

Interesterification of Anhydrous Milk Fat with Rapeseed and/or Linseed Oil: Oxidative Stability

Jean-Michel Giet⁽¹⁾, Mario Aguedo⁽²⁾, Sabine Danthine⁽¹⁾, Michel Paquot⁽³⁾, Annick Thomas⁽⁴⁾, Micheline Vandenberg⁽⁵⁾, Philippe Thonart⁽⁶⁾, Jean-Paul Wathelet⁽²⁾, Christophe Blecker⁽¹⁾, Georges Lognay⁽⁷⁾



Department of Chemistry and Bio-Industries

(1) Laboratory of Food Technology,

(2) Laboratory of General and Organic Chemistry, (4) Laboratory of Numerical Molecular Biophysics, (6) Laboratory of Bio-Industries,

(3) Laboratory of Industrial Biological Chemistry, (5) Laboratory of Animal and Microbial Biology, (7) Laboratory of Analytical Chemistry.

Introduction

Blends of anhydrous milkfat (AMF) and linseed oil (70/30), and AMF, rapeseed oil (RO) and linseed oil (LO), 70/20/10, were submitted to enzymatic interesterification, as reported as part of “*Enrichment of anhydrous milk fat in polyunsaturated fatty acid residues from linseed and rapeseed oils through enzymatic interesterification*” (poster #29846 · Edible Applications Technology Posters session).

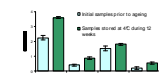
The oxidative stability of the blends, the interesterified (IE) blends and IE blends with 50 ppm α -tocopherol added as antioxidant were studied.

Middle	Composition	Treatment
BB NIE	Binary blend	Non-interesterified
BB IE6h	AMF / LO (70/30)	6-hours interesterification
BB IE6h+AO		interesterification + antioxidant addition
TB NIE	Ternary blend	Non-interesterified
TB IE6h	AMF / RO / LO (70/20/10)	6-hours interesterification
TB IE6h+AO		interesterification + antioxidant addition

Considered fats

6 distinct fats were studied, derived from 2 fat blends: a binary blend (BB) of AMF and LO (70/30) and a ternary blend (TB) containing AMF, RO and LO (70/20/10). Along with the unmodified blends, the interesterified blends and the interesterified blends supplemented with 50 ppm of α -tocopherol as antioxidant were submitted to shelf-life and overaging studies, i.e. storage at 4°C, 25°C and 60°C.

Peroxide value (PV)



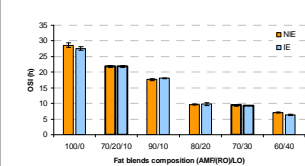
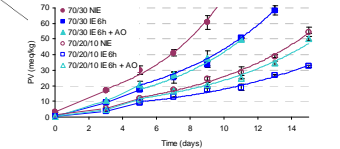
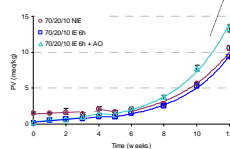
A lowering of PV was observed after the interesterification reaction, maybe due to lipase-catalysed oxidation of the hydroperoxide group, likely followed by its conversion into carbonyls: this process could have been amplified by FFA produced during the reaction, as they have been reported to enhance peroxide decomposition into carbonyl in an acid-catalysed Heck-Criegee rearrangement.

Very little oxidation was observed among the samples stored at 4°C during 12 weeks.

25°C Among the two series (BB and TB), PV range was in the order IE6h+AO > NIE > IE6h. A parallel evolution of NIE and IE6h substrate was observed after about 5 weeks. All curves may be well described and correlated ($R^2 > 0.98$) by an initial linear increase in PV (induction period), followed by a transitional then exponential later-catalyzed phase.

The slope of the linear autooxidation phase is much lower for TB than BB, likely for the same reason than the overall PV evolution, i.e. a lesser susceptibility resulting from a lower content in linoleic acid. The displacement of PUFA from linseed oil triacylglycerols to a saturated viscosity (AMF) may impact on hydrogen transfer and reduce early peroxide formation.

60°C In 3 days PV reach values observed after 8 weeks at 25°C (peroxide formation about 20-fold faster). No significant effect of interesterification was found within BB and a per-oxidant effect was found in TB. At 60°C, the influence of secondary oxidation products, i.e. peroxide conversion into non-peroxide products, was not negligible, as it will be shown.



Oxidative stability index

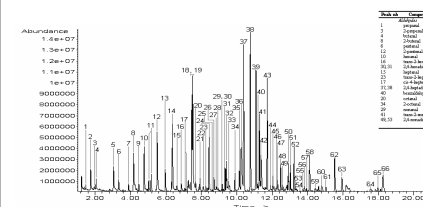
The Rancimat measurements (performed at 100°C) highlight the impact of LO addition to AMF, causing an important decrease in the oxidative stability of the blends.

The introduction of RO in the ternary blend had no plastic properties, as previous work of our team assessed, while preserving a satisfactory oxidative stability.

Still, results obtained from accelerated oxidation conditions are not strictly representative of the process occurring in shelf-life conditions.

The present study focuses on the oxidative stability of interesterified fats under accelerated and non-accelerated storage conditions, closer to normal storage conditions than the Rancimat test.

Solid phase micro-extraction (SPME) : volatile compounds analysis



SPME followed by GC-MS analysis of volatile compounds were performed on the samples stored at 60°C. Trials on samples from the experiments at 4 or 25°C led to SPME file saturation by compounds (not shown), and the detection of very few other compounds (not shown). No effect of triolein addition was observed.

Among more than a hundred volatile compounds detected, 70 were tentatively identified by identified by mass spectra correlation. Most of the compounds were previously reported as autooxidation products of milk fat or vegetable oils.

Carboxylic acids represented a major part of signal integration. Most were short chain fatty acids from AMF. They were found in great amounts at the initial time for IE blends, as products of lipase hydrolysis. Acetic acid along with other short-chain acids (propionic, butyric, hexanoic) were reported as late specific oxidation products of linoleic and linolenic acids and the cause of unpleasant off-flavours.

The increase of acetic acid chromatographic area is correlated with the previous PV measurements ($R^2 > 0.98$ for BB, $R^2 > 0.87$ for TB).

Aldehydes, as hydroperoxides decomposition products, are a major class of odorants. Among those compounds, α -hexenal, 2-hexenal, 2-pentenal, 2,4-hexadienal, 2,4-decadienal, 2-nonenal and 2-heptenal and α -hexanal, 2-pentenal, propenal, heptanal and nonanal were detected in exponentially increasing amounts with storage time.

Chromatographic areas obtained for TB are consistently lower than those observed for BB, except for a sharp increase after 12 days in interesterified TB. We observed higher amounts of heptadecanal, hexadecanal and 2,4-decadienal in both BB than in TB. This is likely a result of the higher content in linoleic and linolenic acids in the binary substrates.

Ketones (e.g. 3,5-volatilized-2-one, 2-heptanone, 2-nonanone and 2-propanone) are another important class of odorants in the field of lipid autooxidation. By considering separately saturated and unsaturated ketones, a consistent decrease in α -nivalones from day 0 to 8 was found. Content in heptanone and pentanone was roughly correlated with the linoleic content in NIE blends (not shown). Minor unsaturated ketones are formed in small amounts increasingly with oxidation time.

The main ketonic compound observed was octanone. This volatile has been proposed as an indicator of milk fat oxidation. As for acetic acid, NIE BB showed very high values, although overcome by the IE TB which displayed enhanced oxidation from day 8 to 12.

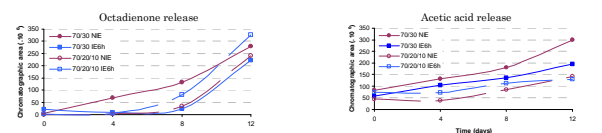
Substrates BB IE6h and TB NIE interestingly provided very similar results. Odorous lactones were detected: δ -octal, decal and decalactones were present in the initial blends and their content decreased with storage time. These three compounds were previously reported as contributing odorants in heated butter. However γ -hexalactone and 2-hexen-4-olide were only detected in oxidized products.

Among the detected alcohols, 1-penten-3-ol and 1-octen-3-ol were the main contributing molecules. Increasing alcohols content was observed for all substrates except BB NIE, which displayed constant values.

A last compound, 1-octen-3-cyclohexene, originated strong signal in highly oxidized samples (not shown). It may be an artefact, however it was not detected on the blank analyses.

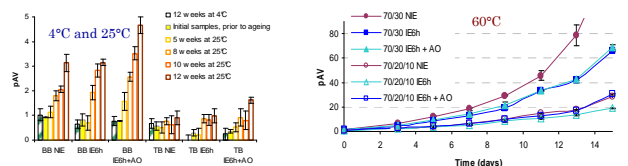
Based on the sum of chromatographic area for aldehydes, ketones, lactones and alcohols (as those compounds are mainly oxidation products), both IE binary and ternary blends displayed a continuous increase in volatile compounds release. However with much lower increase for TB, as a likely result of its lower content in linoleic acid.

In comparison, IE leads to a higher initial content in volatile compounds and, from BB to TB, a comparable and quite stable rate of release until day 8. Surprisingly, IE TB showed a sharp and consistent release of odorous compounds from day 8 to 12, indicating a strong oxidative degradation of the product. It appears difficult to globally and consistently correlate SPME chromatographic areas with other data (substrates composition or results of other methods assessing oxidation). This is likely due to the complexity of the reaction pathways involved in the production of volatile compounds.



Days	BB NIE				BB IE6h				TB NIE				TB IE6h			
	0	4	8	12	0	4	8	12	0	4	8	12	0	4	8	12
Acetic acid	31.2	26.8	28.0	33.6	129.0	224.9	228.5	280.0	62.0	52.0	23.9	18.4	174.9	212.8	63.5	170.0
Octadecanone	0.8	17.0	29.2	33.6	74.6	54.4	112.8	238.2	1.7	1.8	4.8	1.7	1.8	2.3	2.5	10.9
Aldehydes	28.4	17.4	15.5	30.7	114.3	21.5	40.2	184.2	20.0	22.7	51.3	76.3	107.7	24.9	99.2	43.9
Alcohols	0.0	4.1	8.2	14.4	14.3	1.0	1.2	1.3	2.0	19.2	13.9	1.0	1.8	1.0	1.1	7.5
Ketones	0.0	4.1	8.2	14.4	14.3	1.0	1.2	1.3	2.0	19.2	13.9	1.0	1.8	1.0	1.1	7.5
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p-anisidine value (p-AV)

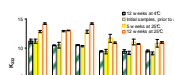


P-anisidine value (p-AV) provides information about secondary oxidation products, i.e. aldehydes.

4°C and 25°C p-AV for the shelf-life study are reported on the left. No significant increase in p-AV was observed after 12 weeks at 4°C, and values remained low for TB at 25°C. The lower p-AV observed for the IE TB was not significantly different within the error range. Very little difference was found between NIE and IE blends. On the other hand, production of secondary oxidation products was important for BB, and took a greater extent in presence of a tocopherol, confirming a pro-oxidant effect, also detectable in TB (IE6h+AO) after 12 weeks.

At **60°C** (on the right) a different behavior was observed, which was closely correlated with PV for BB. For TB the lowest values were obtained for IE6h+AO.

Tocopherols antioxidant mechanism is based on the scavenging of peroxyl radicals by H donation; furthermore, the tocopherol radical is quite stable and promotes termination reactions. In our conditions, α -tocopherol seems to have caused peroxide stabilization (higher PV) and hindered secondary products formation, thus a lower p-AV.



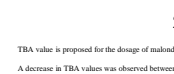
Ultraviolet spectroscopy

Conjugated dienes, main products of linoleic hydroperoxide decomposition, and conjugated trienes, such as ethylene diolates, may be quantified by measuring specific extinction at 232 nm and 268 nm respectively.

Negligible increase was observed at both 232 and 268 nm between the initial substrates and after 12 weeks stored at 4°C. At both wavelengths, specific extinctions of TB were consistently lower than those of BB, their only change with time was a limited shift to higher values, and no significant effect of the treatment was observed within this series. At 232 nm and 268 nm, a shift of specific extinction was observed for IE blends, and both NIE and IE6h+AO displayed similar behavior, e.g. a continuous increase.

For BB, a slight decrease at 232 nm was observed between NIE and IE blends. However, an increase would be expected if the expected peroxide decomposition into carbonyl occurred.

At **60°C** at both wavelengths, relative stagnation was observed during about 7 days, followed by a rapid increase in values. Whereas little differences were found within TB, BB NIE displayed the highest values of K232 and K268, as a result of the higher oxidation rate observed previously.



2-thiobarbituric acid (TBA) value

TBA value is proposed for the dosage of malondialdehyde (MDA), a toxic aldehyde resulting from the oxidation of tri-unsaturated fatty-acids (linoleic acid in particular).

A decrease in TBA values was observed between NIE and IE samples, likely due to existing MDA stripping during the reaction. An increase of about 50% compared to the initial values was observed at the end of storage at 25°C, comparable with the values achieved after 12 weeks at 4°C. BB showed a continuous increase whereas TB displayed first a decrease, attributed to side reactions involving TBA reaction components.

At **60°C**, values reached after 3 days were already higher than those observed after 12 weeks at 25°C. A continuous increase was observed in all cases. Overall TBA values were consistently lower for TB than BB at every temperature, as a result of the lower content in linoleic acid.

	4°C		25°C		60°C	
	Initial values	12 weeks	5 weeks	12 weeks	3 days	13 days
70/30 NIE	0.070 ± 0.006	0.059 ± 0.006	0.067 ± 0.010	0.076 ± 0.009	0.109 ± 0.005	0.160 ± 0.008
70/30 IE6h	0.020 ± 0.004	0.031 ± 0.011	0.051 ± 0.003	0.084 ± 0.003	0.089 ± 0.003	0.112 ± 0.005
70/30 IE6h+AO	0.001 ± 0.003	0.028 ± 0.003	0.043 ± 0.012	0.051 ± 0.004	0.064 ± 0.004	0.075 ± 0.024
70/20/10 NIE	0.028 ± 0.003	0.028 ± 0.003	0.021 ± 0.003	0.036 ± 0.003	0.046 ± 0.006	0.058 ± 0.003
70/20/10 IE6h	0.025 ± 0.000	0.019 ± 0.001	0.012 ± 0.001	0.021 ± 0.002	0.027 ± 0.003	0.040 ± 0.003
70/20/10 IE6h+AO	0.023 ± 0.002	0.017 ± 0.002	0.010 ± 0.001	0.023 ± 0.002	0.036 ± 0.003	0.044 ± 0.010

Jean-Michel Giet, Mario Aguedo, Sabine Danthine, Michel Paquot, Annick Thomas, Micheline Vandenberg, Philippe Thonart, Jean-Paul Wathelet, Christophe Blecker, Georges Lognay, *Interesterification of Anhydrous Milk Fat with Rapeseed and/or Linseed Oil: Oxidative Stability*, submitted to J. Agric. Food Chem.

Conclusions

Peroxides appeared to be the only significant oxidation products after 12 weeks storage at 4°C. As expected, the binary blends (BB) were more sensitive to oxidation than the ternary blends (TB). The BB were associated with increased volatile emission compared to TB.

Interesterification led to variable effects on the oxidation of fat mixture, depending on composition and temperature (beneficial effect on BB, at both 25°C and 60°C, and a rather neutral effect on TB). The IE blends exhibited higher volatile release prior to ageing. A pro-oxidant effect of α -tocopherol addition was observed at 25°C on both BB and TB. At 60°C, an antioxidant effect was observed on TB.