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

Jean-Michel Giet,[†] Mario Aguedo,[‡] Sabine Danthine,[†] Michel Paquot,[§] Annick Thomas,[#] Micheline Vandenbol,[⊥] Philippe Thonart,[∥] Jean-Paul Wathelet,[‡] Christophe Blecker,*[,†] and Georges Lognay[⊗]

[†]Laboratory of Food Technology, [‡]Laboratory of General and Organic Chemistry and [§]Laboratory of Industrial Biological Chemistry and [#]Laboratory of Numerical Molecular Biophysics and [⊥]Laboratory of Animal and Microbial Biology and [∥]Laboratory of Bio-Industries and [⊗]Laboratory of Analytical Chemistry, Department of Chemistry and Bio-Industries, Gembloux Agro-Bio Tech, University of Liège, Passage des Déportés 2, B-5030 Gembloux, Belgium

Blends of anhydrous milk fat (AMF) and linseed oil (70:30) and of AMF, rapeseed oil (RO), and linseed oil (LO) (70:20:10) were submitted to enzymatic interesterification. The oxidative stabilities of the blends, the interesterified (IE) blends, and IE blends with 50 ppm of α-tocopherol added as antioxidant were studied. Samples were stored in open flasks at 60, 25, and 4 °C and periodically submitted to peroxide, *p*-anisidine, and TBA value determinations and UV measurement at 232 and 268 nm. The analysis of volatile compounds was carried out by SPME for the samples stored at 60 °C. Peroxides appeared to be the only significant oxidation products after 12 weeks of 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 the TB. Interesterification led to variable effects on the oxidation of fat mixtures, depending on composition and temperature (beneficial effect on BB, at both 25 and 60 °C, and a rather neutral effect on TB). The IE blends exhibited higher volatile release prior to aging. 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.

KEYWORDS: Linseed oil; rapeseed oil; anhydrous milk fat; enzymatic interesterification; oxidative stability; oven-aging; shelf life

INTRODUCTION

Enzymatic interesterification is a promising technique for the production of structured lipids (*1*). It implies milder conditions than chemical interesterification and thus less product deterioration (*2*). Growing concerns about the health effects of *trans*-fatty acids brought attention to interesterification as an alternative to produce substitutes for hydrogenated vegetable oils (*3*).

The enzymatic interesterification of blends composed of highly saturated solid fats (milk fat or palm stearin, among others) and vegetable oil was proposed (*4*, *5*) for the production of solid, structured lipids, with a high content in long-chain polyunsaturated fatty acids (PUFA) (*6*), the point being that PUFA, which include linoleic (n-6) and linolenic (n-3) acids, are generally recognized as essential to humans and beneficial to health in appropriate amounts (*7*).

However, PUFA, in correlation with their number of unsaturations, are sensitive to autoxidation, which causes off-flavors, due to secondary oxidation products such as volatile aldehydes. Some of these compounds, for example, malondialdehyde, display a significant toxicity (*8*).

*Corresponding author [telephone (0032) 81-62 2328; fax (0032) 81-60 1767; e-mail blecker.c@fsagx.ac.be].

The effects of interesterification (enzyme-catalyzed or chemical randomization) on the oxidative stability of oils and fats are well documented, even though variable results are reported. The loss of antioxidants due to purification processes, by their modification or by the formation of (nonfat) pro-oxidant, was put forward to explain the observed decrease in the oxidative stability of some interesterified triacylglycerols (TAG) (*9*). On the other hand, the fatty acid migration resulting from the interesterification reaction may also lead to an increase in the oxidative stability, attributed to a lower content in highly reactive TAG, containing several PUFA (*10*). Lastly, studies on pure TAG have consistently shown an effect of PUFA position on the glycerol backbone on the oxidative stability (*11*).

Previous work of our team focused on the production of PUFA-rich structured lipids by enzymatic interesterification of anhydrous milk fat (AMF) and linseed oil (LO) blends in microaqueous conditions (*12*).

This study highlighted the excellent plastic properties of interesterified LO-containing blends and acknowledged a decrease in their oxidative stability determined by the Rancimat measurement conducted at 100 °C. Oxidative stability appeared to be inversely correlated with LO content; on the other hand, no significant effect of the interesterification reaction was noted.

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Table 1. Identification and Partial Characterization of the Substrates Considered in This Study

substrate	composition	treatment	FFA (%)	PV (mequiv/kg)
BB NIE	binary blend AMF/LO (70:30)	noninteresterified	0.20 ± 0.00	2.25 ± 0.13
BB IE6h		6 h of interesterification	0.83 ± 0.01	0.34 ± 0.02
BB IE6h+AO		interesterification + antioxidant addition	0.83 ± 0.01	0.44 ± 0.06
TB NIE	ternary blend AMF/RO/LO (70:20:10)	noninteresterified	0.21 ± 0.00	1.51 ± 0.17
TB IE6h		6 h of interesterification	0.74 ± 0.00	0.16 ± 0.11
TB IE6h+AO		interesterification + antioxidant addition	0.74 ± 0.00	0.25 ± 0.05

Nevertheless, a ternary blend involving rapeseed oil (RO) provided most interesting and balanced properties.

Still, results obtained from accelerated oxidation conditions involved in the Rancimat test may not be strictly representative of the process occurring in shelf-life conditions (*13*). This observation motivated the present study, which focuses on the oxidative stability of interesterified fats under accelerated and nonaccelerated storage conditions, closer to normal storage conditions than the Rancimat test (*6*).

MATERIALS AND METHODS

Materials. Lipozyme TL IM was kindly supplied by Novozymes A/S (Bagsværd, Denmark); AMF was purchased from CORMAN SA (Goé, Belgium) and kept at −20 °C until use; LO was obtained from Vandeputte (Mouscron, Belgium); RO was produced by Vandemoortele (Izegem, Belgium) and purchased in a local store. Both were kept in the dark at 4 °C until use. 2-Thiobarbituric acid, *p*-anisidine, and α-tocopherol were purchased from Sigma-Aldrich (St. Louis, MO). Isooctane and 1-butanol were of spectrophotometric grade; all other solvents and reactants were of analytical grade (VWR International, Leuven, Belgium).

Samples Preparation. AMF/LO (70:30) and AMF/RO/LO (70:20:10) blends were prepared for both direct sampling and enzymatic interesterification, performed as previously described (*12*).

Briefly, the immobilized enzyme was conditioned to control hydrolysis byproduct by residence in three successive 600 g RO batches at 70 °C, prior to filtration, rinsing, and introduction in a double-coated batch reactor, containing 600 g of the fat blends. Enzyme concentration was 4% (w/w) and temperature maintained at 70 °C; an agitation rate of 1000 rpm (blade stirrer) and N_2 constant bubbling (1 bar) were applied. The reactor was isolated from light, and the reaction was conducted for 6 h, prior to filtration.

Interesterified batch was divided for direct sampling, and α-tocopherol (50 ppm w/w) was added prior to sampling.

Storage Studies. All fat samples of 5 ± 0.1 g used within the different tests were weighed in open, 25 mL glass flasks with a diameter of 30 mm. A few samples were stored for 12 weeks at 4 °C in a divided box placed in the upper part of a refrigerated chamber.

Shelf-life study was conducted in an LMS 1200 incubator set at 25 ± 0.5 °C. A sufficient number of samples (in 25 mL open flasks) were placed in separate, partly open desiccators according to their composition, to avoid cross-contamination but to permit air renewal. Samples were periodically taken out in triplicate within the 12 week duration of the study and submitted to analysis.

Oven-aging was realized in the same manner as the shelf-life study, in a Memmert ICP800 incubator at 60 ± 0.5 °C. Samples were periodically withdrawn in duplicate during 2 weeks.

Withdrawn samples were flushed with N_2, capped, and stored at −20 °C until analysis.

Analytical Procedures. Free fatty acid (FFA) content, peroxide value (PV), *p*-anisidine value (pAV), 2-thiobarbituric acid value (TBA value), and specific ultraviolet extinction at 232 nm (K_{232}) and at 268 nm (K_{268}) were determined by using AOCS standard methods Ca 5a-40, Cd 8b-90, Cd 18-90, Cd 19-90, and Ch 5-91, respectively (*14*). Those methods were slightly adapted for routine application on small samples.

Solid Phase Microextraction (SPME) Analysis of Samples from the Oven-Aging Test: GC-SPME Analysis and Mass Spectrometry. A divinylbenzene/carboxen/polydimethylsiloxane coated fiber Stable Flex and a manual SPME holder (Supelco Inc., Bellefonte, PA) were used. Before each headspace sampling, the fiber was exposed to the GC inlet for 5 min for thermal desorption at 250 °C. Two grams of thawed (at 50 °C) fat sample was placed in 10 mL vials, and each vial was sealed with a tan PTFE/white silicone septum (Varian Belgium, Sint-Katelijne-Waver, Belgium). The samples were then equilibrated for 10 min at 60 °C. The SPME fiber was exposed for 30 min to each sample maintained at 60 °C, and finally the fiber was inserted into the injection port of the GC. These conditions were chosen on the basis of previous tests (not shown). GC-MS analyses were carried out on an Agilent 5973N gas chromatograph (Agilent Technologies, Palo Alto, CA) coupled to a mass selective detector operating in electron impact mode (ionization voltage = 70 eV). A Varian Factor-Four VF-WAXms capillary column (30 m length, 0.25 mm i.d., 0.25 μm DF) was used (Varian Belgium). The temperature program started at 40 °C for 2 min and then increased at a rate of 5 °C/min to 250 °C, which was maintained for 10 min. Injector, interface, and ion source temperatures were 250, 250, and 230 °C, respectively. Injections were performed in splitless mode, and helium (1 mL/min) was the carrier gas. The identification of molecules was based on comparison of their mass spectra with those contained in Wiley275 databases.

Statistical Analysis. A bilateral Student *t* test (α = 0.05), assuming unknown and distinct variances, was applied to conclude the significance of observed differences.

RESULTS

Raw Materials and Substrate Characterization. The six substrates resulting from these treatments are listed in **Table 1**. FFA content rises after enzymatic interesterification, due to the lipase-catalyzed hydrolysis of fatty acids with residual water in fats (*12*). It nevertheless remained under 1%. The PV of raw AMF, LO, and RO was measured at 1.01 ± 0.20, 1.98 ± 0.14, and 4.83 ± 0.26 mequiv/kg, respectively. These values are in agreement with the manufacturer's specifications, although highly sensitive LO displays early autoxidation evidence. The PVs of the blends are equal to the weighed average of the PV of their constituents.

Interestingly, IE blends present a significantly lower PV (< 0.5 mequiv/kg) when compared to their parent NIE blends. As far as we know, this is the first time that such a decrease of PV is reported in the field of lipid interesterification, even though we worked with substrates presenting initial PVs of > 2 mequiv/kg, which is relatively high for lipid oxidation study.

PV reduction observed after reaction was first considered as a result of the sustained temperature (70 °C) used during the interesterification reaction. This might have enhanced hydroperoxide decomposition into volatile secondary oxidation products, stripped off by N_2 bubbling. This hypothesis was tested by submitting AMF/LO blends to the reaction's conditions, without any added enzyme and in the presence of 4% (w/w) inactivated Lipozyme TL-IM. The absence of lipase activity, that is, nil interesterification degree, was checked by GC as described previously (*12*). In every case, only a weak reduction (< 10% of the initial value) of PV was observed (not shown).

A possible mechanism might be the lipase-catalyzed acylation of the hydroperoxide group by a fatty acid. Indeed, as previously proposed by Baba et al. (*15*), the hydroperoxy group may

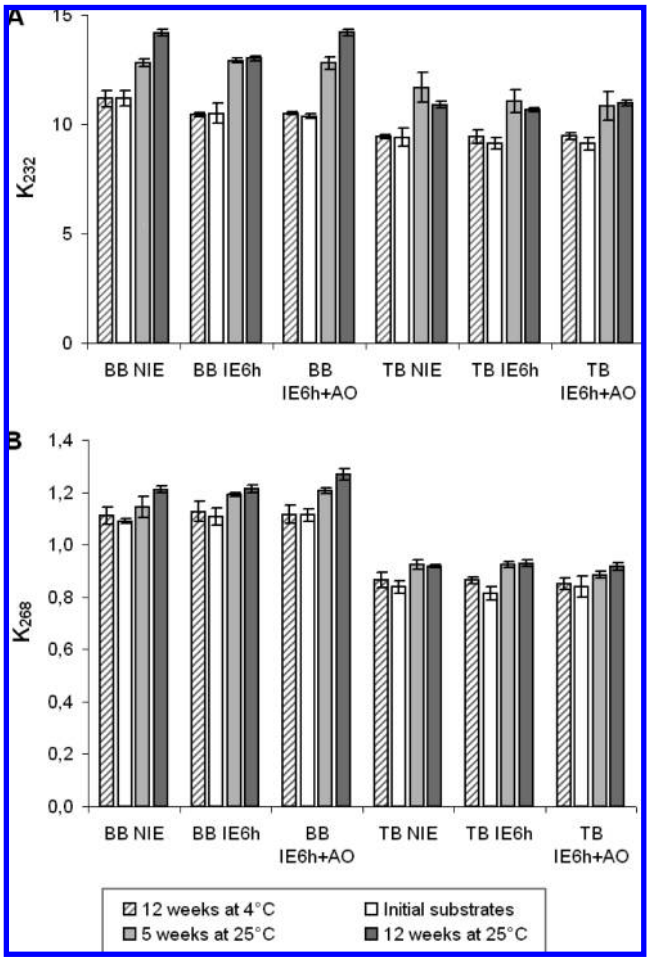

Figure 1. Specific extinction at 232 (**A**) and 268 nm (**B**), for substrates stored for 12 weeks at 4 °C, initial substrates, and after storage at 25 °C during 5 and 12 weeks.

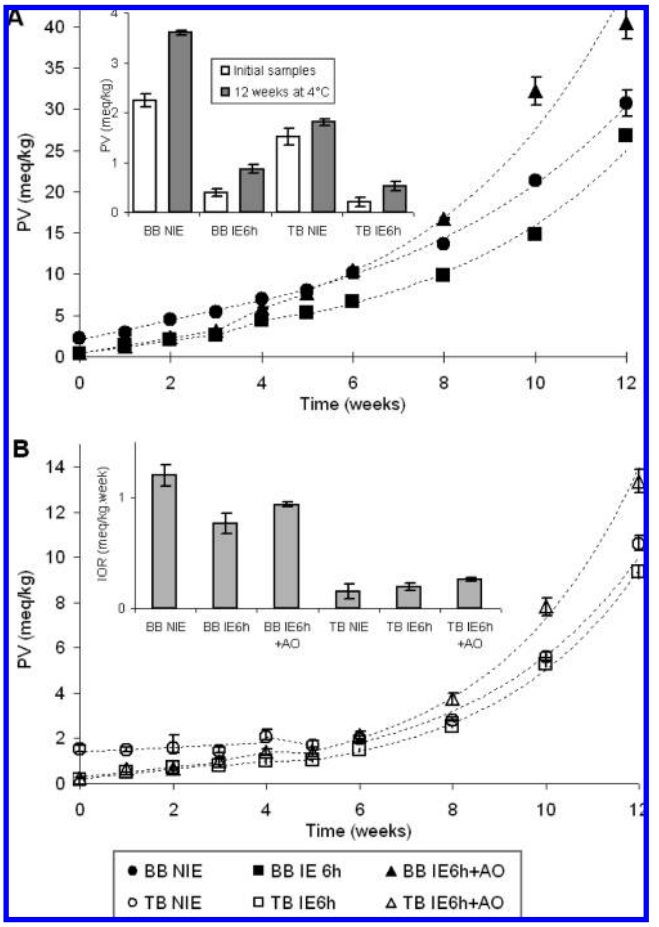

Figure 2. (**A**) PV evolution for BB substrates at 25 °C; (inset) initial PV comparison for both BB and TB substrates, NIE and IE6h, and after 12 weeks at 4 °C. (**B**) PV evolution for the TB; (inset) IOR. Best linear correlation was achieved within the 4 first weeks for BB NIE and all TB and within the 3 first weeks for both interesterified BB.

react within the catalytic site of a lipase, as an alcohol group would do.

$$R_1{-}O{-}O{-}H + R_2{-}O{-}CO{-}R_3 \rightarrow$$
$$R_1{-}O{-}O{-}CO{-}R_3{+}R_2{-}OH$$

Acylated hydroperoxides later evolve into carbonyl.

$$R_1{-}O{-}O{-}CO{-}R_3 \rightarrow R_1 = O + R_3COOH$$

Although a more thorough insight should be addressed to confirm or rebut this proposed pathway of hydroperoxide decay, these reactions would constitute a supplementary origin of FFA within the reaction mixture.

Shelf-Life Studies at 4 and 25 °C. *Specific Extinction at 232 and 268 nm*. The ethylenic hydrogen abstraction, prior to hydroperoxide formation in the early stages of PUFA autoxidation, leads to the rearrangement and conjugation of the double bonds (*16*). This process is accompanied by increased UV absorption at 232 nm (conjugated dienes) and 268 nm (conjugated trienes), measured and expressed as specific extinction at given wavelengths.

Specific extinctions resulting from the shelf-life study are reported in **Figure 1**. At both wavelengths, specific extinctions of TB were consistently lower than those of BB. A negligible increase was observed at both 232 and 268 nm between the initial substrates and after 12 weeks of storage at 4 °C.

At 25 °C, we found no significant effect of the treatments (IE and IE + antioxidant addition) within the TB and only a consistent shift to higher extinction values at both wavelengths. Within BB, the NIE blend showed a steady increase in diene and triene contents, whereas the IE blend was subject to a shift to higher values, as was IE TB.

Peroxide Value. Hydroperoxide formation was followed by iodometric titration (**Figure 2**). Due to its highest content in PUFA, the BB series achieved at all times higher PV than the equivalent TB.

The differences in the initial values were already discussed. **Figure 2A** (inset) reports the PV observed after 12 weeks at 4 °C. Autoxidation remains low in those conditions and of narrower amplitude within TB.

All curves of **Figure 2** may be well described and correlated ($R^2 > 0.98$) by an initial linear increase in PV (induction period), followed by a transitional and then exponential (autocatalytic) phase (*8*).

The slope of the linear autoxidation phase is reported in **Figure 2B** (inset), expressed as an initial oxidation rate (IOR, in mequiv/kg/week).

The IOR was much lower for TB than for BB, likely for the same reason as for the overall PV evolution, that is, a lesser susceptibility resulting from a lower content in linolenic acid. Along with the initial PV reduction, an effect of interesterification was observed within the BB through a lower IOR. In the TB, this effect was not detected, and IOR appeared low and slightly different.

In BB as in TB, the rate of peroxide formation in the exponential phase was comparable from NIE blends to IE blends (except that the NIE curves are shifted to higher values, due to

higher initial PV, and, for BB, higher IOR). On the other hand, in the same autocatalytic region of the graph, α-tocopherol addition clearly increased the rate of oxidation, indicating a significant pro-oxidant activity. This result joins the previous observations on diene formation in BB (IE6h+AO), although further correlations are difficult to establish.

p-Anisidine Value (*pAV*). The pAV provides information about secondary oxidation products, that is, aldehydes. The pAVs for the shelf-life study are reported in **Figure 3**. No significant increase in pAV was observed after 12 weeks at 4 °C.

Several parallels can be made with the PV. As before, the pAV of the BB was consistently higher, at all times, than those related to the corresponding TB. We did not observe an effect of interesterification on secondary oxidative product formation, correlating with the very similar behavior of PV curves of NIE and IE blends. The pro-oxidant action of α-tocopherol addition was clearly confirmed in BB IE6h+AO. It was less noticeable in the equivalent TB, but the general oxidation level was smaller.

2-Thiobarbituric Acid Value (*TBA Value*). The TBA value is proposed for the dosage of malondialdehyde (MDA), a toxic aldehyde resulting from the oxidation of triunsaturated fatty acids (linolenic acid in particular).

TBA values for the shelf-life test are reported in **Table 2**. 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 happened 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 reacting compounds.

Oven-Aging (60 °C). The specific extinctions, at both wavelengths, remained steady for the first 7 days, followed by a quick increase (**Figure 4**). The effect of composition caused TB values to be systematically below BB values. Within the TB, no specific effect of interesterification or α-tocopherol addition can be put forward so far. Within the BB, interesterification caused smaller specific extinction. The only impact of α-tocopherol appeared after 15 days. Very variable correlations between specific extinction and PV were found (R^2 ranging from 0.75 to 0.97 from substrate to substrate).

PV and pAV (**Figure 5**) in these conditions presented huge similitude and were well correlated ($R^2 > 0.98$ in every cases). Some specifics were also observed compared to the previous results obtained at 25 °C.

In 3 days, PV reached values observed only after 8 weeks at 25 °C (peroxide formation about 20-fold faster). The IOR determination (**Figure 5A**, inset), although less precise than in the shelf-life study due to quicker oxidation, indicated an effect of interesterification on BB, but not of α-tocopherol addition. The same applies to TB.

Examination of the whole curves highlighted lower primary and secondary oxidations for interesterified BB and TB. The

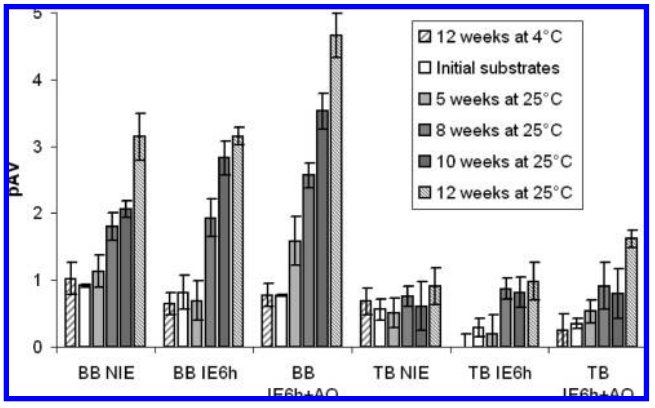

Figure 3. Evolution of the pAV for substrates stored for 12 weeks at 4 °C, initial substrates, and during storage at 25 °C.

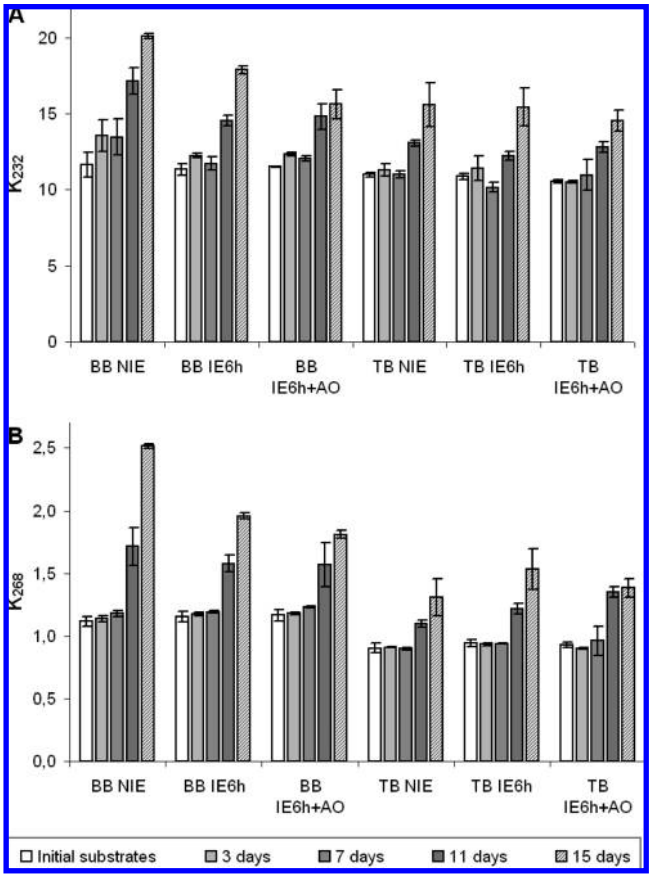

Figure 4. Specific extinction at 232 (**A**) and 268 nm (**B**) for initial substrates (white bars) and after storage at 60 °C for 3 (light gray bars), 7 (medium gray bars), 11 (dark gray bars), and 15 days (finely hatched bars).

Table 2. TBA Values for the Shelf-Life Study (4 and 25 °C) and the Oven-Aging Study (60 °C)

	4 °C		25 °C		60 °C		
	12 weeks	initial value	5 weeks	12 weeks	3 days	7 days	13 days
BB NIE	0.070 ± 0.006	0.050 ± 0.006	0.067 ± 0.010	0.076 ± 0.009	0.109 ± 0.005	0.160 ± 0.008	0.215 ± 0.006
BB IE6h	0.050 ± 0.004	0.037 ± 0.004	0.045 ± 0.011	0.053 ± 0.003	0.068 ± 0.003	0.098 ± 0.000	0.112 ± 0.005
BB IE6h+AO	0.051 ± 0.003	0.038 ± 0.003	0.043 ± 0.012	0.058 ± 0.006	0.064 ± 0.004	0.078 ± 0.024	0.085 ± 0.005
TB NIE	0.028 ± 0.003	0.026 ± 0.003	0.021 ± 0.003	0.036 ± 0.003	0.046 ± 0.006	0.058 ± 0.005	0.070 ± 0.004
TB IE6h	0.025 ± 0.000	0.019 ± 0.001	0.012 ± 0.001	0.028 ± 0.002	0.027 ± 0.003	0.040 ± 0.005	0.044 ± 0.001
TB IE6h+AO	0.023 ± 0.002	0.017 ± 0.002	0.010 ± 0.001	0.032 ± 0.002	0.036 ± 0.005	0.044 ± 0.010	0.053 ± 0.002

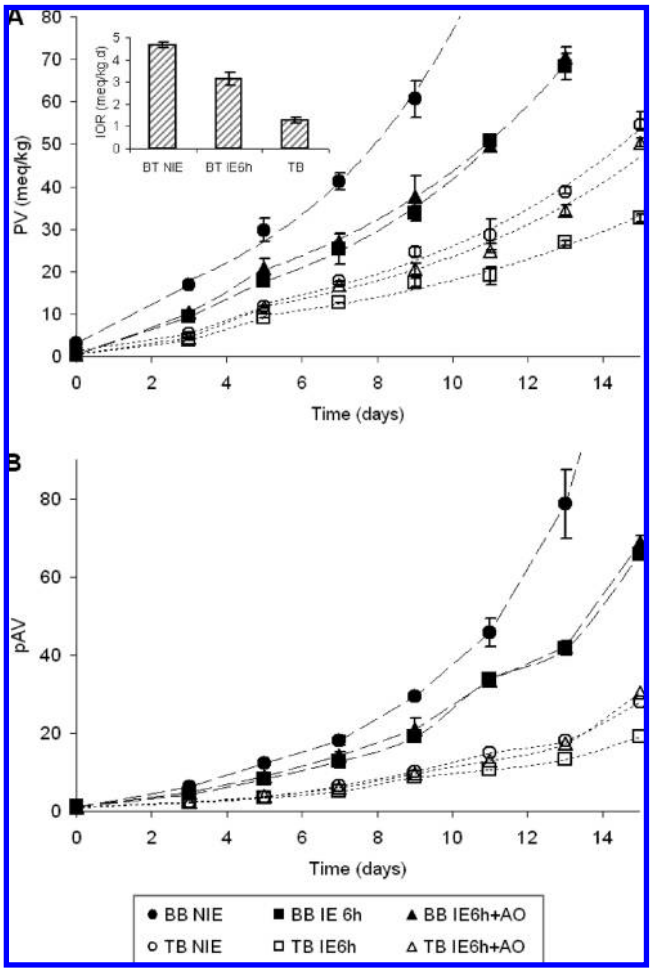

Figure 5. (**A**) PV evolution for all substrates at 60 °C; (inset) IOR of BB NIE, average of BB IE6h and IE6h+AO, and average IOR of all TB (calculation based on the initial value and after 3 days). (**B**) paV evolution for all substrates (the legend is common).

effect of α-tocopherol addition ranged from no detectable effect (BB) to weakly pro-oxidant (TB).

The same observations can be made concerning the TBA: beneficial effect of interesterification in lowering the TBA values, further increased by the tocopherol addition, variable depending on the nature of the blend.

SPME. SPME followed by GC-MS analysis of volatile compounds was performed on the samples stored at 60 °C. Trials on samples from the experiments at 4 or 25 °C led to SPME fiber saturation by carboxylic acids and the detection of very few other compounds (not shown).

Interesterified substrates with added antioxidant were characterized by chromatograms with volatile profiles very close to those from the IE substrates, without added α-tocopherol. The number of compounds and their signal intensities were not significantly different in both cases (not shown). Because no effect of α-tocopherol addition was observed, only the effects of composition and interesterification are discussed.

Among more than a hundred volatile compounds (not shown), about 70 were identified and are listed in **Table 3**. Most of the compounds were previously reported as autoxidation products of milk fat or vegetable oils (*8*). In contrast with the work by Lund and Hølmer, in our experimental conditions no alkanes were detected in the headspace of analyzed samples (*17*).

Carboxylic acids represented a major part of signal integration (**Table 4**). Most were short-chain fatty acids from AMF (*12*). 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 (*18*) and the cause of unpleasant off-flavors.

The increase of acetic acid chromatographic area is reported in **Figure 6A**. It is correlated with the previous PV measurements (**Figure 5A**; $R^2 > 0.98$ for BB, $R^2 > 0.87$ for TB).

Aldehydes, as hydroperoxide decomposition products, are a major class of odorants. Among those compounds, *n*-alkenals (2,4-heptadienal, 2-butenal, 2-pentenal, 2,4-hexadienal, 2,4-decadienal, 2-nonenal, and 2-heptenal) and *n*-alkanals (hexanal, pentanal, propanal, heptanal, and nonanal) were detected in exponentially increasing amounts with storage time. The total amount of aldehydes (sum of the chromatographic area) was quite well correlated with the corresponding pAV (**Figure 5B**; $R^2 > 0.95$). Chromatographic areas obtained for TB are consistently lower than observed in BB, except for a sharp increase after 12 days in interesterified TB.

We observed higher amounts of heptadienal, hexadienal, and 2,4-decadienal in BB than in TB. This is likely a result of the higher content in linoleic and linolenic acids in the binary substrates (*8*).

Ketones (e.g., 3,5-octadien-2-one, 2-heptanone, 2-nonanone, and 2-propanone) are another important class of odorants in the field of lipid autoxidation. Considering separately saturated and unsaturated ketones, a consistent decrease in *n*-alkanones form day 0 to 8 was found. Content in heptanone and pentanone was roughly correlated with the linseed content in NIE blends (not shown). Monounsaturated ketones are formed in small amounts increasingly with oxidation time. The main ketonic compound observed was octadienone. This volatile has been proposed as an indicator of milk fat oxidation (*19*). Its formation through time is reported in **Figure 6B**. As for acetic acid, NIE BB showed very high values. Substrates BB IE6h and TB NIE interestingly provided very similar results.

Odorous lactones were detected: δ-octa-, deca-, and dodecalactones 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 (*19*). However, γ-hexalactone and 2-hexen-4-olide were detected only in oxidized products.

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

A last compound, 1-acetylcyclohexene, induced strong signals in highly oxidized samples (not shown). It may be an artifact; however, it was not detected on the blank analyses.

On the basis of the sum of chromatographic areas 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 compound release. Much lower initial values were obtained for TB, as a likely result of its lower content in linolenic 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 (substrate composition or results of other methods assessing oxidation). This is likely due to the complexity of the reactional pathways involved in the production of volatile compounds (*8*).

Table 3. Volatile Compounds Detected in the Headspace of Substrates, Tentatively Identified by Mass Spectra Correlation

peak	compound	peak	compound	peak	compound
	Aldehydes		**Ketones**		**Acids**
1	propanal	2	2-propanone	36	acetic
3	2-propenal	27	3-hydroxy-2-butanone	42	propionic
4	butanal	5	2-pentanone	46	butyric
8	2-butenal	7	1-penten-3-one	55	pentanoic
6	pentanal	51	4-methyl-3-penten-2-one	59, 63	pentenoic
12	2-pentenal	9	3-hexanone	62	hexanoic
10	hexanal	52	2-cyclohexen-1-one	64	2-ethylhexanoic
16	*trans*-2-hexenal	14	2-heptanone	65	heptanoic
30, 31	2,4-hexadienal	32	3-octen-2-one	68	octanoic
15	heptanal	39, 43	3,5-octadien-2-one	69	2,4-hexadienoic
23	*trans*-2-heptenal	48	1-phenylethanone	71	nonanoic
17	*cis*-4-heptenal	28	2-nonanone	72	decanoic
37, 38	2,4-heptadienal	45	2-undecanone	73	9-decenoic
40	benzaldehyde	60	2-tridecanone	75	benzoic
20	octanal			76	lauric
34	2-octenal		**Alcohols**	77	myristic
29	nonanal	26	2-propanol		
41	*trans*-2-nonenal	24	3-pentanol		**Lactones**
49, 53	2,4-nonadienal	21	*trans*-2-penten-1-ol	54	γ-hexalactone
44	2,6-nonadienal	22	*cis*-2-penten-1-ol	58	2-hexen-4-olide
47	2-decenal	13	1-penten-3-ol	66	δ-octalactone
50, 61	2,4-decadienal	57	*trans*-cyclopentene-3,4-diol	70	δ-decalactone
56	3-dodecen-1-al	25	1-hexanol	74	δ-dodecalactone
		33	2-hexen-1-ol		
		67	phenol		**Miscellaneous**
		35	1-octen-3-ol	18, 19	1-acetylcyclohexene
		11	2-octen-3-ol		

Table 4. Integrated Chromatographic Area (0×10^6) for Volatile Families Detected in the Headspace of Fat Samples before Aging (0) and after 4, 8, and 12 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
acids	315.2	261.6	208.0	356.6	1829.0	224.9	224.5	288.0	62.0	52.0	129.8	181.4	1740.9	212.8	636.5	1700.8
aldehydes	61.8	171.0	280.2	338.6	74.6	56.4	115.8	208.2	10.7	21.2	48.8	197.6	12.8	30.3	58.2	1099.3
ketones	203.4	179.4	155.5	319.7	114.3	23.5	40.2	268.2	29.0	22.7	51.3	76.3	107.7	24.9	99.2	431.9
n-alkanones	188.8	99.9	11.0	17.5	90.8	11.9	8.7	28.9	29.0	19.3	13.9	33.0	105.5	19.0	11.1	72.5
alkenones	0.0	4.1	8.2	14.4	0.3	2.8	6.4	13.4	0.0	0.2	1.8	1.5	0.0	1.9	3.8	13.9
octadienone	4.2	68.8	133.4	279.3	22.1	7.6	22.9	221.5	0.0	2.6	33.7	241.0	2.2	3.5	81.7	325.7
δ-lactones	14.6	13.8	1.0	2.7	32.4	3.0	1.0	2.0	1.3	1.5	1.1	2.2	14.1	5.6	9.5	23.2
other lactones	0.0	18.3	6.1	18.4	0.7	1.5	4.0	9.2	0.0	0.3	1.0	7.0	0.0	0.5	1.7	36.3
alcohols	21.2	22.0	22.9	21.0	0.6	9.6	15.8	17.2	0.3	4.4	8.3	40.8	0.0	4.0	8.4	72.6
total[a]	301.0	383.4	465.8	700.3	222.6	94.1	176.8	504.8	41.4	50.0	110.6	323.8	134.6	65.3	177.0	1663.3

[a] Total of aldehydes, ketones, lactones, and alcohols.

DISCUSSION

The effect of composition appears to be quite obvious, that is, the TB, containing less LO and thus less highly unsaturated PUFA, presented a lesser oxidative susceptibility at all tested temperatures; this observation is in agreement with previous Rancimat measurements. We assessed a higher volatile emission from BB than from TB, along with higher TBA values.

An impact of the interesterification was observed through a significant reduction of the initial PV. Concerning the BB, a lower initial oxidation (peroxide formation) rate was noted at both 25 and 60 °C. This is likely the result of the dispersion of PUFA from LO highly unsaturated TAG, as proposed by Kimoto et al. This behavior could be linked to the interesterification process itself, which redistributes the FA within the TAG, modifying the accessibility of the PUFA. Previous works of our team (*12*, *20*) highlighted the disappearance of low melting point TAG in AMF/oil blends in the course of the interesterification reaction. This can explain the higher oxidative stability observed during the initiation phase of autoxidation. However, the effect remained undetectable in TB at 25 °C.

During the autocatalytic phase of autoxidation at 25 °C, no evidence of a positive or negative effect of interesterification on BB or TB was found, except a shift to lower values of the curves reflecting oxidation. At 60 °C, however, oxidation of the IE products was found to be slower than that of NIE blends.

It was proposed that higher temperature increases the rate of hydroperoxide decomposition (thus enhancing autoxidation) but causes lower oxygen solubility in steady oil, so that peroxide neoformation is hindered (*21*).

The contradictory effect of temperature brought to light the beneficial effect of interesterification. The redistribution (dispersion) of PUFA from LO and, to a smaller extent, from RO, due to interesterification, significantly slowed the rate of

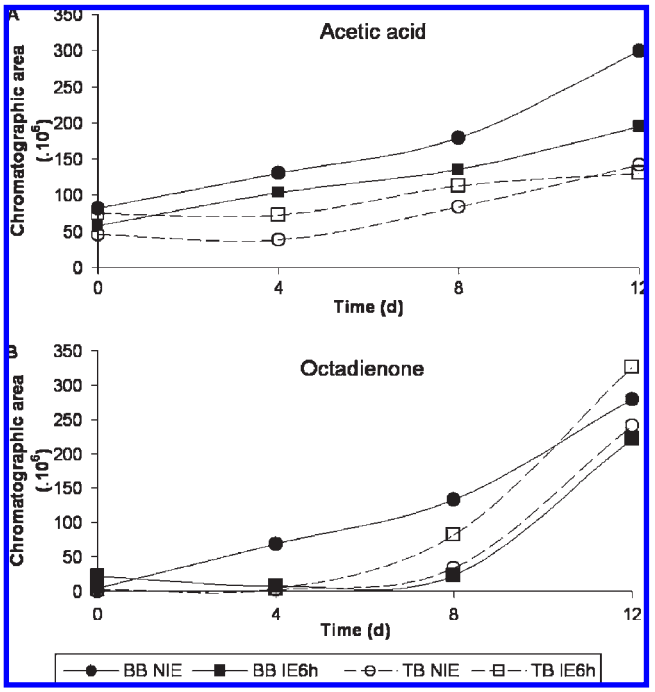

Figure 6. (**A**) Acetic acid release in time at 60 °C. (**B**) Octadienone release (the legend is common).

autoxidation compared to the native NIE blends. In the presence of excess oxygen and higher temperature (Rancimat test at 100 °C), no effect of interesterification was noted (*12*).

Frega et al. (*22*) suggested a pro-oxidant effect of FFA. The present study did not pinpoint such an effect.

The α-tocopherol addition caused an apparent pro-oxidant effect at 25 °C. After Kamel-Eldin and Appleqvist (*21*), if α-tocopherol may enhance peroxide decomposition, these authors rejected the idea of a significant pro-oxidant effect per se in bulk oil, preferring a synergy between α-tocopherol and transition metal ions, which would be the real pro-oxidant agents.

The apparent pro-oxidant effect of α-tocopherol addition was undetectable at 60 °C for BB, in agreement with the findings of Marinova and Yanishlieva (*23*) on the reduction of the pro-oxidant effect of tocopherol addition with increasing temperature. The pro-oxidative effect itself involves in particular the formation of superoxide radical $O_2^{\bullet -}$, hindered at higher temperature by low oxygen solubility.

In TB, the presence of added tocopherol nullified the observed positive effects of interesterification. The effect of tocopherol addition in a pro- or antioxidant action is also related to its initial concentration and initial PV of model substrates (*24*) and closely related to the composition of substrate (*21*). Divergences in the effect of α-tocopherol as antioxidant, depending on the experimental conditions, were already reported (*25*).

This study brings new evidence of the complex dependence of oxidative stability with composition and temperature effect.

From a practical point of view, we can so far confirm the potential of PUFA enrichment of AMF by selected vegetable oils by interesterification as a potent method of structured lipid production, although keen attention must be given to the oxidative state of raw oils and to storage conditions. The addition of α-tocopherol appears to be unnecessary to enhance storage stability at low temperature.

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