these oils.

Koidis, Triantafillou, and Boskou (2008) examined cloudy olive oil (freshly produced before full precipitation), obtained from a processor in Greece, for total aerobic microbiota, lactic acid bacteria, yeasts, and molds using the same media as Ciafardini and Zullo (2002a and 2002b). They followed the evolution of these counts in two cloudy samples and one commercial oil sample at 15-day intervals for 90 days, sampling both the upper portion and lower portion of the stored oil. Initial counts for total plate counts, lactic acid bacteria and yeast ranged from at or below the limit of detection (assumed to be approximately 1 or 2 log CFU/ml) to ~3 log CFU/ml. In general, aerobic plate counts decreased in most upper portion samples and remained unchanged in the bottom portion samples. With the exception of lactic acid bacteria in the bottom portion of one cloudy sample, counts did not increase. Yeast counts also were higher in the bottom portion than in the upper portion, but remained below 1000 CFU/ml. Yeasts and lactic acid bacteria declined to nondetectable levels at 60 days in the upper portion but continued to be detected for 90 days (end of study) in the bottom portion. The yeasts identified were *Candida guillermondii*, *C. parapsilosis*, *C. lusitaniae*, *C. famata*, *C. albicans* 1, and *Rhodotorula mucilaginosa* 2. During storage, mold counts increased from less than 1 log to ~1.5 log CFU/ml. The molds identified belonged to the genera *Helicosporium*, *Alternaria*, *Penicillium*, and *Aspergillus*.

Components of olive oil affecting microbial growth and survival

Olive oil differs from other edible oils not only in its fatty acid composition, but also in the presence of minor bioactive compounds because it is consumed unrefined. All of the types of olive oil contain essentially the same fatty acids, but not the same concentrations of minor components such as triterpene acids and alcohols, α-tocopherol, squalene, phenolic acids, lignans, and polyphenols. Major phenolic compounds in olive oil are generally classified as simple phenols (e.g., hydroxytyrosol, tyrosol, vanillic acid); secoiridoids (e.g., oleuropein glucoside), dialdehydic forms of oleuropein; and polyphenols and flavonols.

Freshly-produced olive oil contains small amounts of water naturally emulsified in the oil. The amount of water depends upon the processing methods. Greek researchers (Koidis, Triantafillou, and Boskou, 2007) examined freshly produced (cloudy) olive oils and commercially filtered olive oils and found water contents ranging from 0.17 to 0.49% for the cloudy oils and 0.08 and 0.09% for the commercial oils.

Microorganisms were trapped in the water droplets in the cloudy oils. The size of the water droplets was 1 to 5 µm in the freshly produced oil; after 1 month sedimentation, a sample from the bottom portion of the oil showed a higher concentration of droplets, but the droplets remained approximately the same size. The small size of these droplets limit nutrient availability and "space" for microorganisms. The best estimate available for the pH of the aqueous phase comes from the mill waste. For two-phase extraction systems in Spain, the pH of the mill waste has been reported as 5.0 to 5.5 (range for four samples taken at different times) by Cayuela et al. (2008) and as 4.5 by Cerrone et al. (2010).

The possibility that olive oil has antimicrobial properties has been recognized for many years. An Italian study published in 1970 noted that, in Italy, imported tuna was commonly canned in olive oil at heat treatments that would not be expected to be effective against *Clostridium botulinum* "without significant spoilage or public health risk" (Dallyn and Everton, 1970). They demonstrated that a water-soluble component in some olive oils significantly reduced the heat resistance of spores of *Clostridium sporogenes* (putrefactive anaerobe 3679 – a typical surrogate for *C. botulinum*). The presence of an inhibitor of lactic acid bacteria in green olives was reported in the 1960s (Fleming and Etchells, 1967; Fleming, Walter, and Etchells,1969) and Juven and Henis (1970) identified oleuropein as one component of the observed antimicrobial activity in olives. Several studies since then have examined the antimicrobial properties of oleuropein (Koutsoumanis et al., 1998; Tassou and Nychas, 1994 and 1995; Tranter et al., 1993).

Medina et al. (2006) studied the antimicrobial activity of different edible oils, and found that oils from olive fruit had strong bactericidal action against both gram positive and gram negative bacteria. Greater than 4 log reductions were observed within 1 h of exposure with Picual and Arbequina virgin olive oils for single strains of *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella enterica*, *Yersinia* sp. and *C. perfringens*. Reductions of single strains of *E. coli* (unknown serotype) and *Shigella sonnei* were 1-2 or 2-4 log, respectively during the same time frame. No reductions were observed with sunflower or corn oil. None of the oils, including olive oil, were inhibitory to the yeast *Candida albicans*. The phenolic compounds in the olive oils were identified and their concentrations estimated. The dialdehydic form of decarboxymethyl oleuropein and ligstroside aglycons, hydroxytyrosol, and tyrosol were the phenolic compounds that statistically correlated with bacterial survival.

Virgin olive oil and olive oil purchased from a retail market in Spain were tested for antimicrobial effects against foodborne pathogens. Greater than 4-log reductions of one or two strains of *S. aureus*, *E. coli* O157:H7, *L. monocytogenes*, *Shigella sonnei*, *S.* Enteritidis, or *Yersinia* sp. were achieved after 5 min of exposure to the virgin olive oil. Reductions in the olive oil (not virgin) ranged from greater than 4 log (*S. aureus* and *Yersinia* sp.) to less than 1 log (*L. monocytogenes*) (Medina et al., 2007).

Turkish extra virgin olive oils and refined oils (olive, hazelnut, and canola) were evaluated for antimicrobial activity against single strains of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* Enteritidis (Karaosmanoglu et al., 2010). The refined oils were selected because they have very similar fatty acid compositions to extra virgin olive oil but their phenolic concentrations are different. With the extra virgin olive oils, populations of the pathogens decreased from 5 log CFU/ml to below the limit of detection within 1 h of exposure to two different extra virgin olive oils (highest and lowest total phenolic contents of nine evaluated). Less than 0.4-log CFU/ml reductions were observed for refined olive, canola, or hazelnut oils. Using initial populations of 5.7 log CFU/ml and 5-min treatment times, substantial differences in activity were observed among the individual variety olive oils (smaller reductions observed for oils with lower total phenolic content).

Individual phenolic compounds isolated from Turkish extra virgin olive oils, including cinnamic acid, ferulic acid, 4-hydroxybenzoic acid, luteolin, syringic acid, tyrosol, vanillic acid, and vanillin were

evaluated for their antimicrobial activity. Most were able to inhibit the growth of *L. monocytogenes* or *S.* Enteritidis to some degree. Inhibition corresponded to concentration of the phenolic compound.

Medina et al. (2009) compared the bactericidal effects of several olive phenolic compounds with other food phenolic compounds and with synthetic disinfectants glutaraldehyde and ortho-phthalaldehyde against *Pseudomonas* fluorescens, *S. aureus*, *Enterococcus faecalis*, and *E. coli*. Olive compounds with a dialdehydic structure exhibited strong bactericidal activity, and in the presence of organic material, stronger bactericidal activity than the synthetic disinfectants. The ability of phenolic fractions from extra virgin olive oil to inhibit the growth of two strains of lactic acid bacteria, one strain of yeast and one mold was evaluated Keceli and Robinson, 2002). Inhibition of growth was dependent on concentration, pH (greater impact at lower pH), and microorganism (variable impact on the lactic acid bacteria, no inhibition of the mold).

Variability in components affecting survival of bacterial pathogens

The water content of olive oil is known to depend on the method of processing used to obtain the oil and the length of time that the oil is stored for sedimentation. Less well known are the changes in oil components that have antimicrobial activity. Several studies have shown that the composition of the phenolic fraction varies with cultivar, ripeness, climatic conditions, and oil extraction processes; these have been reviewed by Gallina-Toschi et al. (2005). For example, Patumi et al. (2002) studied the effect of irrigation differences on the characteristics of olives and olive oil, including phenolic compounds. The content of total polyphenols in the fruit and in the oil decreased with increasing levels of irrigation. The individual phenolic compounds measured also declined, with the exception of hydroxytyrosol, which increased. Cerretani et al. (2005) demonstrated differences in phenolic content for cultivars (Ghiacciolo and Nostrana di Brisighella) and with extraction technique (low-scale mill compared to industrial mill).

Aflatoxins and other mycotoxins in olive oil

Aflatoxins are metabolites produced by the molds *Aspergillus flavus*, *A. parasiticus*, and *A. nomius*. These compounds are potent carcinogens, teratogens, and mutagens and are a serious hazard to human and animal health. Ochratoxins are produced by various species of *Aspergillus* and *Penicillium* molds; Ochratoxin A is the most toxic. Molds grow on plant materials, including olives, held under warm humid conditions, although toxigenic molds did not grow equally well on all varieties of Greek olives (Ghitakou et al., 2006). Virgin and extra virgin olive oils are consumed without undergoing the refining steps used to remove aflatoxins from other vegetable oils. Ferracane et al. (2007) analyzed 30 samples of virgin oils from southern Italy and Morocco, and reported 0.1 to 17.0 ng/g (ppb) ochratoxin A (with mean 0.7) in 80% of samples assayed, and 0.54 to 2.50 ng/g (ppb) aflatoxin B in 10% of samples. Daradimos, Marcaki, and Koupparis (2000) analyzed 50 samples of Greek olive oil for aflatoxin B1, and found it in 72% of samples tested, with a range of 2.8 to 15.7 ng/kg (0.0028 to 0.0157 ng/g (ppb)). Current tolerance levels set by the European Community for most food products are 2 ppb aflatoxin B1 and 4 ppb total aflatoxins, although edible oils are not specifically addressed (EC, 2006).

Safety of products containing olive oil

Virgin olive oil in milk- or egg-based mayonnaises in combination with lemon juice reduced populations of inoculated *Salmonella* Enteritidis and *L. monocytogenes* by approximately 3 log CFU/g in 30 min

(Medina at al., 2007). Mayonnaise made with sunflower oil with or without lemon juice and mayonnaise made with virgin olive oil alone did not result in reduced populations of S. Enteritidis. Mayonnaise prepared with egg yolk, acetic acid, and oil (extra virgin olive oil, blended olive oil, or sunflower oil) was inoculated with *Salmonella* Enteritidis PT 4 and held at 20°C for 0, 24, 48, and 72 h (Radford et al., 1991). No viable *S.* Enteritidis were recovered from the extra virgin olive oil mayonnaise samples after 72 h, and the death rate was significantly faster than for mayonnaises prepared with blended olive oil or sunflower oil. The blended olive oil and sunflower oils contained insignificant amounts of phenolic compounds. The pH of all the mayonnaises evaluated was 4.3.Populations of *L. monocytogenes* were reduced by greater than 2 log CFU/g on lettuce when dressed with virgin olive oil, or virgin olive mixed with either lemon juice or vinegar (Medina et al., 2007).

A botulism outbreak in 1989 was associated with a garlic-in-olive oil product that had been stored at room temperature (Morse et al., 1990). The implicated product was prepared by mixing chopped garlic, ice water, and extra virgin olive oil and was labeled "keep refrigerated" in small print. The index patient had stored the product at room temperature for approximately three months before opening it, and then placing it in the refrigerator. He had used small quantities in cooked items before using it to prepare garlic bread. The garlic bread was wrapped in aluminum foil and heated at 300°F for 20 min before serving. Three individuals became ill with botulism.

References

Artajo, L.-S., M.-P. Romero, M. Suarez, and M-J. Motilva. 2007. Partition of phenolic compounds during the virgin olive oil industrial extraction process. Eur. Food Res. Technol. A 225 (5-6): 617-625.

Cayuela, M.L., P.D. Millner, S.L.F. Meyer, and A. Roig. 2008. Potential of olive mill waste and compost as biobased pesticides against weeds, fungi, and nematodes. Sci. Total Environ. 399: 11-18.

Cerretani, L., A. Bendini, A. Rotondi, G. Lercker, and T.G. Toschi. 2005. Analytical comparison of monovarietal virgin olive oils obtained by both a continuous industrial plant and a low-scale mill. Eur. J. Lipid Sci. Technol. 107: 93-100.

Cerrone, F., M. del Mar Sanchez-Peinado, B. Juarez-Jimenez, J. Gonzalez-Lopez, and C. Pozo. 2010. Biological treatment of two-phase olive mill wastewater (TPOMW, alpeorujo): Polyhydroxyalkanoates (PHAs) production by *Azotobacter* strains. J. Microbiol. Biotechnol. 20 (3): 594-601.

Ciafardini, G., G. Cioccia, G. Peca, and B.A. Zullo. 2004. Transfer of selected yeasts to oil through olive inoculation. Ital. J. Food Sci. 16 (1): 105-111.

Ciafardini, G., and B.A. Zullo. 2002a. Microbiological activity in stored olive oil. Intl. J. Food Microbiol. 75: 111-118.

Ciafardini, G., and B.A. Zullo. 2002b. Survival of micro-organisms in extra virgin olive oil during storage. Food Microbiol. 19: 105-109.

Dallyn, H., and J.R. Everton. 1970. Observations on the sporicidal action of vegetable oils used in fish canning. J. Appl. Bacteriol. 33: 603-608.

Daradimos, E., P. Marcaki, and M. Koupparis. 2000. Evaluation and validation of two fluorometric HPLC methods for the determination of aflatoxin B1 in olive oil. Food Addit. Contamin. Part A 17 (1): 65-73.

Di Giovacchino, L., S. Sestili, and D. Di Vincenzo. 2002. Influence of olive processing on virgin olive oil quality. Eur. J. Lipid Sci. Technol. 104: 587-601.

European Commission (EC) Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Off. J. Eur. Union. 2006;L 364:5–24.

Ferracane, R., A. Tafuri, A. Logieco, F. Galvano, D. Balzano, and A. Ritieni. 2007. Simultaneous determination of aflatoxin B1 and ochratoxin A and their natural occurrence in Mediterranean virgin olive oil. Food Addit. Contam. Part A 24 (2): 173-180.

Fleming, H.P., and J.L. Etchells. 1967. Occurrence of an inhibitor of lactic acid bacteria in green olives. Appl. Microbiol. 15 (5): 1178-1184.

Fleming, H.P., W.M. Walter, Jr., and J.L. Etchells. 1969. Isolation of a bacterial inhibitor from green olives. Appl. Microbiol. 18 (5): 856-860.

Flynn, D. 2011. University of California, Davis, personal communication.

Gallina-Toschi, T., L. Cerretani, A. Bendini, M. Bonoli-Carbognin, and G. Lercker. 2005. Oxidative stability and phenolic content of virgin olive oil: An analytical approach by traditional and high resolution techniques. J. Sep. Sci. 28: 859-870.

Ghitakou, S., K. Koutras, e. Kanellou, and P. Markaki. 2006. Study of aflatoxin B1 and ochratoxin A production by natural microflora and Aspergillus parasiticus in black and green olives of Greek origin. Food Microbiol. 23: 612-621.

International Olive Council (IOC). 2010. Trade Standard Applying to Olive Oils and Olive-Pomace Oils. Available at http://www.internationaloliveoil.org/estaticos/view/222-standards. Accessed on 3/23/2011.

Juven, B., and Y. Henis. 1970. Studies on the antimicrobial activity of olive phenolic compounds. J. Appl. Bacteriol. 33: 721-732.

Karaosmanoglu, H., F. Soyer, B. Ozen, and F. Tokatli. 2010. Antimicrobial and antioxidant activities of Turkish extra virgin olive oils. J. Agric. Food Chem. 58: 8238-8245.

Keceli, T., and R.K. Robinson. 2002. Antimicrobial activity of phenolic extracts from virgin olive oil. Milchwissenschaft 57: 436-440.

Koidis, A., E. Triantafillou, and D. Boskou. 2008. Endogenous microflora in turbid virgin olive oils and the physiochemical characteristics of these oils. Eur. J. Lipid Sci. Technol. 110: 164-171.

Koutsoumanis, K., C.C. Tassou, P.S. Taoukis, and G.-J.E. Nychas. 1998. Modelling the effectiveness of a natural antimicrobial on *Salmonella* Enteritidis as a function of concentration, temperature and pH, using conductance measurements. J. Appl. Microbiol. 84: 981-987.

Medina, E., M. Brenes, A. Garcia, C. Romero, and A. de Castro. 2009. Bactericidal activity of glutaraldehyde-like compounds from olive products. J. Food Prot. 72: 2611-2614.

Medina, E., A. de Castro, C. Romero, and M. Brenes. 2006. Comparison of the concentrations of phenolic compounds in olive oils and other plant oils: Correlation with antimicrobial activity. J. Agric. Food Chem. 54: 4954-4961.

Medina, E., C. Romero, M. Brenes, and A. de Castro. 2007. Antimicrobial activity of olive oil, vinegar, and various beverages against foodborne pathogens. J. Food Prot. 70(5):1194-1199.

Morse, D.L., L.K. Pickard, J.J. Guzewich, B.D. Devine, and M. Shayegani. 1990. Garlic-in-oil associated botulism: episode leads to product modification. Am. J. Pub. Health 80(11):1372-1373. Available at http://ajph.aphapublications.org/cgi/reprint/80/11/1372 Accessed on July 8, 2011.

Patumi, M., R. d'Andria, V. Marsilio, G. Fontanazza, G. Morelli, and B. Lanza. 2002. Olive and olive oil quality after intensive monocone olive growing (*Olea europaea* L., cv. Kalamata) in different irrigation regimes. Food Chem. 77: 27-34.

Radford, S.A., C.C. Tassou, G.J.E. Nychas, and R.G. Board. 1991. The influence of different oils on the death rate of *Salmonella* Enteritidis in homemade mayonnaise. Letters Appl. Microbiol. 12:125-128.

Tassou, C.C., and G.J.E. Nychas. 1994. Inhibition of *Staphylococcus aureus* by olive phenolics in broth and in a model food system. J. Food Prot. 57: 120-124.

Tassou, C.C., and G.J.E. Nychas. 1995. Inhibition of *Salmonella* Enteritidis by oleuropein in broth and in a model food system. Letters Appl. Microbiol. 20: 120-124.

Tranter, H.S., C.C. Tassou, and G.J.E. Nychas. 1993. Effect of the olive phenolic compound, oleuropein, on growth and enterotoxin B production by *Staphylococcus aureus*. J. Appl. Bacteriol. 74: 253-260.

U.S. Department of Agriculture (USDA). 2010. United States Standards for Grades of Olive Oil and Olive-Pomace Oil. Available at http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELDEV3011889 Accessed on 3/23/2011.

Zullo, B.A., and G. Ciafardini. 2008. Lipolytic yeasts distribution in commercial extra virgin olive oil. Food Microbiol. 25 (8): 970-977.

Zullo, B.A., G. Cioccia, and G. Ciafardini. 2010. Distribution of dimorphic yeast species in commercial extra virgin olive oil. Food Microbiol. 27:1035-1042.

Annotated Bibliography

Artajo, L.-S., M.-P. Romero, M. Suarez, and M-J. Motilva. 2007. Partition of phenolic compounds during the virgin olive oil industrial extraction process. Eur. Food Res. Technol. A 225 (5-6): 617-625.

Oleuropein and ligstroside are secoiridoids (type of monoterpene) present in olive paste at crushing. The concentration of these compounds changes with time of malaxation (mixing or kneading of the paste). Oleuropein was present in the crushed olive paste early in the production season, but declined to trace levels late in the production season. Concentration of oleuropein declined during malaxation and was nondetectible in the oil and wet pomace phases after centrifugation. However derivatives of this class of compounds (3,4-DHPEA-EDA and p-HPEA-EDA) were present in the oil phase (and have been shown by Medina et al., 2009, to have bactericidal activity) if the precursors are present in the olive paste. Other investigators have shown that the composition of the phenolic fraction depends on the cultivar, climatic conditions during growth, degree of maturation, and the technology used for oil extraction.

Baiano, A., C. Terracone, G. Gambacorta, and E.L. Notte. 2009. Changes in quality indices, phenolic content and antioxidant activity of flavored olive oils during storage. J. Am. Oil Chem. Soc. 86:1083-1092.

The effects of spices and herbs on the quality, phenolic and antioxidant activity of olive oils were examined at 0, 6, and 9 months of storage. Olive oil samples were made from Peranzana olives from Apulia, Italy. The spices and herbs used for flavoring were lemon, garlic, oregano, cayenne hot pepper, and rosemary. An unflavored olive oil sample was used as a control. A marked decline in total phenolic content was observed for the unflavored oil during storage as well as for all of the flavored oils except for the hot-pepper flavored oil (which showed an increase at 6 months and a decrease at 9 months). The composition of the phenolics changed over time as well. The amounts of tyrosol and hydroxytyrosol increased, while the amounts of 3,4-DHPEA-EDA and p-HPEA-EDA (degradation products of oleuropein and ligstroside) decreased markedly between 6 and 9 months.

Cerretani, L., A. Bendini, A. Rotondi, G. Lercker, and T.G. Toschi. 2005. Analytical comparison of monovarietal virgin olive oils obtained by both a continuous industrial plant and a low-scale mill. Eur. J. Lipid Sci. Technol. 107: 93-100.

Analytical comparison included total phenols, o-diphenols, simple phenols (hydroxytyrosol and tyrosol), secoiridoid derivates (dialdehydic form of deacetoxy-oleuropein aglycon, aleuropein aglycon and ligstroside aglycon). The level of total phenols, simple phenols, and o-diphenols was significantly greater for both varieties studied when oil was extracted in a low-scale mill compared to an industrial mill. The level of secoiridoid derivates was significantly higher for the Ghiacciolo cultivar but not the Nostrana di Brisighella cultivar when extracted in the low-scale mill.

Ciafardini, G. and B.A. Zullo. 2002. Microbiological activity in stored olive oil. Intl. J. Food Micro. 75:111-118.

The purpose of this study was to determine whether the hydrolysis of oleuropein that occurs during decanting and storage of olive oil is catalyzed by enzymes from the olives or from microorganisms present on the fruit. Olive oil samples used were from the Leccino olives in Italy. Microbiological analysis included enumeration of bacteria (PCA and MRS for lactic acid bacteria), yeasts (Sabouraud medium), and molds (oxytetracyclin glucose yeast extract agar with antibiotics). Microscopic observation showed the presence of microorganisms in the water droplets and sediments in the olive oil. These microorganisms were present throughout the period of preservation. Yeasts were found to be the most abundant in the olive oil samples, while bacteria and molds were not found. These yeasts were identified as *Saccharomyces cerevisiae* and *Candida wickerhamii*. They can break down oleuropein due to producing β-glucosidase. This enzyme can also be produced by the olive fruit. After a few months, the bitterness disappears in the olive oil due to this enzyme. The absence of lipases in the isolated yeasts lead the authors to believe the yeasts contribute in a positive way to the organoleptic quality of the oil.

Ciafardini, G. and B.A. Zullo. 2002. Survival of micro-organisms in extra virgin olive oil during storage. Food Micro. 19:105-109.

Olive oil studied was obtained from Leccino variety olives from central Italy, processed at low temperatures. Microorganisms, primarily yeasts, were found on the sediment particles and in water droplets in newly produced olive oil, and persisted for the 150 days of the experimental period in unfiltered oil. The decline in numbers observed during

the first month of sedimentation could be attributed to the migration of suspended particles to the bottom of the vessel. Filtered oil contained fewer yeasts, which can be attributed to mechanical removal of suspended particles. Fungi belonging to the genus *Aspergillus* were occasionally found; bacteria were not found. Total polyphenol concentrations were monitored throughout the experimental period, and declined in both filtered and nonfiltered oil, reaching the same concentration after 150 days. Sterile olive oil was inoculated with *Candida wickerhamii* strain 6/c (previously isolated from the same type of oil) to determine its growth and survival characteristics. The inoculum was suspended either in sterile water or vegetation water from the olives. The yeasts grew rapidly in the inoculated oil for the first 3 days, reaching higher populations when inoculated in sterile water.

Dallyn, H. and J.R. Everton. 1970. Observations on the sporicidal action of vegetable oils used in fish canning. J. Appl. Bact. 33:603-608.

This study investigated sporicidal activity of oils. They used *Clostridium sporogenes* putrefactive anaerobe PA 3679 to test in olive and groundnut oils. The spores were inoculated on strips of paper and held at 110oC for 15 minutes in olive oil, mineral oil, or steam. Spores held in mineral oil or steam remained viable, but those held in olive oil did not. Extraction of olive oil with water removed the factor responsible for this effect. Survivor curves for spores heated in aqueous extracts of olive oil and groundnut oil showed more rapid destruction in the oil extracts than in phosphate buffer; olive oil extract was the most effective. The identity of the water-soluble component was not determined, but it was speculated than an autoxidation product from the oil might be responsible.

Di Giovacchino, L., S. Sestili, and D. Di Vincenzo. 2002. Influence of olive processing on virgin olive oil quality. Eur. J. Lipid Sci. Technol. 104: 587-601.

This paper is included because it provides data on the effects of processing on total phenol content. No data on composition of phenolics or secoiridoids.

Fleming, H.P., and J.L. Etchells. 1967. Occurrence of an inhibitor of lactic acid bacteria in green olives. Appl. Microbiol. 15 (5): 1178-1184.

Presence of an inhibitor of several species of lactic acid bacteria was demonstrated by the presence of inhibition zones surrounding tissue which had been cut from frozen olives and implanted in seeded nutritive agar. The inhibitor was ethanol-soluble and stable when heated at 100°C in aqueous solution. Activity of the inhibitor was greatly reduced when the pH was adjusted to 10, held for 10 min, then readjusted to 4.7. Freezing the olives increased the size of the zone of inhibition compared to unfrozen olives.

Fleming, H.P., W.M. Walter, Jr., and J.L. Etchells. 1969. Isolation of a bacterial inhibitor from green olives. Appl. Microbiol. 18 (5): 856-860.

Inhibitor of lactic acid bacteria was isolated from olives. Identity not established.

Gallina-Toschi, T., L. Cerretani, A. Bendini, M. Bonoli-Carbognin, and G. Lercker. 2005. Oxidative stability and phenolic content of virgin olive oil: An analytical approach by traditional and high resolution techniques. J. Sep. Sci. 28: 859-870.

A review of literature that includes data on secoiridoids in virgin olive oils from different olive cultivars and degree of ripeness and different processing techniques.

Juven, B., and Y. Henis. 1970. Studies on the antimicrobial activity of olive phenolic compounds. J. Appl. Bact. 33: 721-732.

Follow-up to earlier work by these authors and Fleming et al. in which antimicrobial activity was extracted from olives with ethyl acetate, and one component of the ethyl acetate extract was identified as oleuropein. In this study, the effect of oleuropein on growth of yeasts (*Saccharomyces cerevisiae, S. oviformis, S. carlsbergensis, Candida albicans, C. tropicalis, C. krusei*, and *Pichia membranaefaciens*), the yeast-like mold *Geotrichum candidum*, and molds (*Trichoderma lignorum*, Rhizopus sp., Rhizoctonia solani, Aspergillus niger, Phoma sp., and Penicillium cyclopium) were examined. Only G. candidum, *Rhizopus* sp., and *Rhizoctonia solan*i were partially inhibited by 0.2% oleuropein. The inhibitory effect of oleuropein on Lactobacillus plantarum was found to be affected by the concentration of organic nitrogenous constituents and sodium chloride in the medium.

Karaosmanoglu, H., F. Soyer, B. Ozen, and F. Tokatli. 2010. Antimicrobial and antioxidant activities of Turkish extra virgin olive oils. J. Agric. Food Chem. 58:8238-8245.

> Nine types of Turkish olive oils and three refined oils (olive, hazelnut, and canola oil) were tested for antimicrobial activity against *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Enteritidis. Using a low initial population (5 x 103 CFU/ml), all of the extra virgin olive oils tested showed bactericidal activity (no survivors after 1 h). Two extra virgin olive oils, one refined olive oil, canola and hazelnut oil were tested using a higher initial population (1 x 105 CFU/ml). No survivors remained after 1 h exposure to the extra virgin olive oils, whereas the refined olive oil and canola and hazelnut oils showed little or no antimicrobial activity. Phenolic compounds tested were cinnamic acid, ferulic acid, 4-hydroxybenzoic acid, luteolin, syringic acid, tyrosol, vanillic acid, and vanillin. Growth inhibition of *E. coli* O157:H7 ranged from approximately 1.0% (1 mg/kg tyrosol) to 10.5% (0.5 mg/kg vanillin).

Keceli, T., and R.K. Robinson. 2002. Antimicrobial activity of phenolic extracts from virgin olive oil. Milchwissenschaft 57: 436-440.

> Phenolic extract from commercial virgin olive oil was examined for antimicrobial activity when Lactobacillus delbrueckii subsp. bulgaricus and *Streptococcus thermophilus*, *Kluyveromyces marxianus* and *Penicillium frequentens* were grown in broth culture. Major phenolics identified in the extract were hydroxytyrosol and tyrosol. However some components, including derivatives of oleuropein, may have been present but could not be identified. Growth of the lactic acid bacteria, particularly *S. thermophilus*, was delayed in the presence of phenolic extract at pH 6.9. Growth of the yeast *K. marxianus* was inhibited more at pH 4.0 than at pH 6.9. Phenolic extracts of olive oil did not greatly affect growth of the mold *P. frequentens*.

Koidis, A., E. Triantafillou, and D. Boskou. 2008. Endogenous microflora in turbid virgin olive oils and the physiochemical characteristics of these oils. Eur. J. Lipid Sci. Tech. 110:164-171.

> Newly produced olive oil containing 0.17 to 0.49% water, naturally emulsified in the oil, was examined microscopically and by cultural methods. Microscopy examination indicated the presence of water droplets 1 to 5 µm in diameter and suspended solid particles ranging from 5 to 60 µm in size. Microbiologically, yeasts, molds and bacteria were found in the olive oil samples. The molds found were identified to the *Helicosporium*, *Alternaria*, *Penicillium* and *Aspergillus* genera. The yeasts were identified as *Candida guillermondii*, *C. parapsilosis*, *C. lusitaniae*, *C. famata*, *C. albicans* 1 and *Rhodotoula mucilaginosa* 2. The total aerobic counts and lactic acid bacteria counts ranged from 2 to 3 log CFU/ml initially. At 30, 60, and 90 days, samples were taken from the top portion and the bottom portion of the storage vessel. Total aerobic and lactic acid bacteria counts were fairly constant in the bottom portion, but decreased to nondetectable (lactic acid bacteria) and 1 to 2 logs (total aerobic flora) in the upper portion after 60 days.

Koutsoumanis, K., C.C. Tassou, P.S. Taoukis, and G.-J.E. Nychas. 1998. Modelling the effectiveness of a natural antimicrobial on *Salmonella* Enteritidis as a function of concentration, temperature and pH, using conductance measurements. J. Appl. Microbiol. 84: 981-987.

> Growth of S. enteritidis in BHI medium with three concentrations of oleuropein (0, 0.2, and 0.8%) at three temperatures and 4 pH values showed oleuropein had a bacteriostatic effect. The growth rate was slower in the presence of oleuropein.

Medina, E., M. Brenes, A. Garcia, C. Romero, and A. de Castro. 2009. Bactericidal activity of glutaraldehyde-like compounds from olive products. J. Food Prot. 72: 2611-2614.

> The Minimal Bactericidal Concentration of glutaraldehyde and glutaraldehyde-like compounds against *S. aureus*, *E. faecalis*, *E. coli* and *P. fluorescens* were determined. Bactericidal activities of olive glutaraldehyde-like compounds (the dialdehydic form of decarboxymethylelenolic acid (EDA), EDA linked to tyrosol (TyEDA), or EDA linked to hydroxytyrosol (HyEDA) were more effective than other food phenolic substances studied.

Medina, E., A. de Castro, C. Romero, and M. Brenes. 2006. Comparison of the concentrations of phenolic compounds in olive oils and other plant oils: Correlation with antimicrobial activity. J. Agric. Food Chem. 54: 4954-4961.

Oils evaluated were virgin olive oil (three of each of five olive varieties, total 15 oils); three olive oils (mixture of virgin olive oil and refined olive oil); three pomace olive oils (mixture of virgin olive oil and refined pomace oil); two sunflower oils, two corn oils, two rapeseed oils, one soybean oil and one cotton oil. Microorganisms on which selected oils were evaluated for antimicrobial activity were *Enterococcus faecalis*, *Enterococcus faecium*, *Clostridium perfringens*, *Streptococcus mutans*, *Listeria monocytogenes*, *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Staphylococcus aureus*, *Salmonella enterica*, *Escherichia coli*, *Yersinia* sp. 5057655, *Bacteroides* sp. 667, *Shigella sonei* and *Candida albicans*. None of the vegetable oils studied showed antimicrobial activity except those obtained from olive fruits. Virgin olive oils were most effective and pomace olive oils least effective as antimicrobials. Among the microorganisms tested, the yeast *C. albicans* was the only one that was not affected by any of the oils tested. The virgin olive oils were less effective against *E. coli* and *S. sonnei* than against the other bacteria. All of the oils were examined for polyphenols by HPLC. The dialdehydic form of decarboxymethyl oleuropein and ligstroside aglycons, hydroxytyrosol and tyrosol, were the phenolic compounds that statistically correlated with bacterial survival. Experiments were also performed with buffer that had been equilibrated with olive oils. The compounds that had antimicrobial activity were polar compounds that diffused from oil to buffer, and regression analysis showed good correlation between concentration of active compounds in buffer and cell viability.

Medina, E., A. Garcia, C. Romero, A. de Castro, and M. Brenes. 2009. Study of the anti-lactic acid bacteria compounds in table olives. Int. J. Food Sci. Tech. 44: 1286-1291.

Growth of LAB in brines of olives not treated with NaOH is variety-dependent. The most active antimicrobial compound, the dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol, was not detected in fresh olive fruits but was formed during brining from hydrolysis of oleuropein, and this reaction was enzymatically catalyzed. Heating the olives inactivates the enzyme, leading to an accumulation of oleuropein in olives and brines, inhibition of the formation of antimicrobials, and growth of Lactobacillus pentosus in olive brines.

Medina, E., C. Romero, M. Brenes, and A. D. Castro. 2007. Antimicrobial activity of olive oil, vinegar, and various beverages against foodborne pathogens. J. Food Prot. 70(5):1194-1199.

The foodborne pathogenic strains used were *Salmonella enterica* serovar Enteritidis, *Listeria monocytogenes* and *Staphylococcus aureus*, and *E. coli* O157:H7, *Yersinia* sp. and *S. sonnei* JCP. The researchers found that all pathogens survived after 5 min in the fruit juices with no reduction greater than 1 log. Milk, yogurt drink, Coca-Cola, and coffee extracts also failed to show rapid bactericidal effects. Wine (red and white), green tea, black tea, vinegar, and olive oil extracts showed bactericidal effects against these pathogens; of these, vinegar showed the strongest effect. Aqueous extract of virgin olive oil was nearly as effective, giving a >5-log reduction of *S. aureus* and *Yersinia* sp. and >4 log reduction for the remaining pathogens. Phenolic composition of the virgin olive oil and olive oil used in the experiment are presented. Mayonnaises and salads used as food models showed the effects of the food matrix on bactericidal effectiveness. For example, egg mayonnaises were made with virgin olive oil and sunflower oil, with and without lemon juice. When lemon juice was present in the mayonnaise with virgin olive oil, S. Enteritidis was below the limit of detection (3 log CFU/g reduction) in 30 min. This effect was not seen when sunflower oil was used. However in the absence of lemon juice, virgin olive oil was no more effective than sunflower oil at reducing the population of the pathogen.

Montedoro, G., M. Servili, M. Baldioli, R. Selvaggini, E. Miniati, and A. Macchioni. 1993. Simple and hydrolysable compounds in virgin olive oil. 3. Spectroscopic characterizations of the secoiridoid derivatives. J. Agric. Food Chem. 41: 2228-2234.

Four new phenolic compounds were separated by HPLC. The compounds were two (3,4-dihydroxyphenyl) ethanol derivatives and two (p-hydroxyphenyl) ethanol derivatives. The compounds identified were an isomer of oleuropein aglycon, the dialdehydic form of elenolic acid linked to (3,4-dihydroxyphenyl)ethanol, and the dialdehydic form of elenolic acid linked to (p-hydroxyphenyl)ethanol.

Morse, D.L., L.K. Pickard, J.J. Guzewich, B.D. Devine, and M. Shayegani. 1990. Garlic-in-oil associated botulism: episode leads to product modification. Am. J. Pub. Health. 80(11):1372-1373.

This article examines a botulism outbreak in February 1989 to make assertions for changes of flavored oil production. Three cases of botulism outbreak occurred from the ingestion of garlic-in-olive oil used to make garlic bread at a home dinner. The garlic-in-olive oil was stored at room temperature prior to opening and using. This led the outbreak to occur from spore germination of *C. botulinum*. The bottle of the garlic-in-olive oil product stated that it should be refrigerated, but the warning was in small print and most likely not noticeable to the consumer. Since room temperature can create

ideal conditions for growth of spores and toxin production, the FDA and New York State Department of Agriculture and Markets have banned companies from manufacturing garlic-in-oil products without microbial inhibitors or acidifying agents. It was clear that "refrigerate only" products were not safe due to the dangers of consumers not following the instructions.

Radford, S.A., C.C. Tassour, G.J.E. Nychas, and R.G. Board. 1991. The influence of different oils on the death rate of Salmonella enteritidis in homemade mayonnaise. Letters App. Micro. 12:125-128.

Mayonnaise was prepared with egg yolk, acetic acid, and oil, using one of the following: virgin olive oils (Italian or Greek), a proprietary blended olive oil, or sunflower oil. The population of *Salmonella* Enteritidis PT 4 inoculated into the prepared mayonnaises, was determined after 0, 24, 48, and 72 hours at 20oC. Profiles of the phenolic compounds were determined by HPLC. No viable *S.* Enteritidis were recovered from extra virgin olive oil samples incubated for 72 h, and the death rate was significantly faster than for blended olive oil or sunflower oil. Blended olive oil gave a death rate that was significantly faster than sunflower oil in two trials but not significantly different in two trials. The blended olive oil and sunflower oils contained insignificant amounts of phenolic compounds.

Romero, C., E. Medina, J. Vargas, M. Brenes, and A.D. Castro. 2007. In vitro activity of olive oil polyphenols against Helicobacter pylori. J. Agric. Food Chem. 55:680-686.

This article examines the effect olive oil has on *Helicobacter* pylori, a bacterium known to cause gastrointestinal diseases such as peptic ulcers and some types of gastric cancer. Olive oil samples were from the olive varieties of Picual, Manzanilla, Cornicabra, Hojiblanca, and Arbequina; eight strains of *H. pylori* were used. Phenolic compounds were shown to diffuse from olive oil into simulated gastric juice, and to be stable in the acidic environment for hours. The dialdehydic form of decarboxymethyl ligstroside aglycon showed the strongest bactericidal effect. Concentrations of phenolic compounds are given for the five monovarietal olive oils studied.

Tassou, C.C., and G.J.E. Nychas. 1994. Inhibition of *Staphylococcus aureus* by olive phenolics in broth and in a model food system. J. Food Prot. 57: 120-124.

Oleuropein (80% purity) and phenolics extracted from olives with 80% ethanol in water were tested for bacteriostatic effect in broth and reconstituted milk respectively. Inhibition in broth was influenced by the initial inoculum size, pH of the media, and concentration of oleuropein. Enterotoxin B production was inhibited by the addition of phenolics from olives to reconstituted milk at levels of 0.5% (toxin production was 50% of amount produced in control), 1.5% (toxin production was 2.5% of control), and 2% (toxin production 0.25% of control).

Tassou, C.C., and G.J.E. Nychas. 1995. Inhibition of *Salmonella* Enteritidis by oleuropein in broth and in a model food system. Letters Appl. Microbiol. 20: 120-124.

The inhibitory effect of commercial oleuropein was tested against *Salmonella* Enteritidis in a coliform broth and in reconstituted mile (model food system). Inhibition in broth was influenced by initial inoculum size, pH of the medium and concentration of the additive. No inhibition was evident in the model food system.

Tranter, H.S., C.C. Tassou, and Nychas, G.J.E. 1993. Effect of the olive phenolic compound, oleuropein, on growth and enterotoxin B production by *Staphylococcus aureus*. J. Appl. Bacteriol. 74: 253-260.

Low concentrations (0.1%) of oleuropein delayed growth of *S. aureus* in two media as indicated by changes in conductance. Higher concentrations (0.4-0.6%) inhibited growth completely.

Zanichelli, T.A. Baker, M.N. Clifford, and M.R. Adams. 2005. Inhibition of *Staphylococcus aureus* by oleuropein is mediated by hydrogen peroxide. J. Food Prot. 68: 1492-1496.

Inhibition of *S. aureus* by oleuropein shown to be largely due to hydrogen peroxide production by oleuropein when tryptone in the underlying medium is oxidized.

Zullo, B.A., G. Cioccia, and G. Ciafardini. 2010. Distribution of dimorphic yeast species in commercial extra virgin olive oil. Food Micro. 27:1035-1042.

This article examined the yeast found in olive oils from 23 producers in Italy. Some yeasts are useful for breaking down bitterness compounds and improving organoleptic characteristics, but some are harmful because they produce lipases that hydrolyze the triglycerides, which affects the organoleptic and nutritional properties of olive oil. Among the unwanted yeasts are dimorphic yeast species, some of which are considered opportunistic pathogens because they have been isolated from immunocompromised hospital patients. Of the 23 samples analyzed, 14 contained yeasts, of which six contained dimorphic yeasts. No yeasts were found in nine samples. Twenty dimorphic yeast isolates were studied by two identification methods. Four were identified by both techniques as *C. guilliermondii* and *C. parapsilosis*, which are opportunistic pathogens. Four isolates were identified biochemically as C. guilliermondii, but ribosomal sequencing results indicated that these were *C. diddensiae*, a nonpathogenic species. The remaining 12 isolates could not be identified biochemically; ribosomal D1/D2 sequencing results could identify similarities to known species but none were identical.