Antibacterial Potential of Heated Polyunsaturated Oils

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INTRODUCTION

The process of discarding biomass residues in a sustainable way with minimal environmental impact has led to the concept of biorefining which is increasingly present in the forest, agriculture and agri-food industries, in general. It definitely forms a significant part of the response to the growing scarcity of resources combined with the increase in the global population and pollution. It results in the reuse and maximum transformation of biomass by proposing alternatives to the landfilling and incineration of residues, by-products and co-products of these industries.

Pesticides are found in 90% of the streams and 55% of the groundwater manly because of agricultural activities, weeding and industrial sites. Airborne contamination would be found around farms to pose a risk to non-targeted organisms, primarily to users in a professional context (Baldi *et al*., 2013; van der Werf, 1997). Furthermore, the development of resistance of pathogens to chemical pesticides is also drawing attention (Russell, 2004). Thus the need for alternative methods such as the use of antimicrobial substances from natural sources, that may be less toxic to human health, and, thanks to the action of multiple compounds, less prone to resistance development.

Saturated (palmitic, stearic), monounsaturated, omega-3 and omega-6 fatty acids as well as some vegetable oils such as olive oil have been tested by for their antimicrobial potential (Desbois and Smith, 2010; De Carvalho *et al*., 2008, Dilika *et al*., 2000). The main conclusions were that linoleic acid would be more effective than oleic acid, in turn, more effective than the unsaturated fatty acids of smaller carbon chains. Saturated fatty acids had no significant antibacterial effect.

Used oils from frying operation form residues that could be valorized for a second life. These thermally treated oils contain *trans* fatty acid isomers which evolve towards low levels of cyclic fatty acid monomers (CFAM) during frying operations (Cherif *et al*., 2019, Destaillats and Angers, 2005; Sébédio and Juaneda, 2006). CFAM are known to interact significantly with enzymes involved in the liver metabolism in the rat (Mboma *et al*., 2018). However, data on their potential interactions with bacterial enzymes are scarce or non existant

HYPOTHESIS

Polyunsaturated oils that are heat treated under non-oxidative conditions, contain *trans*, conjugated and cyclic isomers of fatty acids that confer to those oils an antibacterial effect.

OBJECTIVE

In our on-going work on the valorization of rejected or low grade food ingredients and products, we are searching for new usages for used (frying) oils. The objective of the present work was to determine the antibacterial potential of heated polyunsaturated linoleic oils (safflower and sunflower) that contain *trans* and/or cyclic fatty acids on four model bacteria: *Escherichia coli*, *Pseudomonas syringae* pv. tomato, *Xanthomonas campestris* pv. vitians and *Micrococcus luteus.*

MATERIALS AND METHODS

Vegetable oils

Safflower oil and linseed oil were heated at different temperatures (200, 240 and 275 °C) after saponification or not. A commercial sample of Sunflower oil from a Burundi factory, that had been refined at 240 °C for 6 h was also tested. Thus, the oils samples contained different levels of *trans*, cyclic and conjugated fatty acid isomers.

Antibacterial tests

The bacteriostatic and bactericidal effects of the oils were determined according to Delisle-Houde *et al.* (2017), under sterile conditions using 96-well microplates (Sarstedt AG & Co., Nümbrecht, Germany). The bacteria under study, *Escherichia coli, Micrococcus luteus*, *Pseudomonas syringae* pv. *tomato* and *Xanthomonas campestris* pv. *vitians,* were cultured in Tryptic Soy Broth nutrient broth (TSB, Becton, Dickinson and Company, Sparks, MD) for 16 hours at 28 °C for *P. syringae* pv. *tomato* and *X. campestris* pv. *vitians* or 37 ° C for *E. coli* and *M. luteus*. In each of the wells, 10 µl of either of the bacterial cultures were added to 90 µl of the various oils tested. The control wells contained 90 µl of physiological saline (0.9% NaCl) sterile. The microplates were incubated (24 h) at 28 or 37 °C and 10 µl of 2,3,5-triphenyl-2H-tetrazolium (1 mg/ml, Ward's Science, Rochester, NY) were subsequently added to each well. The absence of red staining in the well indicated that the oil inhibited bacterial growth (bacteriostatic effect, Fig. 1). Subsequently, the contents (100 µl) of each wells were plated on Trypto-casein soy agar (TSA, Becton, Dickinson and Company) in petri dishes. The agar plates were then incubated at 28 or 37 °C. After 48 hours of incubation, they were examined for the presence or absence of bacterial growth, the latter indicating a bactericidal effect. Each oil was tested in triplicate..

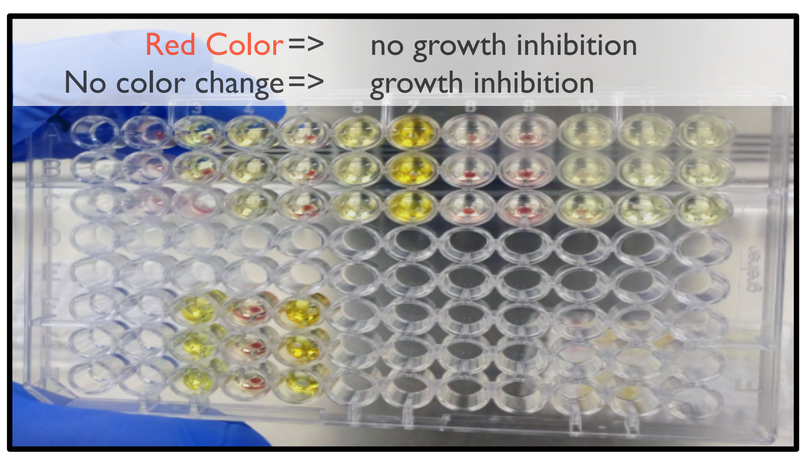

Figure 1. Effect of heat treated vegetable oils on bacterial growth. Incubation for 24h at optimal temperature and addition of 2,3,5-triphenyl-2H-tetrazolium.

RESULTS AND DISCUSSION

The results for bacteriostatic tests are presented in Table 1. They indicate that the temperature of the heat treatment would play a role in the preparation of the oils to be used as antimicrobial agents. The oils treated at 275 °C showed an inhibitory effect on Gram– bacteria, even if the treatment time was short (0,17 d = 4h). The other factor to be considered would be the temperature-time pair to which the oils were subjected. This would account for the differences in efficiency noted between the oils treated at 240 °C for 3 days and at 200 °C for 4 days, the first being effective unlike the latter, despite a longer heat treatment.

Oil characterization showed that, in general, a longer time resulted in a higher acid value (AV). The results for the saponified safflower oil sample suggested that oils with highest AV should possess a bacteriostatic effect. This is the case, the oils with AV of 35-45% exhibited a bacteriostatic effect on Gram- bacteria but not on Gram+ *M. Luteus*, unlike the saponified sample.

But the AV is not the only factor in oils having a bacteriostatic effect, as a low AV safflower oil sample (275 ˚C for 0,17 d) with an AV of 3.0, had a bacteriostatic effect. Could the level in *trans* FA contribute to the bacteriostatic effect, as suggested by Kabara *et al.* (1972)? In general, samples with higher *trans* FA had a bacteriostatic effect, but not all.

The results from these exploratory experiments suggest that, in addition to high AV and *trans* FA, other compounds such as minor FA isomers or degradation products, yet to be identified, or a combination of compounds might cause the observed bacteriostatic effect.

No bactericidal effect was observed (data not shown).

Table 1. Bacteriostatic potential of heat treated safflower, sunflower and linseed oils (+ bacterial growth; – bacteriostatic effect)

	Physiological water	Safflower Oils							Saponified Safflower Oil	Sunflower oil[4]	Linseed Oil
Heat treatment (˚C)	NA[1]	NA	200	200	240	**240**	**240**	**275**	**200**	240	**275**
Treatment time (day)	NA	NA	0,17	4	0,17	**3**	**4**	**0,17**	**3**	0,25	**0,17**
Bacterial Growth											
E. coli[5]	+	+	+	+	+	–	–	–	–	+	–
P. syringea pv. *tomato*[5]	+	+	+	+	+	–	–	–	–	+	–
X. campestris pv. *vitians*[5]	+	+	+	+	+	–	–	–	–	+	–
M. luteus[6]	+	+	+	+	+	+	+	+	–	+	+
Acid Number (%)	NA	0,11	0,44	2,79	2,77	**33,2**	**45,4**	**3,0**	**NA**	2,29	**4,44**
Fatty Acid (%)											
9*c*,12*c* -18:2	NA	78,5	77,8	64,7	76,8	**49,5**	**44,3**	**70,8**	**75,4**	35,4	**15,0**
9t,12*c* -18:2	NA	0,1	0,1	0,5	0,4	**4,2**	**8,9**	**3,6**	**0,7**	5,8	**1,0**
9*c*,12*t* -18:2	NA	0,1	0,0	0,6	0,5	**4,3**	**9,0**	**4,0**	**0,1**	5,9	**1,9**
9t,12*t* -18:2	NA	0,0[2]	0,0	0,2	0,1	**0,9**	**1,1**	**0,3**	**2,0**	0,4	**0,3**
CFAM[7]	NA	n.d.[3]	n.d.	0,1	0,0	**0,3**	**0,2**	**0,0**	**0,0**	0,0	**0,3**

[1] Non applicable; [2] value lower than 0,05%; [3] not detected; [4] commercial sunflower oil sample, refined for 6 h at 240 ˚C; [5] Gram– bacteria; [6] Gram+ bacteria; [7] cyclic fatty acid monomers.

CONCLUSION

- Saponified safflower oil (free fatty acids) that had been heat treated at 200 °C for 3 days had a **bacteriostatic effect** on all 4 bacteria (both **Gram+ and Gram-**);
- Heat treated safflower oil (240°C for 3 and 4 days) and heat treated linseed oil (275°C for 4 h) had a **bacteriostatic effect** on **Gram-** bacteria;
- Other heat treated oils and the control untreated oil had **no bacteriostatic effect** on any of the tested bacteria;
- There was **no bactericidal** effect on the tested bacteria by any of the oils;
- In general, heat treated oils with higher levels of acidity (33-45%) and/or *trans* fatty acid (9-20%) exhibited a bacteriostatic effect mainly on Gram- bacteria, except for sunflower oil;
- Minor fatty acid isomers or degradation products, yet to be identified, might cause the bacteriostatic effect.

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