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Ozonation of sunflower oils: Impact of experimental conditions on the composition and the antibacterial activity of ozonized oils

Sophie Moureu [a,b], Frédéric Violleau [a,b,*], Djamila Ali Haimoud-Lekhal [c], Anne Calmon [a,b]

[a] Université de Toulouse, INP-Ecole d'Ingénieurs de Purpan, Laboratoire de Chimie Agro-Industrielle, 75, voie du TOEC, BP 57611, 31076 Toulouse Cedex 03, France
[b] INRA, UMR 1010 CAI, 31030 Toulouse, France
[c] Université de Toulouse, INP-Ecole d'Ingénieurs de Purpan, Equipe Systèmes de Productions Agricoles, 75, voie du TOEC, BP 57611, 31076 Toulouse Cedex 03, France

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ABSTRACT

Ozone can react with vegetable oils to produce ozonized oils which have antimicrobial properties and can be used in dermatology. The aim of this study was to evaluate the influence of ozonation conditions and of the initial fatty acid composition on iodine index (II), peroxide index (IP), acidity value (AV) of ozonized sunflower oils. The antibacterial activity of these products against the three bacterial strains that are more often involved in mastitis (*Staphylococcus aureus*, *Escherichia coli* and *Streptococcus uberis*) was also evaluated. In that purpose, two different sunflower oils have been studied: a "classical" oil (55% linoleic acid, 35% oleic acid) and a "high oleic" oil (90% oleic acid). Both were ozonized with or without water during different times (from 1 to 7 h). Results show that the addition of water has a direct impact on the increase in IP (up to 2600 meq of active oxygen/kg of oil with water and 430 without) and AV but does not influence the kinetic of the decrease in II. Minimal inhibitory concentrations were ranging from 1.25 to 40 mg/mL and the antibacterial activity of oils ozonized with water was better than the one of oils ozonized alone. These results are an open door to new applications of ozonized oils.

1. Introduction

Ozonized oils present interesting antibacterial activity. These compounds are produced by reaction between ozone and vegetable oils. Indeed, ozone, one of the most powerful oxidant, reacts with the carbon–carbon double bonds of unsaturated fatty acid according to the mechanism described by Criegee (1975) to form different oxygenated species such as ozonides, aldehydes and peroxides.

The ozonation of different vegetable oils, such as olive, sunflower, canola or coconut oil, under different conditions have been studied (Díaz et al., 2005, 2006b; Díaz-Gómez et al., 2009; Omonov et al., 2010; Sadowska et al., 2008; Valacchi et al., 2011). However, the comparison between the results remains difficult due to the large amount of parameters influencing the reaction (*e.g.* ozone flow rate and concentration, use of solvent). As far as we know, there has not been a comparative study of the effect of ozonation on the oils of two varieties of the same oilseed crops under different conditions. Sunflower oils obtained from different hybrids seem to be good subject matters due to their different fatty acid profiles. Indeed, oleic acid represents 10–50% of the fatty acid composition in traditional hybrids, 50–70% in mid oleic hybrids and more than 70% in high oleic hybrids (Sadras and Villalobos, 1994). In each case, oleic (one carbon–carbon double bond – C18:1) and linoleic (two carbon–carbon double bonds – C18:2) acids account for nearly 90% of the fatty acid content. However, the number of carbon–carbon double bonds can be different from one hybrid to another. Therefore, during the ozonation, the amount of unsaturation could impact the composition and antibacterial activity of ozonized oils.

Ozonized vegetable oils have been studied for their antimicrobial activity (Geweely, 2006; Lezcano et al., 1999; Montevecchi et al., 2013; Sechi et al., 2001) and their use in dermatology (Falcón Lincheta et al., 1998; Valacchi et al., 2013; Vasquez Daud et al., 2011). These works have led to the commercialization of cosmetics or drugs containing ozonized oils. It is the case of the Oleozon® (ozonized sunflower oil) which has been developed in the Ozone Research Center of Cuba and registered for the topical treatment of

* Corresponding author at: Université de Toulouse, INP-Ecole d'Ingénieurs de Purpan, Laboratoire de Chimie Agro-Industrielle, 75, voie du TOEC, BP 57611, 31076 Toulouse Cedex 03, France. Tel.: +33 5 61 15 29 78.
E-mail address: frederic.violleau@purpan.fr (F. Violleau).

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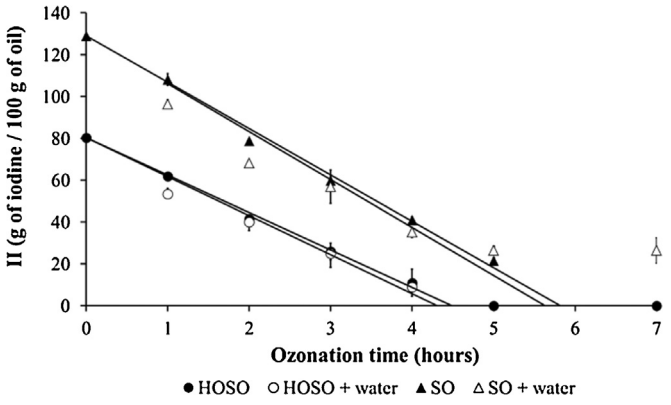

Fig. 1. Evolution of iodine index with increasing reaction time in different ozonation conditions (1 column).

epidermophytosis (Menéndez et al., 2008; Moleiro Mirabal et al., 2006). Yet only a few studies deal with their veterinary use (Camps-Ramírez et al., 2006a,b; González Alvarez et al., 2007; Jo et al., 2005).

A major endemic disease of dairy cattle is mastitis, which is an inflammation of the mammary gland and udder tissue. It usually occurs as an immune response to bacterial invasion and the main mastitis-causing pathogens are *Staphylococcus* aureus, *Escherichia coli* and *Streptococcus uberis* (Bradley, 2002). Antibiotics are the most commonly used treatment for this disease but they are more and more decried (traces in milk, not ecological . . .). Organic dairy farmers often use homeopathy for treatment or holistic approach as alternatives to antibiotic treatments (Lefevre et al., 2008). Ozonized vegetable oils could be a new way to prevent and/or to treat mastitis. It has been shown that ozonized sunflower oil has an antibacterial activity against *S. aureus* and *E. coli* (Díaz et al., 2006a, b; Díaz-Gómez et al., 2009; Rodrigues et al., 2004; Sechi et al., 2001) but the effect of this product on *S. uberis* has never been studied.

The aim of this study is to highlight the influence of ozonation conditions (*e.g.* reaction duration, addition of water) and of the initial fatty acid composition on iodine index (double bonds quantification), peroxide index (active oxygen quantification), acidity value (acidic compounds quantification) of ozonized sunflower oils. The antibacterial activity of these products against *S. aureus*, *E. coli* and *S. uberis* has also been studied. In that purpose a "classical" and a "high oleic" sunflower oils were ozonized with or without water (protic media, environmentally friendly) for different time and were characterized.

2. Material and methods

2.1. Samples and reagents

"High oleic" sunflower oil (HOSO) was obtained from a local producer of oil (France) and the "classical" sunflower oil (SO) was from a local grocery store (Carrefour, France). Both were refined oils. Solvents (*e.g.* acetic acid, cyclohexane) were purchased from Carl Roth (France) and used without further purifications (A.C.S. grade). Wijs reagent, toluene, potassium iodide, sodium thiosulfate and Tween-80 were from Fisher Scientific (France). Potassium hydroxide, trimethylsulfonium hydroxide (TMSH), *tert*-butyl methyl ether (TBME) and fatty acid methyl ester standards were from Sigma–Aldrich (France). Mueller Hinton broth was purchased from Carl Roth (France).

Table 1
Main fatty acids composition of non-ozonized oils. Q9

Fatty acid composition (%)	HOSO	SO
	mean ± SD	mean ± SD
Palmitic acid (C16)	3.11 ± 0.06	5.52 ± 0.09
Stearic acid (C18)	2.18 ± 0.04	3.28 ± 0.02
Oleic acid (C18:1)	89.10 ± 0.11	34.73 ± 0.27
Linoleic acid (C18:2)	3.78 ± 0.13	54.75 ± 0.08

2.2. Bacterial strains

Three strains were tested: *S. aureus* (CIP 76.25), *E. coli* (CIP 76.24) and *S. uberis* (CIP 105450). The microorganisms were obtained from the Institut Pasteur (Paris, France).

2.3. Preparation of ozonized sunflower oil samples

Ozone was generated by a Triogen device (LAB2B Ozonia, Suisse) supplied with pure oxygen. Experimentally, 50 g of oil or an emulsion made of 50 g of oil and 5 g of ultra-pure water were placed in a reactor. Ozone (gas flow rate: 30 L/h, $[O_3] \approx 65$ mg/L) was bubbled through the oil for different times: 1–7 h. Water (27 °C) was recirculated through the double wall of the reactor during all the ozonation process. Each ozonation reaction was realized in duplicate. After production, ozonized oils were stored in opaque polypropylene flasks at 4 °C.

2.4. Determination of the fatty acid composition of oils

Non-ozonized sunflower oils were analysed using gas chromatography after transesterification with TMSH. Solutions at ≈50 mg/mL of oils were prepared in TBME. In an insert, 50 μL of the solution were mixed with 50 μL of TMSH. One microliter of this mixture Q5 was splitless injected. The temperature of the injector was set at 250 °C.

Analysis were performed with a Clarus 600 GC (Perkin Elmer) chromatographic system, in a ZB-FFAB capillary column (30 m × 0.25 mm × 0.25 μm film thickness, Phenomenex), with a flame ionization detector at 230 °C. The carrier gas was hydrogen at 1.2 mL/min. The column temperature was programmed for 5 min at 70 °C, then to reach 160 °C at 5 °C/min, from 160 °C to 220 °C at 2 °C/min and to stay at 220 °C for one minute. External fatty acid methyl ester standards were used to identify components by comparing their relative retention time. Fatty acid content was expressed as relative area percentage.

2.5. Characterization of oils

Iodine index, peroxide index and acidity value were determined using a titration device 916 Ti-Touch (Metrohm, France) for non-ozonized and ozonized oils.

Table 2
Equations of the trend curves for the evolution of iodine index with reaction time in different ozonation conditions.

HOSO	HOSO + water	SO	SO + water
$II = -18.67t + 80.34$ $R^2 = 0.919$	$II = -17.89t + 80.34$ $R^2 = 0.995$	$II = -22.18t + 129.1$ $R^2 = 0.993$	$II = -22.92t + 129.1$ $R^2 = 0.844$

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