Changes of Sunflower Oil Polyenoic Fatty Acids under High Temperatures

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Abstract: Heat induced *cis-trans* isomerisation of sunflower oils depending on temperature, reaction time and original content of linoleic acid was investigated. The content of isomeric fatty acids was determined by gas chromatography and the content of polymers by gel permeation high-performance liquid chromatography. The content of *trans* fatty acids increased with time and with temperature and a rate of *cis-trans* isomerisation and polymerisation depends on the temperature according to Arrhenius equation. The content of polymers was significantly lower in sunflower oil with high content of oleic acid because of the low concentration of linoleic acid in oil. In both oils the content of conjugated linoleic acid initially increased depending on time and temperature, however after certain time the stationary state occurred. Polymerisation of polyenoic fatty acids takes place directly with heat induced *cis-trans* isomerisation.

Keywords: *cis-trans* isomerisation; polymerisation; *trans* fatty acid; conjugated linoleic acid

INTRODUCTION

Double bonds of unsaturated fatty acids (FA) can be either subject to geometrical isomerisation with forming *trans* isomers of fatty acids (TFA) or positional isomerisation with shifting of double bond in carbon chain. Dienoic FA usually provide a conjugated isomers (Velíšek 2002). Conjugated isomers of octadecadienoic acid are collectively known as conjugated linoleic acid (CLA) (Larqué *et al.* 2001). Isomerisation of double bonds of FA are effectively induced by heating up 200°C, so the TFA are formed especially by physical refining and deodorisation of plant oils. Their presence negatively affects a final nutritious quality of refined oils (Ackman *et al.* 1974; O'Brien 2004; Čmolík *et al.* 2007).

Under high temperatures also polymerisation and cyclisation of FA probably occurs. Generating polymers and cyclic FA are together with TFA deleterious. Therefore, the thermal isomerisation and polymerisation of FA should be more studied in term of technological and in particular of nutritional aspect.

The aim of this study was to investigate heat induced *cis-trans* isomerisation and polymerisation of standard sunflower oil (SSO) and high oleic acid sunflower oil (HOASO) depending on temperature, reaction time and original content of linoleic acid in oil.

MATERIALS AND METHODS

For experiments were chosen two refined sunflower oils differing in content of oleic and linoleic acid and not containing linolenic acid. SSO was obtained from Setuza, a.s. (Czech Republic), production 2007 (Σ C18:2 61.35%, *t* C18:2 1.65%, linoleic acid 59.69%; Σ C18:1 19.53%, *t* C18:1 0.00%; polymers 0.00%). HOASO was obtained from Palma-Tumys, Bratislava, a.s. (Slovakia), production 2007 (Σ C18:2 9.78%, *t* C18:2 0.20%, linoleic acid 9.58%; Σ C18:1 77.78%, *t* C18:1 0.00%; polymers 0.00%).

Laboratory isomerisation. Samples of investigated plant oils (ca 40 g) were parallel heated repeatedly twice in couple of glass laboratory reactors under argon atmosphere in hot-flue thermostat at temperatures 200, 220, 230, 240 and 250°C for time lasting up to 170 hours. Reactors were equipped by air-leak tubes for gas intake. Argon flow was just minimal for a weak agitation of heated oils. Accurate temperature in thermostat was scanned by a digital thermometer F200 for the whole time of isomerisation according to computer programme F200E. Samples for analyses were taken of heated oils in certain intervals (twice per day).

Analysis of composition of fatty acids isomers. Contents of individual isomeric FA were determined by capillary gas chromatography (Agilent Technology, 6890N) with flame ionisation detection (CGC/FID) according to ISO 15304:2002. Fatty acid methyl esters (FAME) were prepared by standard method (ISO 5509:2000), pentadecanoic acid was used as internal standard to obtain content of FA in absolute values (% w/w). For analysis the capillary column SP^{TM} 2560 (Supelco, Bellefonte), 0.25 mm × 100 m, film thickness 0.2 µm was used. The conditions of analysis were: hexane solution of FAME (1%) was used for the injection (1 µl), split injection (1:50) at 220°C; flow of carrier gas (He) 1 ml/min; analysis at 175°C for 120 min; FID detection at 250°C, flow of H_2 40 ml/min, air flow 450 ml/min and make-up gas (N_2) flow 45 ml/min.

Determination of content of polymeric lipids. Polymers of FA were determined by gel permeation high-performance liquid chromatography (Agilent 1100 Series, Agilent Technologies) with evaporative light scattering detection (GP-HPLC/ELSD). Sample of heated oil (10 mg) was diluted in 10 ml of tetrahydrofuran (THF) and 5 µl of the solution was injected on column PLgel 5 µm, 10 nm, 300 × 7.5 mm (Agilent) with separation capacity up to 4000 g/mol. THF was used as a mobile phase with flow 0.6 ml/min. Analysis was running at 25°C for 15 min, ELSD detection at 50°C. Results were introduced as a sum of all polymer lipids without resolution of polymerisation degree in % rel.

RESULTS AND DISCUSSION

The content of individual TFA increased with the time and with the temperature in both investigated oils, as it was supported by expressed degree of isomerisation (DI) depending on reaction time (Figure 1). DI of octadecenoic (C18:1), resp. octadecadienoic acid (C18:2) was expressed as a ratio between total content of *trans* isomers and total content of all isomers of C18:1 (*c*- and *t*-), resp. C18:2 (Kemény *et al.* 2001). In both oils the $DI_{C18:2}$ was significantly higher then the $DI_{C18:1}$. $DI_{C18:2}$ were same in both oils, but $DI_{C18:1}$ was lower in HOASO. Range of *cis-trans* isomerisation of C18:2 thus is not depend on original content of linoleic acid, but the higher is original content of oleic acid, the lower is range of isomerisation of C18:1 in oil. The main products of *cis-trans* isomerisation of original linoleic acid are mono-*trans* isomers (9*c*,12*t*- and 9*t*,12*c*-).

Dependance of content of *trans* C18:2 (*c,t*-, *t,c*-, *t,t*-) and CLA and *trans* C18:1 on reaction time was linear in all temperatures with average correlation coefficients explicited in Table 1. This dependance was linear only at the start of reaction but after a certain time a stationary state occurs at temperatures 240 and 250°C. It can be caused by determined retardation of original linoleic

Table 1. Correlation coefficients (*R*) for linear dependance of contents of *trans* isomers on reaction time for SSO and HOASO

		200°C	220°C	230°C	240°C	250°C
SSO	*cis-trans* C18:2[a]	1.00	1.00	1.00	1.00	1.00
	trans-cis C18:2	1.00	1.00	1.00	1.00	1.00
	trans-trans C18:2	0.92	0.98	0.95	0.99	0.99
	conjugated C18:2	0.93	0.96	0.96	0.98	0.94
	trans C18:1[b]	–	0.90	0.69	0.98	0.99
HOASO	*cis-trans* C18:2	1.00	0.99	1.00	0.99	0.99
	trans-cis C18:2	1.00	0.99	1.00	0.99	0.99
	trans C18:1	0.79	0.88	0.96	0.95	1.00

[a]octadecadienoic acid, [b]octacedenoic acid

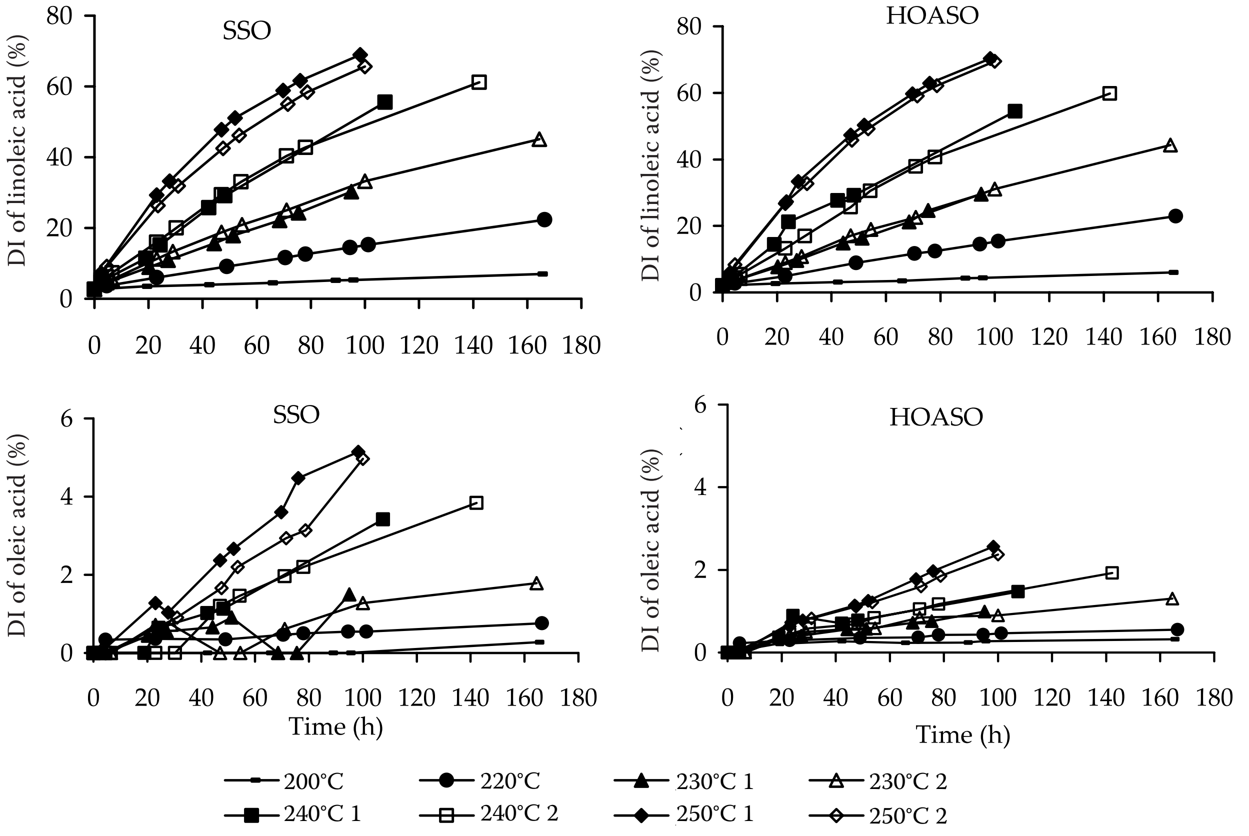

Figure 1. Time course of the degree of isomerisation (DI) of linoleic and oleic acid in SSO and HOASO at controlled temperatures (200–250°C)

acid decrease. To verify this hypothesis a dependance between linoleic acid content and $DI_{C18:2}$ was examined. Obtained dependance was linear with correlation coefficients 0.11, 0.93, 0.99, 0.99, 1.00 for SSO and 0.46, 0.96, 0.99, 0.98, 1.00 for HOASO in order of temperature (200, 220, 230, 240, 250°C) and thus the hypothesis about correlation between $DI_{C18:2}$ and it is content in oil was supported. Regarding the decrease of original C18:2 the *cis-trans* isomerisation corresponds with the kinetics of the first order (Wolff 1993; Hénon *et al.* 1999), but regarding the increase of TFA the isomerisation is close to zero order.

The content of CLA increased with time at all of investigated temperatures in both oils (Figure 2A). This increase was initially a linear function of time, but after a certain time the stationary state occurred because the forming conjugated isomers were probably consumed in the polymerisation reaction. The higher is temperature, the faster is forming of CLA.

The rate and the degree of *cis-trans* isomerisation increase with the heating time and temperature according to Arrhenius equation (Wolff 1993; Hénon *et al.* 1999).

$$k = Ae^{-E/RT}$$

In order to verify an Arrhenius formula an exponential dependance of rate constant of isomerisation on reciprocal absolute temperature ($1/T$) was investigated for all isomeric FA and polymers. Values of rate constants were found out of time dependance of contents of individual isomeric acids and polymers. Values of correlation coef-

Table 2. Correlation coefficients (R) for exponencial dependance of rate of an increase of isomeric FA and polymers content on isomerisation temperature

	SSO	HOASO
cis-trans C18:2[a]	1.00	1.00
trans-cis C18:2	1.00	1.00
trans-trans C18:2	1.00	–
conj. C18:2	0.99	0.47
trans C18:1[b]	0.98	0.96
Polymers	0.95	–

[a]octadecadienoic acid, [b]octacedenoic acid

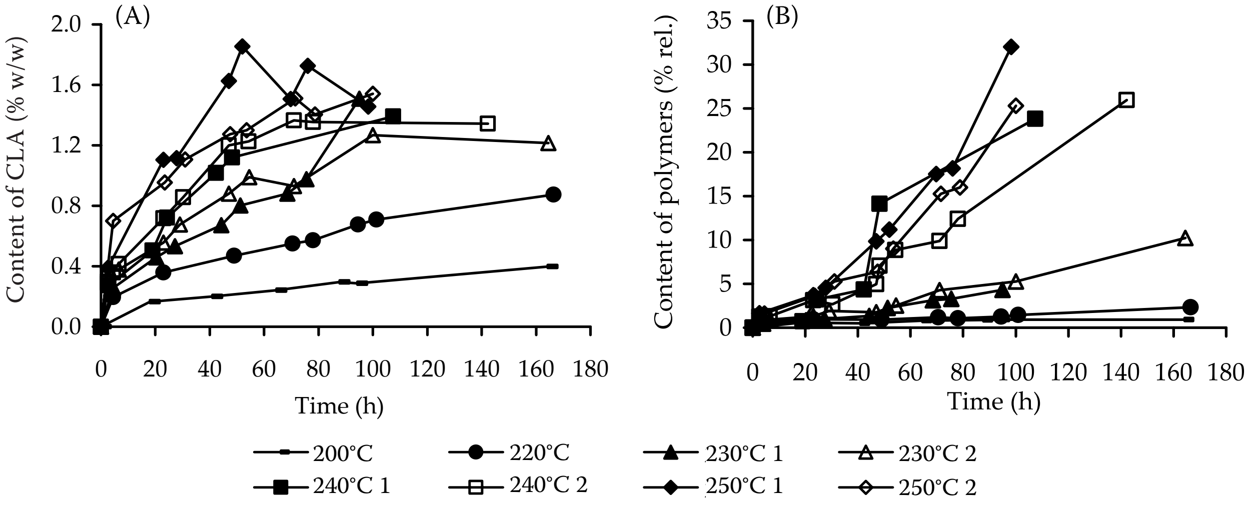

Figure 2. Time course of the content of CLA (A) and polymers (B) at controlled temperatures (200–250°C) for SSO

ficients obtained from exponential functions of temperatures are introduced in Table 2. Arrhenius equation was validated for rate of forming of all *trans* isomers, CLA and polymers in SSO. In HOASO this dependance was proved only for *c,t* and *t,c* C18:2 and *t* C18:1 because of the lower isomerisation and polymerisation degree.

Range of polymerisation increased with time (Figure 2B) in particular in SSO. In HOASO the content of polymers was significantly lower because of the low concentration of original linoleic acid in oil. The content of polymers initially linearly increased according to content of CLA. When forming of CLA comes to a stationary phase, the content of polymers begins to increase rapidly probably because of consuming of a newly formed conjugated isomers for polymerisation.

CONCLUSIONS

Content of TFA, CLA and polymers increased with the time and temperature. A linear correlation between $DI_{C18:2}$ and its content in oil was obtained. Polymerisation of polyenoic FA takes place directly with *cis-trans* isomerisation with temperature dependance according to Arrhenius equation like all of TFA and CLA.

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