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The process of isomerisation of plant oils rich in linoleic acid aimed at obtaining linoleic acid isomers (CLA)*

Proces izomeryzacji olejów roślinnych bogatych w kwas linolowy w celu otrzymania izomerów kwasu linolowego (CLA)

Słowa kluczowe: izomery kwasu linolowego, CLA, izomeryzacja, oleje: makowy, słonecznikowy, winogronowy

Badano proces izomeryzacji kwasu linolowego 9c,12c$C_{18:2}$ w następujących olejach: winogronowym (zawierającym 67,81% kwasu linolowego), makowym (70,1% kwasu linolowego) i słonecznikowym (58,2% kwasu linolowego). Proces prowadzono w roztworze glikolu etylenowego lub w glicerynie, w temperaturze 185 ± 5°C, katalizator NaOH w ilości 25% (m/m), czas reakcji 3 godz. W produkcie oznaczano skład kwasów tłuszczowych, zwłaszcza w obszarze izomerów kwasu linolowego, metodą chromatografii gazowej.

W wyniku przeprowadzonych badań stwierdzono, że stopień izomeryzacji kwasu linolowego zawartego w badanych olejach, mierzony stosunkiem sumy uzyskanych izomerów 9c,11t+10t,12c$C_{18:2}$ do zawartości kwasu linolowego w oleju 9c,12c$C_{18:2}$ jest znacząco wyższy dla glikolu etylenowego niż dla gliceryny.

Produkt izomeryzacji oleju winogronowego zawiera ok. 62% sumy izomerów 9c,11t+10t,12c, oleju makowego ok. 61%, a oleju słonecznikowego ok. 50%. Zawartość niepożądanego izomeru 11c,13t w produkcie izomeryzacji oleju winogronowego wynosi 3,4%, natomiast dla pozostałych olejów: słonecznikowego i makowego 5,1–5,6%.

Key words: CLA, isomerisation, plant oils: poppy seed, sunflower, grape seed

The subject of analyses was the process of isomerisation of linoleic acid 9c,12c$C_{18:2}$ in the following oils: grape seed containing 67.81% of linoleic acid, poppy seed containing 70.1% of linoleic acid and sunflower containing 58.2% of linoleic acid. The process was conducted in a solution of ethylene glycol or glycerine, in temperature of 185 ± 5°C, catalyst NaOH in amount of 25% (m/m), and reaction time 3 hours. A composition of fatty acids, especially within a range of linoleic acid isomers was determined in a product using gas chromatography method.

As a result of conducted analyses it was demonstrated that a degree of isomerisation of linoleic acid contained in studied oils, measured as a ratio of a sum of obtained isomers 9c,11t+10t,12c$C_{18:2}$

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to the content of linoleic acid 9c,12c$C_{18:2}$ in oil is considerably higher for ethylene glycol comparing to glycerine.

The product of isomerisation of grape seed oil contains about 62% of a sum of isomers 9c,11t+10t,12c, contains about 61% of poppy seed oil, while in the case of sunflower oil it is about 50%. The content of undesirable isomer 11c,13t in a product of isomerisation of grape seed oil is 3.4%, while for other oils, i.e. sunflower and poppy seed it is 5.1–5.6%.

Introduction

Isomers of linoleic acid containing sets of conjugated double bonds have gained a lot of interest during the last two decades due to their biological activity. They constitute a whole family of isomers, from 6,8 isomer to 13,15 $C_{18:2}$ isomer. The biggest importance is attributed to isomers 9c,11t and 10t,12c.

Isomer 9c,11t was described by Keppler already in the sixties of a previous century, however only at the beginning of the eighties it attracted more interest. The activity of 9c,11t isomer in decreasing of mutagenesis was demonstrated in research on grilled meat fat by the end of the seventies (Ip et al. 1991, Pariza et al. 1979). Further research proved anticarcinogenic and antiatherosclerosis activity of that isomer (Belury 2002, Bhattacharya et al. 2006, Ip et al. 1991).

Described by Keppler, 9c,11t isomer is created as an intermediate product of microbiological isomerization of linoleic acid 9c,12c$C_{18:2}$, and desaturation of oleic acid with presence of linoleic acid isomerase with *Butyrivibrio fibrisolvens* in conditions of rumen and intestines microflora of ruminants (Corl et al. 2001, Griinari et al. 2000, Keppler et al. 1966).

The mixture of isomers of linoleic acid containing conjugated double bonds, where one of them is in *trans* form, is generally referred as CLA (*conjugated linoleic acid*), and its main component, 9c,11t isomer is described as rumenic or bovine acid.

The results of numerous studies prove biological activity of 9c,11t and 10t,12c isomers (Pariza et al. 2001). For example, 9c,11t and 10t,12c isomers inhibited the development of cancer cells of breast, stomach, skin cancer or leukemia (Belury 2002, Ha et al. 1990, Ip et al. 1991, Lipkowski et al. 2003, Park et al. 2000). 10t,12c isomer appeared to be more active towards cancer cells of large intestine and colon (HT-29 and MIP 101), while towards prostate cells both isomers — 9c,11t and 10t,12c showed a moderate activity (Palombo et al. 2002).

The natural source of positional and geometric isomers of linoleic acid (CLA) are fatty products of ruminant origin, mainly milk and meat fat. The content of CLA isomers, mainly 9c,11t $C_{18:2}$ in milk fat depends on animal species and range within wide limits from about 0.4 to about 2.0%, and in special cases, e.g. in sheep milk fat even up to 3% (Fritsche et al. 1999, Gwardiak et al. 2000, Patkowska-Sokoła et al. 2000).

In connection with low content of CLA isomers in natural products, the research on their enrichment in bioactive isomers (Walisiewicz-Niedbalska et al. 2001) and on development of methods of their synthesis (Walisiewicz-Niedbalska et al. 2002) were conducted.

Synthetic CLA isomers are obtained by isomerisation of linoleic acid with presence of a strong base (NaOH or KOH), or by dehydration of 12-hydroxyoleic acid derivative (Yang et al. 2002). As a result of isomerisation of linoleic acid — the main component of sunflower oil and poppy seed oil, a mixture of the two main isomers 9c,11t and 10t,12c are obtained (Walisiewicz-Niedbalska et al. 2002) and 11c,13t and 8t,10c are obtained (Berdeaux et al. 1998, Ma et al. 1999, Yu et al. 2003).

The aim of this work was to examine the possibility of application of plant oils containing linoleic acid 9c,12c$C_{18:2}$ (grape seed and sunflower oil) for obtaining of linoleic acid with conjugated double bonds, mainly 9c,11t and 10t,12c. The composition of linoleic acid isomers obtained in the process of grape seed oil isomerisation was compared with poppy seed, and sunflower oils. Also an influence of reaction environment, i.e. ethylene glycol and glycerine on other than 9c,11t and 10t,12c isomers of linoleic acid formation, was determined. Isomerisation process was conducted in known and earlier described way (Walisiewicz-Niedbalska et al. 2002).

Materials and Methods

Raw materials

In the investigation grape seed oil, poppy seed oil and sunflower oil were used. Poppy seed oil was obtained by the pressing of seeds of low-morphine variety Michałko and was used in an unrefined form. Grape seed oil and sunflower oil were bought in a supermarket in a refined form. Fatty acid composition of used oils is presented in Table 1.

The reaction environment was: pure ethylene glycol or pure pro analysis distilled glycerine produced by Trzebinia Refinery. As a catalyst – sodium hydroxide pure p.a. was used. Hexan pure p.a. was used as a solvent.

Procedure

Isomerisation process was carried out within already known method (Walisiewicz-Niedbalska et al. 2002) in a reactor of volume 5 l. Glass reactor heated by electricity was equipped with a cover with 3 connections in which were inserted stirrer, thermometer and raw materials supplying pipe changed later with a cooler. The cooler was used to remove water produced in the reaction. Distillation of solvent was carried out on Büchi evaporator in temperature 60–70^{o}C and pressure 50–70 mbar.

Table 1

Characteristics of raw materials — *Charakterystyka surowców*

	Poppy seed oil *Olej makowy*	Sunflower oil *Olej słonecznikowy*	Grape seed oil *Olej winogronowy*
Acid value, mgKOH/g	0.6	0.1	0.2
Unsaponificable matter, % (m/m)	0.9	0.3	0.3
Fatty acid composition, % (m/m)			
$C_{14:0}$	–	0.1	0.1
$C_{16:0}$	9.2	7.9	7.7
$C_{17:0}$	0.1	–	0.1
$C_{18:0}$	2.0	6.1	3.8
$C_{16:1}$	0.1	0.1	0.1
$9cC_{18:1}$	16.0	23.9	17.4
$7cC_{18:1}$	1.3	1.5	1.0
$9c,12cC_{18:2}$	70.1	58.2	67.8
n.i.*/	0.1		0.4
$9c,12c,15cC_{18:3}$	0.8	1.0	0.5
$C_{20:0}$	0.1	0.2	0.2
$C_{20:1}$	0.1	0.1	0.3
$C_{22:0}$	–	0.1	–
$C_{22:1}$	–	0.1	–
n.i.**/	0.1	0.7	0.6

n.i.*/ not identified linoleic acid isomers — *niezidentyfikowany izomer kwasu linolowego*
n.i.**/ not identified — *niezidentyfikowane*

In to the reactor 2500 g of ethylene glycol or glycerine were introduced, heated up to 130^{o}C and then 120 g of NaOH was added slowly. After its dissolving, 500 g of plant oil were added. Then the mixture was heated up 185±5^{o}C and the isomerisation process was carried out for 3 hours.

After that, reaction mixture was cooled down to the temperature of about 70^{o}C, water at the temperature of about 70^{o}C was added and then created sodium soaps were acidified with hydrochloric acid. About 100 ml of hexane was added in order to better separation of fatty acids to the mixture. After the settling, a water layer was separated, and organic layer was washed with water untill the removal of mineral acid. Organic fraction was dried with sodium sulfate, solvent was distilled off on an evaporator and the obtained product was directed to analysis.

The scheme of the process is presented on Figure 1.

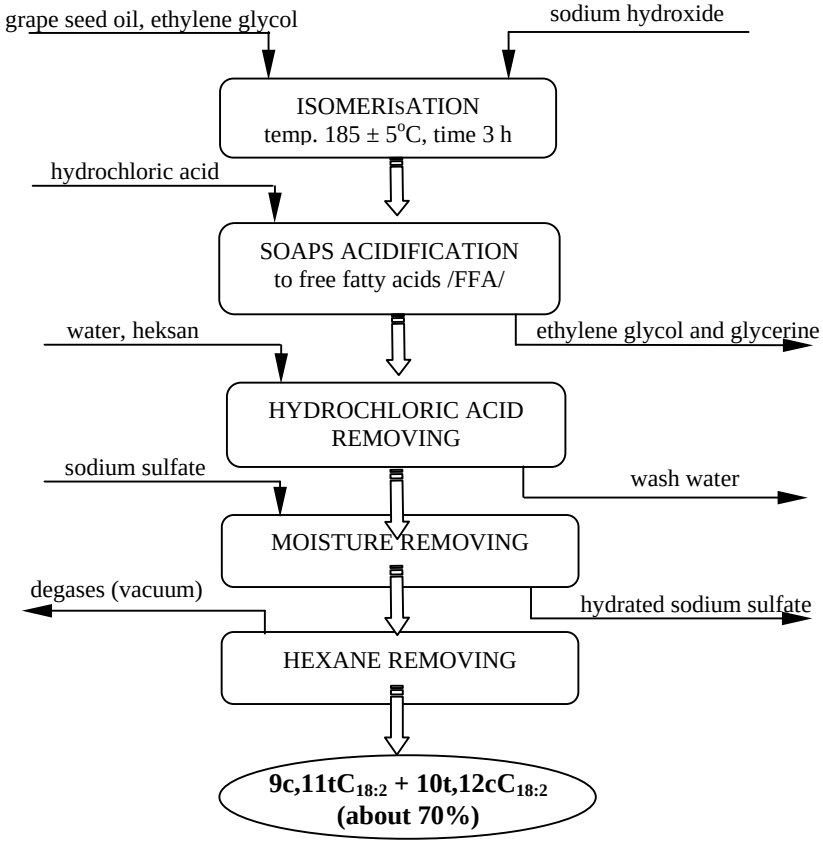

Fig. 1. Scheme of obtaining linoleic acid isomers: 9c,11t and 10t,12c$C_{18:2}$ by isomerisation reaction of linoleic acid of grape seed oil — *Schemat otrzymywania mieszaniny izomerów kwasu linolowego 9c,11t i 10t,12c w procesie izomeryzacji kwasu linolowego oleju z pestek winogron*

Analysis

Fatty acid determination was carried out by GC on Hewlett Packard II apparatus with flame ionisation detector (FID) and SUPELCO column SP-2560, 100 m × 0.25 mm × 0.2 um. Methyl esters were obtained following AOCS Official Methods 1f-96.

The temperature of the column was 170°C, of the injector 200°C and of the detector 250°C; helium was used as the gas carrier. The qualitative analysis was carried out by comparing the retention times of the components with standards and confirming the identification by the GC/MS method. Octadecadienoic acid conjugated methyl ester SIGMA product, 0-5632 isomers were used as standards.

The identification of positional isomers was performed by the GC/MS method, using the specific fragmentation of fatty acid derivatives carried out with 2-amino-2-methyl-1-propanol (DMOX). The separation of fatty acid methyl esters into FAME-*cis* and FAME-*trans* isomer fractions was performed by thin-layer chromatography (TLC-Ag+). A toluene-hexane mixture (85:15 v/v) was used as a developing system; 2.7-dichlorofluorescein was used as the developer, while visualization was carried out in visible light.

Fractions of FAME-*trans* and FAME-*cis* were carried out into derivatives with 4,4-dimethyloxazoline. Obtained DMOX derivatives were analyzed using GC/MS method. GC and GC/MS analyses of each sample were repeated three times. Presented results are calculated as average scores. Their relative standard deviation (RSD) does not excess 1.5%.

Results and Discussion

Positional and geometric isomers of linoleic acid (9c,11t and 10t,12c$C_{18:2}$) were obtained in a process of alkaline isomerisation of that acid contained in plant oils: poppy seed, sunflower and grape seed. The process was conducted as described in an earlier work: temperature 185 ± 5^{o}C, time of reaction 3 hours, reaction environment – ethylene glycol or glycerine, and poppy seed oil as a raw material (Walisiewicz-Niedbalska et al. 2002)

Results of an isomerisation reaction of poppy seed, sunflower and grape seed oils conducted in an environment of ethylene glycol or glycerine are presented in Table 2.

Selective degree of an isomerisation, expressed as a ratio of a sum of isomers 9c,11t+10t,12c$C_{18:2}$ in a product to the content of linoleic acid 9c,12c$C_{18:2}$ in a raw material, for reaction conducted in ethylene glycol was 0.87–0.92, while for glycerine it was 0.72–0.86 (Table 2). That is probably due to the fact, that glycerine added to a system together with glycerine released in a reaction increased concentration of the products inhibiting reaction. However, conduction of a reaction in glycerine possesses such an advantage, that the product is of straw colour, as opposed to light-brown colour obtained when using ethylene glycol.

The product of an isomerisation of linoleic acid from poppy seed oil that contained 32.7% 9c,11t$C_{18:2}$ and 35.1% 10t,12c$C_{18:2}$ was described in an earlier paper (Walisiewicz-Niedbalska et al. 2002). The present paper presents results of an isomerisation of poppy seed oil, and oils from grape seeds and sunflower. The product of an isomerisation was analysed using gas chromatography method in changed conditions (column 100 m instead of 50 m long) that allowed a more precise separation of isomers of linoleic acid during chromatographic division.

Table 2

Alkaline isomerisation of linoleic acid of plant oils in an environment of ethylene glycol (EtGlyc) or glycerine (Glyc). Temperature 185 ± 5°C, time 3 hours — *Wyniki izomeryzacji alkalicznej kwasu linolowego olejów roślinnych w środowisku glikolu etylenowego (EtGlyc) lub gliceryny (Glyc). Temperatura 185 ± 5°C, czas 3 godz.*

Isomer of linoleic acid	Content — Zawartość, % (m/m)					
	poppy seed oil *olej makowy* 9c,12c$C_{18:2}$ – 70.1%		sunflower oil *olej słonecznikowy* 9c,12c$C_{18:2}$ – 58.2%		grape seed oil *olej winogronowy* 9c,12c$C_{18:2}$ – 67.8%	
	EtGlyc	Glyc	EtGlyc	Glyc	EtGlyc	Glyc
9c,11t$C_{18:2}$	32.9	26.4	26.3	19.6	32.1	27.1
10t,12c$C_{18:2}$	28.3	23.8	23.7	17.4	30.0	26.8
Isomerisation ratio $\frac{\text{9c,11t+10t,12c}C_{18:2}\text{ (in product)}}{\text{9c,12c }C_{18:2}\text{ (in raw material)}}$	0.87	0.72	0.92	0.86	0.92	0.79

Table 3

Results of plant oils isomerisation: temperature 185 ± 5°C, catalyst NaOH, in an environment of ethylene glycol (EtGlyc), reaction time 3 h — *Wyniki izomeryzacji kwasu linolowego olejów: makowego, z pestek winogron i słonecznikowego w środowisku glikolu etylenowego, temperatura 185 ± 5°C, czas 3 godz.*

Fatty acids *Kwasy tłuszczowe*	Content — *Zawartość* % (m/m)					
	poppy seed oil *olej makowy*		sunflower oil *olej słonecznikowy*		grape seed oil *olej winogronowy*	
	before	after reaction	before	after reaction	before	after reaction
Linoleic acid, 9c,12c $C_{18:2}$	70.1	1.5	58.2	1.6	67.8	1.2
Conjugated linoleic acids (CLA)						
9c,11t		32.9	–	26.3	–	32.1
10t,12c	–	28.3	–	23.7	–	30.0
11c,13t	–	5.6	–	5.1	–	3.4
others CLA isomers	–	1.8	–	1.5	–	1.1
Σ 9c,11t+10t,12c$C_{18:2}$	–	61.2	–	50.0	–	62.1
Σ CLA isomers	–	68.6	–	56.6	–	66.6
Linolenic acid, 9c,12c,15c $C_{18:3}$	0.8	0.5	1.0	0.7	0.5	0.4
Isomerisation ratio $\frac{\text{Σ CLA isomers (in product)}}{\text{9c,12c (in raw material)}}$	0.98		0.97		0.98	
Selective isomerisation ratio $\frac{\text{9c,11t+10t,12cC18:2 (in product)}}{\text{9c,12c (in raw material)}}$	0.87		0.86		0.92	

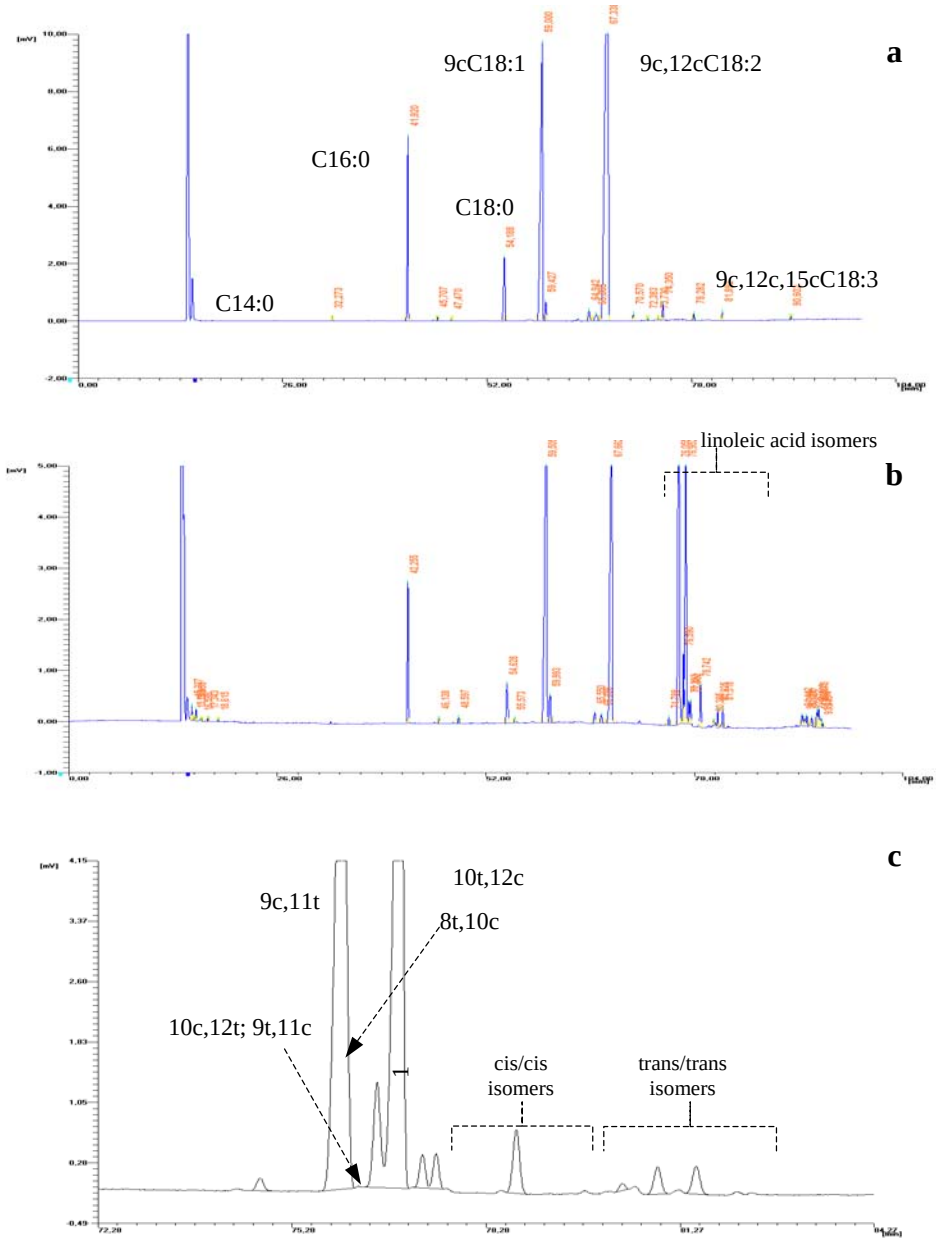

Fig. 2. GC chromatograms of FAME of grape seed oil: before (a), after reaction (b) and ME of linoleic acid isomers fragment (c) — *Chromatogramy CG; EM kwasów oleju winogronowego przed reakcją (a), po reakcji izomeryzacji (b) oraz fragment chromatogramu obejmujący EM kwasu linolowego i jego izomery (c)*

Results of an isomerisation of linoleic acid of oils are presented in Table 3 and on Figure 2. Figure 3 presents sample chromatograph GC of grape seed oil before (a) and after isomerisation reaction (b) and a fragment of a chromatograph including linoleic acid 9c,12cC$_{18:2}$ and its isomers (c).

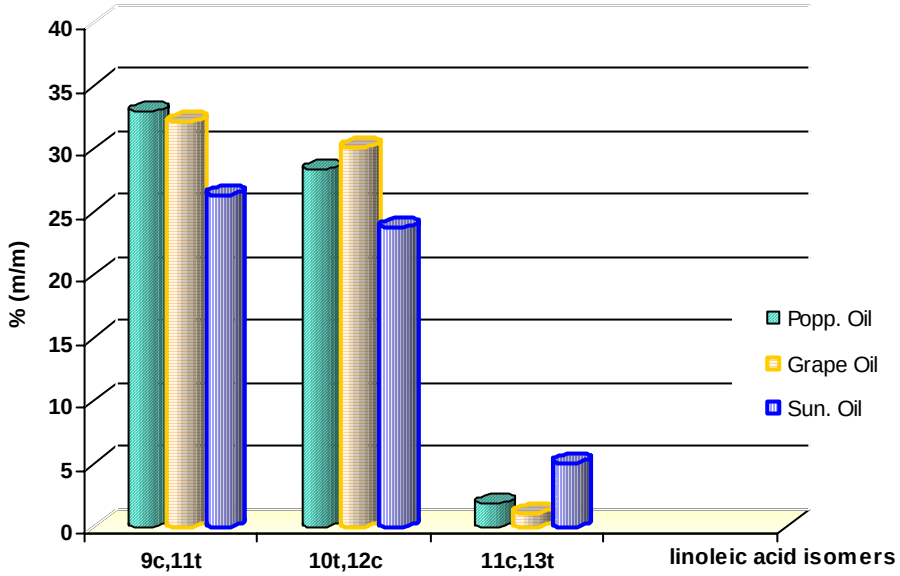

Fig. 3. Content of linoleic acid isomers 9c,11t and 10t,12cC$_{18:2}$ and isomer 11c,13t in a product of isomerisation reaction of poppy seed oil (Popp. Oil), grape seed oil (Grape Oil) and sunflower oil (Sun. Oil). Reaction temperature 185 ± 5°C, time 3 h, catalyst NaOH, environmental ethylene glycol — *Zawartość izomerów kwasu linolowego 9c,11t I 10t,12c oraz 11c,13t$_{C18:2}$ w produkcie izomeryzacji kwasu linolowego olejów: makowego (Popp. Oil), z pestek winogron (Grape Oil) i słonecznikowego (Sun. Oil). Temperatura reakcji 185 ± 5°C, czas 3 g., katalizator – NaOH, środowisko reakcji – glikol etylenowy*

The degree of an isomerisation of linoleic acid measured as a ratio of a sum of isomers of linoleic acid 9c,11t+10t,12cC$_{18:2}$ in a product to a content of linoleic acid 9c,12cC$_{18:2}$ in a raw material for each of the three oils is on a similar level, i.e. 0.98–0.97. In turn, selective degree of an isomerisation of linoleic acid measured as a ratio of a content of a sum of 9c,11t+10t,12cC$_{18:2}$ in product to a content of linoleic acid 9c,12cC$_{18:2}$ in raw material was similar for poppy seed and sunflower oils 0.87 and 0.86, and for grape seed oil it was 0.92. The big difference, reaching 0.11, between isomerisation degree and selective isomerisation degree for poppy seed and sunflower oils testifies to a formation of other linoleic acid isomers than desirable 9c,11t and 10t,12cC$_{18:2}$ during reaction. In the case of grape seed oil however, with a difference of both isomerisation degrees on a level of 0.006, the

amount of created undesirable isomers of linoleic acid is almost two-fold lower: 4.5% in relation to 7.4 and 6.6% for poppy seed and sunflower oils, respectively.

Thus, besides the main isomers 9c,11t and 10t,12c$C_{18:2}$, other isomers are formed during an isomerisation process. In the described conditions of chromatographic (100 m long column) separation, an isomer 11c,13t (Fig 3 b and c) was distinguished as a separate peak among them. In previous analyses (Walisiewicz-Niedbalska et al. 2002) it was interpreted together with 10t,12c$C_{18:2}$ isomer. Faintly visible dislocation on a peak responding to isomer 9c,11t$C_{18:2}$ (Fig. 3c) was observed at the same time, that points to a separate peak, which according to literature data (Roach et al. 2002) may be interpreted as an isomer 8t,10c$C_{18:2}$. In a product of an isomerisation described in the previous paper (Walisiewicz-Niedbalska et al. 2002) isomer 11c,13t and also 8t,10c$C_{18:2}$ were not separated that was due to other analytical conditions, and especially an application of 50 m long column.

According to the obtained results, the product of isomerisation of grape seed oil contains 61.2% (m/m) CLA isomers (9c,11t + 10t,12c), of sunflower oil 50.0% and of poppy seed oil 61.2% (m/m). Content of a sum of 9c,11t and 10t,12c isomers in commercial products ranges from 59.3 to 95.9% (Chouinard et al. 1999, Gnädig et al. 2001, Yu et al. 2003,). Differences in a content of CLA isomers in a product of an isomerisation in the present study and also in literature data are due to the content of linoleic acid in raw material and to selectivity of an isomerisation process.

The content of 11c,13t isomer in commercial products obtained in a synthetic way was from undetectable amount to 11.4% (Chouinard et al. 1999), and even to 22.75% (m/m) (Gnädig et al. 2001). The content of linoleic acid isomers, except for the main ones, i.e. 9c,11t and 10t,12c, ranges from undetectable values to 13.4% (m/m) (Chouinard et al. 1999, Gnädig et al. 2001, Yu et al. 2003). The content of 11c,13t isomer in obtained isomerisation product ranges from 3.4% (m/m) (grape seed oil) to 5.6% (m/m) (poppy seed oil).

Conclusion

The product of isomerisation of linoleic acid 9c,12c $C_{18:2}$ from grape seed oil contains about 61% of CLA isomers (9c,11t — 32.1% and 10t,12c — 30.0%), and a content of other isomers of linoleic acid is about 4.5%, including about 3.4% of 11c,13t isomer. Fatty acids composition is comparable to commercial preparations.

Grape seed oil may be a good raw product for CLA isomers obtaining.

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