Valuable furan fatty acids in soybeans and soy products

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Abstract

Furan fatty acids (FuFAs) are valuable minor compounds in our food with excellent antioxidant properties. Naturally occurring FuFAs are characterised by a central furan moiety with one or two methyl groups in β -/ β' -position of the heterocycle (monomethyl- or M-FuFAs and dimethyl- or D-FuFAs). Comparably high concentrations of D-/M-FuFAs were reported in soybeans, but soy is often consumed as a processed product, such as full-fat soy flour and flakes, soy drink, tofu and texturised soy protein (TSP). Due to the chemical lability of D-/M-FuFAs, e.g. in the presence of light or oxygen, a degradation during the processing is possible. For this purpose, freshly harvested soybeans ($n=4$) and differently processed soybean products ($n=22$) were analysed on FuFAs. Three FuFAs, i.e. 11-(3,4-dimethyl-5-pentylfuran-2-yl)-undecanoic acid (11D5), 9-(3,4-dimethyl-5-pentylfuran-2-yl)-nonanoic acid (9D5), and 9-(3-methyl-5-pentylfuran-2-yl)-nonanoic acid (9M5), were identified and quantified in all fresh soybeans and most of the processed soy products ($n=20$). A trend towards lower D-/M-FuFA contents in higher processed products was observable. Lower FuFA concentrations were usually accompanied with a decrease of the share of the less stable D-FuFAs (9D5, 11D5) in favour of the M-FuFA 9M5. Furthermore, one or two 3,4-nonmethylated furan fatty acids (N-FuFAs), i.e. 8-(5-hexylfuran-2-yl)-octanoic acid (8F6) and partly 7-(5-heptylfuran-2-yl)-heptanoic acid (7F7), were detected in all processed products, but not in the freshly harvested soybeans. Our results indicate that D-/M-/N-FuFAs may serve as suitable markers for both, careful manufacturing processes and adequate storage conditions of soy products.

Keywords Furan fatty acids · Soy · 8-(6-Hexylfuran-2-yl)-octanoic acid · Processed food · Degradation

Introduction

Furan fatty acids (FuFAs) are a class of valuable minor fatty acids, characterised by a central furan building block within the acyl chain [1, 2]. Typically, the furan moiety is substituted with one methyl group (monomethyl-FuFAs, M-FuFAs) in β -position or two methyl groups in β - and β' -position (dimethyl-FuFAs, D-FuFAs). The carboxyalkyl chain is usually odd numbered (7–13 carbon atoms), while the terminal alkyl residue typically consists of a propyl or a pentyl group [1]. D-/M-FuFAs are biosynthesised by plants [1, 3–6], bacteria [1, 7, 8] and algae [1, 9]. Important and representative FuFAs are 11-(3,4-dimethyl-5-pentylfuran-2-yl)-undecanoic acid (11D5), 9-(3,4-dimethyl-5-pentylfuran-2-yl)-nonanoic

acid (9D5) and 9-(3-methyl-5-pentylfuran-2-yl)-nonanoic acid (9M5) [2].

D-/M-FuFAs are excellent radical scavengers and antioxidants, because one D-/M-FuFA molecule is able to trap two radicals (e.g. hydroxyl, alkoxy or peroxy radicals) [10, 11], while they are converted via oxidative ring opening into a dioxoene [1]. D-/M-FuFAs can effectively protect lipids from lipid peroxidation and organisms against oxidative stress and associated diseases [1, 8, 10–13]. The antioxidative capacity of FuFAs correlates with number of methyl groups on the furan ring (i.e., D-FuFAs > M-FuFAs) [12]. Furthermore, anti-inflammatory effects were reported for FuFAs [14].

Despite these documented positive health effects, data on FuFAs in plant derived food are scarcely found in scientific literature. Typical total FuFA concentrations in plants ranged between 0.1 and 60 mg/kg fresh weight (literature values were partly transferred into fresh weight base for uniformity) [15–18]. Frequently, FuFA concentrations were determined after transesterification and expressed as contribution to

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the total fatty acid methyl esters (FAMES) [15, 19]. These amounts were low, but comparable with those of other lipophilic antioxidants like vitamin E [20]. Comparably high FuFA concentrations of 16–65 mg/100 g FAMES were detected in soybeans [19, 21]. Wu et al. reported FuFA contents of 16–49 mg/100 g FAMES (9D5 and 11D5) in soybeans harvested in autumn [19]. By analysis of 56 soybean varieties grown in Puerto Rico, Wu et al. outlined that the content of D-FuFAs was strongly affected by variety, environment, season and maturity [19]. For instance, soybeans harvested in springtime were richer in FuFAs than soybeans harvested in autumn (e.g. 17 vs. 27 mg/100 g FAMES for the same variety and growing site) [19]. Guth and Grosch found comparable values of 26–42 mg/100 g FAMES in soybean oil extracted from soybeans in their laboratory [22].

In addition, 3,4-nonmethylated furan fatty acids (N-FuFAs) were scarcely reported to occur in food samples [23]. N-FuFAs were not biosynthesised like D-/M-FuFAs but were described as secondary oxidation products of conjugated linoleic acids (CLAs) in model reactions [24, 25]. N-FuFAs were mainly formed during photooxidation in presence of singlet oxygen via a cyclic endoperoxide as an intermediate [25–27]. However, N-FuFAs were described in food only once, namely in fish and fish feed from a zero discharge aquaculture [23]. Vetter et al. reported that N-FuFAs were present in fish feed [23]. This indicated that they could be a quality marker of food. In this context, it was possible that soy products on a different level of processing differed in the level of D-/M-FuFAs on one hand and N-FuFAs on the other hand. Therefore, soybean products seemed to be a superior matrix to investigate this topic due to high FuFA concentrations and the wide range of products on the market.

Hence, the goal of the study was to investigate D-/M-FuFA concentrations and patterns in differently processed soy products (soy flour, flakes, drink, texturised soy protein (TSP) and tofu). Because of the presence of anti-nutritive substances [28], soybeans are mainly consumed after processing in form of soy flour, flakes, drink, TSP or tofu. During the manufacturing of full-fat soy flour, soybeans are only cleaned, peeled, ground and partly roasted [29]. Compared to that, soybeans are steamed, rolled out and toasted to produce full-fat soy flakes [29, 30]. Soy drink and tofu production includes soaking of chopped beans in water, followed by homogenisation and pasteurisation of the resulting suspension [29]. Then, calcium sulphate is added, and the precipitated tofu is squeezed [29]. TSP is produced from isolated soy protein, e.g. from the residue of oil extraction, which is texturised [29]. Apart from the soy flour production, all processes include a manufacturing step of homogenisation soybeans in the presence of water (drink, tofu, TSP depending on the chosen process) or at least the application of vapour (flakes). Wakimoto et al. [14] as well as Schödel and Spittler [31] reported a degradation of D-/M-FuFAs in

aqueous solutions. Furthermore, D-/M-FuFAs are degraded when exposed to light [18, 32–34]. Both properties indicated that the D-/M-FuFA content of soy products may probably differ from the reported values in soybeans. These analyses were complemented with the screening of the samples on the possible presence of N-FuFAs. For these purposes, four or five samples of each product group were analysed with gas chromatography coupled to mass spectrometry (GC/MS) after transesterification and an enrichment step using silver ion chromatography [15].

Materials and methods

Chemicals

Ethanol, methanol and *n*-hexane (all HPLC grade) were ordered from Th. Geyer (Renningen, Germany). Concentrated sulphuric acid (96–98%, for analysis *p.a.*) and diethyl ether (for synthesis, $\geq 99\%$) were from Carl Roth (Karlsruhe, Germany). Sodium chloride ($\geq 98.9\%$), silver nitrate ($\geq 99.5\%$, for analysis *p.a.*) and silica gel 60 were purchased from Sigma Aldrich (Steinheim, Germany). 2,2,4-Trimethylpentane (*i*-octane, for pesticide residue analysis) was ordered from Fluka Analytics (Seelze, Germany).

Standards

The 11D5 ethyl ester (11D5-EE, purity $> 99\%$) was isolated from fish oil ethyl esters [35]. The 11D5 methyl ester (11D5-ME, purity $> 99\%$) was obtained by transesterification of 11D5-EE [36]. The 9M5 was extracted with *n*-hexane from latex gloves according to Müller et al. [32]. The *n*-hexane extract was first ethylated using 1% sulphuric acid in ethanol [32] and hydrogenated in accordance with Hauff et al. [37]. The resulting FuFA esters were purified twice using silver ion chromatography [32]. The purity of 9M5-EE was 98% according to GC/MS analysis. The 9M5-ME (purity $> 98\%$) was isolated in a similar way, using sulphuric methanol instead of ethanol. A semi-quantitative 9D5-EE and 11-(3,4-dimethyl-5-propylfuran-2-yl)-undecanoic acid ethyl ester (11D3-EE) mix standard was prepared as described elsewhere [35, 38] followed by silver ion chromatography [32]. The respective 9D5-ME and 11D3-ME mix standard was prepared via transesterification [36]. The share of 9D5 and 11D3 was 13% and 86%, respectively, in both mix standards, the purity for the sum of D-FuFAs was $> 98\%$. Free 8-(6-hexylfuran-2-yl)-octanoic acid (8F6, purity 95%) was purchased from Santa Cruz Biotechnology (Dallas, TX, USA) and ethylated as well as methylated [36]. The internal standard mix (ISTD mix) consisted of 11D5-EE (ISTD I, mass fraction $\omega = 3.1 \mu\text{g/mL}$), 9M5-EE (ISTD II, $\omega = 2.6 \mu\text{g/mL}$) and 8F6-EE (ISTD III, $\omega = 3.2 \mu\text{g/}$

mL). Myristic acid (14:0, purity > 98%) was obtained from Fluka (Steinheim, Germany), ethylated and used as ISTD IV ($\omega = 0.1$ mg/mL 14:0-EE) [36].

Samples

Fresh soybean samples (harvested in autumn 2018) were obtained from a cooperative society in Baden-Wuerttemberg, South Germany (varieties *Lenka*, *Sultana*), from the State Plant Breeding Institute, University of Hohenheim (Stuttgart, Germany; variety *Idefix*) and from a farmer (Ludwigsburg, Germany; unknown variety). In addition, samples of soy flour (full-fat, $n = 5$), soy drinks ($n = 5$), TSP ($n = 4$), soy flakes (full-fat, $n = 4$) and tofu ($n = 4$) were purchased at German retail market.

Sample preparation and transesterification

Soy drink and tofu were freeze-dried using the LYOVAC GT 2-System (Leybold-Heraeus, Hürth, Germany) [39]. Dry samples were finely ground by means of an IKA A11 basic laboratory mill (IKA, Staufen, Germany). A sample amount corresponding with ~ 20 mg fat was weighed into a 20 mL amber glass test tube and transesterified according to Krauß et al. [39] except the following changes: the reaction time was extended to 2 h and 2 mL *n*-hexane were used for the FAME extraction. The resulting FAME solution was used for FuFA enrichment as well as for gravimetric determination of FAMES.

Silver ion chromatography

The preparation of silica gel impregnated with silver nitrate was performed according to Vetter et al. [15] with minor changes. Twenty grams of activated silica gel 60 was slowly added to a solution of 5 g AgNO_3 in ~ 100 mL ultrapure water (Purelab classic, Elga, Lane End, United Kingdom) under permanent stirring. After 15 min of stirring [15], the water was carefully removed using a rotary evaporator [40] (60 °C, 150 mbar). Afterwards, the coated silica gel was activated for 24 h at 130 °C. Columns were packed using a slurry of 660 ± 20 mg 1% deactivated AgNO_3 -silica gel [15] and 5 mL *n*-hexane. This suspension was filled into a Pasteur pipette plugged with glass wool. After conditioning the column with 2 mL of eluent I (*n*-hexane/diethyl ether; 99.5/0.5 (v/v) [36]), an aliquot of ~ 0.5 mg FAMES and 10 μL of ISTD mix (i.e. 31 ng 11D5-EE, 26 ng 9M5-EE, 31 ng 8F6-EE) were placed on the column. The fractionation was performed according to Wendlinger et al. [36] with slight changes; e.g. the volume of eluent II (*n*-hexane/diethyl ether; 97/3 (v/v)) which was increased from 15 to 30 mL. This adjustment allowed the simultaneous quantification of D-/M-FuFAs and N-FuFAs in fraction II. Fraction II was reduced to ~ 1 mL,

transferred to a 2 mL vial and supplemented with ~ 50 μL *i*-octane as a keeper. Then, the volume of fraction II was reduced to 100 μL under a gentle stream of nitrogen and 400 ng ISTD IV was added. All samples were analysed in duplicate. All steps were performed in an environment as dark as possible and using amber glass to reduce degradation of analytes sensitive to oxidation [36].

Gas chromatography with mass spectrometry (GC/MS)

An HP 6890 series II plus gas chromatograph equipped with an HP 6890 autosampler was used in combination with a 5973N MSD mass spectrometer (all Agilent, Waldbronn, Germany). One microliter of sample and standard solutions were injected using a splitless injector held at 250 °C. Separations were performed on an Rtx-2330 capillary column (90% biscyanopropyl, 10% cyanopropylphenyl polysiloxane; 60 m length $\times$ 0.25 μm internal diameter $\times$ 0.1 μm film thickness; Restek, Bellefonte, PA, USA) [36]. Helium 5.0 (Westfalen, Münster, Germany) was used as the carrier gas at 1.2 mL/min. The oven program was modified from Wendlinger et al. [36]. After 1 min at 60 °C, the temperature was first ramped at 13 °C/min to 150 °C, second at 3 °C/min to 225 °C and third at 20 °C/min to 250 °C (hold time 7 min). FuFA-ME and -EE were quantified in the selected ion monitoring (SIM) mode (dwell time for each ion: 35 ms) after a solvent delay of 7 min. Eight ions, i.e. m/z 95.0, 109.1, 123.1, 137.1, 151.2, 165.2, 179.2 and 193.2 were measured throughout the run. Additionally, the corresponding molecular ions of FuFA-ME/-EE and fragment ions of ISTD IV were measured in four time windows (TW): (TW 1, 7.0–16.5 min): m/z 88.1, 101.1, 238.2, 252.2, and 266.2; (TW 2, 16.5–24.5 min): m/z 280.2, 294.2, 308.2, 322.3 and 336.3; (TW 3, 24.5–29.0 min): m/z 322.3, 336.3, 350.3, 364.4, and 378.4; (TW 4, 29.0–41.2 min): m/z 364.4, 378.4, 392.4, 406.4 and 420.4. For additional peak identification, sample solutions were measured by GC/MS in full scan mode (m/z 50–650). The transfer line was held at 270 °C, and the temperature of the quadrupole and the ion source were set at 150 °C and 230 °C, respectively.

FuFA identification, quantification and quality control

FuFAs were identified by means of their mass spectra as reported elsewhere [15, 41, 42]. The type of FuFA (D-, M- or N-FuFA) was determined by means of the McLafferty ion, i.e. m/z 123.1 for D-FuFAs, m/z 109.1 for M-FuFAs, and m/z 95.0 for N-FuFAs. With knowledge of the type of FuFA, the base peak, which is formed by allylic cleavage in the carboxyalkyl chain, allows the determination of the length of the alkyl chain (Table 1). These two ions and the

Table 1 Abbreviation, retention time (t_R) and relative abundances of GC/MS-SIM ions of all FuFAs determined in this study and for the internal standards I–III which were used for quantification

Substance (IUPAC name)	Abbr. ^a	t_R ^b	McLafferty ^c	Base peak ^d	[M] ⁺	ISTD ^e
<i>Reference standards and analytes (methyl ester)</i>						
8-(5-Hexylfuran-2-yl)-octanoic acid-ME	8F6-ME	22.5	95.0 (38%)	165.2 (100%)	308.2 (23%)	8F6-EE (ISTD III)
7-(5-Heptylfuran-2-yl)-heptanoic acid-ME ^f	7F7-ME	22.6	95.0 (40%)	179.2 (100%)	308.2 (29%)	8F6-EE (ISTD III)
9-(3-Methyl-5-pentylfuran-2-yl)-nonanoic acid-ME	9M5-ME	23.0	109.1 (13%)	165.2 (100%)	322.3 (16%)	9M5-EE (ISTD II)
9-(3,4-Dimethyl-5-pentylfuran-2-yl)-nonanoic acid-ME	9D5-ME	24.1	123.1 (17%)	179.2 (100%)	336.3 (28%)	11D5-EE (ISTD I)
11-(3,4-Dimethyl-5-pentylfuran-2-yl)-undecanoic acid-ME	11D5-ME	27.6	123.1 (17%)	179.2 (100%)	364.4 (28%)	11D5-EE (ISTD I)
<i>Internal standards (ethyl ester)</i>						
8-(5-Hexylfuran-2-yl)-octanoic acid-EE	8F6-EE	23.2	95.0 (35%)	165.2 (100%)	322.3 (24%)	
9-(3-Methyl-5-pentylfuran-2-yl)-nonanoic acid-EE	9M5-EE	23.6	109.1 (13%)	165.2 (100%)	336.3 (16%)	
11-(3,4-Dimethyl-5-pentylfuran-2-yl)-undecanoic acid-EE	11D5-EE	28.3	123.1 (18%)	179.2 (100%)	378.4 (31%)	

^aAbbreviation according to Vetter et al. [16]^bRetention time (min)^cMcLafferty ion which was used for differentiation of N-/M-/D-FuFAs^dBase peak which was used for quantification^eInternal standard used for quantification^fRetention time and ratio of SIM ions were calculated from samples not from a reference standard

molecular ion ($[M]^+$) enable the calculation of the length of the carboxyl chain [15, 41, 42]. The identity of 9M5-ME and 11D5-ME was verified by comparing the retention times in samples with those of authentic 9M5-ME and 11D5-ME standards. A semi-qualitative standard of 9D5-ME (see above) was used to verify the presence of 9D5 in samples. Later on, abundance ratios of the three GC/MS-SIM ions mentioned above were determined to verify D-/M-FuFAs in the samples (tolerated absolute deviation $\pm 3\%$, Table 1). Similar abundance ratios of the GC/MS-SIM ions as in 8F6-ME were requested for the assignment of other N-FuFAs (tolerated absolute deviation $\pm 6\%$, Table 1). In all cases, the base peak was used as quantification ion (Table 1). All N-FuFAs were quantified with 8F6-EE, all M-FuFAs with 9M5-EE and all D-FuFAs with 11D5-EE (Table 1). The recovery rate was determined with an external matrix standard (ISTD-Mix spiked in sample matrix) for each product group and each compound of the ISTD-Mix. This matrix standard was prepared by processing one sample of each product group without adding ISTD mix prior the enrichment step. The ISTD mix was then added right before analysis together with ISTD IV. Data were corrected with the calculated recovery rate of the corresponding D-, M- and N-FuFA in the ISTD. Based on a final volume of 100 μ L, limits of detection (LOD, signal-to-noise ratio = 3) of the methyl esters were 3 μ g/g FAMES for N-FuFAs and M-FuFAs and 2 μ g/g FAMES for D-FuFAs. Limits of quantification (LOQ, signal-to-noise ratio = 10) of the methyl esters were 5 μ g/g FAMES for N-FuFAs, 3.5 μ g/g FAMES for M-FuFAs and 2.5 μ g/g FAMES for D-FuFAs. Concentrations of N-FuFAs

in the range between LOD and LOQ were set to LOD/2 when calculating the total N-FuFA content.

Results and discussion

D-/M-FuFAs in soybeans

Four soybean samples planted in the south of Germany and harvested in autumn 2018 contained D-/M-FuFA concentrations of 14–21 mg/100 g FAMES. The bulk (13–21 mg/100 g FAMES) originated from D-FuFAs (Table 2). These FuFA concentrations were at the lower end of the values reported by Wu et al. and Guth and Grosch for D-FuFAs in soybeans harvested in autumn some 25 years ago (Table 2) [19, 21, 22, 43]. 9D5 was the most abundant FuFA in our samples, 11D5 in those of Guth and Grosch (Table 2) [22]. This indicated the inspection of different varieties in the previous and present study. In agreement with that, Wu et al. reported varying amounts and ratios of 9D5 and 11D5 as well as < 10% contribution of 9M5 to the total FuFA content in 56 soybean varieties [19]. Accordingly, 9M5 concentrations (0.6 mg/100 g FAMES) and contributions (3–4%) were also low in our samples (Table 2).

D-/M-FuFAs in processed soy products

Full-fat soy flour, which was the least-processed soy product in this study, was the richest product group in D-/M-FuFAs (17–30 mg/100 g FAMES) (Fig. 1, Table 2). Concentrations in soy flour were ~ 50% higher than in the

Table 2 D-/M-FuFA concentrations [mg/100 g FAMES] and share [%] of 9M5, 9D5 and 11D5 in all soybean samples along with the corresponding mean, median and the literature values [17, 19, 20]

	<i>n</i> ^a	D-/M-FuFA concentration [mg/100 g FAMES]				Share [%]		
		9M5-ME	9D5-ME	11D5-ME	Sum	9M5	9D5	11D5
Bean 1		0.6	9.4	3.8	14	4	68	28
Bean 2		0.6	10	6.8	18	3	58	38
Bean 3		0.6	16	4.7	21	3	75	22
Bean 4		0.6	14	4.2	19	3	75	22
Median	4	0.6	12	4.5	18	3.3	71	25
Mean	4	0.6	13	4.9	18	3.4	69	28
Wu et al. (autumn) [17]	56	^b	3–29	6–27	16–49 ^c	< 10	^b	^b
Wu et al. (spring) [17]	6	^b	^b	^b	27–65 ^c	< 10	^b	^b
Guth and Grosch [20]	5	0.7–2.3	13.1–17.0	13.2–22.9	26.3–42.2	2.4–5.5	40.3–46.4	49.4–54.3
Guth et al. [19]	6	^b	18.3–22.5	9–13.2	28.1–35.7 ^c	^b	^b	^b

^aNumber of samples^bData not reported^cSum of 9D5 and 11D5 **Fig. 1** Concentration range and median value of D-/M-FuFAs (sum of 9M5-ME, 9D5-ME and 11D5-ME) in mg/100 g FAMES) in soy products of different processing stages

(independent) soybean samples of this study. This indicated that soy flour was produced from different soybean varieties or that soybeans were harvested in another season with a higher FuFA content. Comparably high D-/M-FuFA concentrations were also detected in full-fat soy flakes (11–22 mg/100 g FAMES). Compared to soybean samples, soy flour and flakes showed similar ratios of 9D5 (53–58%), 11D5 (34–39%) and slightly higher 9M5 contributions (7–12%, Table 2).

In contrast, D-/M-FuFA concentrations in more strongly processed food items (soy drink, tofu and TSP) were generally < 10 mg/100 g FAMES (Fig. 1, Table 2). The wide range with partly very low D-/M-FuFA concentrations in soy drinks of 0.9–9.7 mg/100 g FAMES could be due to partial loss of FuFAs during the processing. In support of this indication, the FuFA pattern in soy drinks differed from the one in beans, flour and flakes. For instance, the two soy drink samples with particularly low FuFA concentrations (0.9 and 4.6 mg/100 g FAMES) did not contain D-FuFAs but only 9M5 (Fig. 2a, c). However, 9M5 was on a similar level as in soy flour and flakes (1.3–3.1 mg/100 g FAMES). This unique D-/M-FuFA pattern can only be explained by substantial loss of D-FuFAs during processing or storage. In agreement with that, D-FuFAs are known to be less stable than M-FuFAs [2, 11]. Also, the three other soy drink samples contained 9M5 in the expected concentration range (1.3–2.0 mg/100 g FAMES). The higher total FuFA content was due to the presence of D-FuFAs albeit at lower concentrations than in soybeans, soy flour and flakes. Hence, an elevated share of 9M5 in these samples (20–41%, Fig. 2c) can be used as an indicator for partial loss of D-FuFAs. The 9D5 (34–47%) was slightly more abundant than 11D5 (25–33%) which indicated no (pronounced) preferred loss of individual D-FuFAs.

Similarly, the FuFA concentration in tofu samples 1 and 2 (1.3–3.2 mg/100 g FAMES) were much lower than in tofu samples 3 and 4 (Fig. 2b). Tofu 1 and 2 were dominated by 9M5 (29–56%), while tofu 3 and 4 were richer in D-FuFAs. The total FuFA concentrations in tofu 3 and 4 (16–17 mg/100 g FAMES) were similar to those of soybeans and soy flakes (Table 3). Tofu 1, 2 and 4 had a transparent packaging, while the packaging of tofu 3 protected the

Fig. 2 Concentrations [mg/100 g FAMES] (a, b) and shares [%] (c, d) of 9M5-ME, 9D5-ME and 11D5-ME in soy drinks (a, c) and tofu samples (b, d)

product from light. Partial degradation of D-FuFAs by light [32] may have played a role for tofu 1 and 2. If exposure to light played a role, the time of exposure may be the reason for the differences between tofu 1 and 2 (low in D-FuFAs) and tofu 4 (high in D-FuFAs). This may not only include the time between the harvest and processing but also the time in supermarkets.

The total FuFA content in TSP was constantly low (2.4–3.3 mg/100 g FAMES) due to lower contents of all three D-/M-FuFAs. However, the share of 9M5 was only slightly increased (10–25%), while 9D5 (48–53%) and 11D5 (27–39%) were still the main FuFAs in TSP (Fig. 2b, d). In this case, the concentrations of all three FuFAs (including 9M5, ≤ 0.3 mg/100 g FAMES in TSP 2–4) were much lower than in soybeans. This indicated that the low total FuFA content in TSP 2–4 was only to a small extent caused by degradation of D-/M-FuFAs. An almost equal depletion of all three FuFAs during the manufacturing process may be rather due to fractionation (e.g. by loss of specific lipid

classes during the defatting process of the soy protein) than to degradation. TSP 1 had a higher level and an elevated share of 9M5 (0.8 mg/100 g FAMES; 25% 9M5). This indicated a higher extent of FuFA degradation during processing of TSP 1 (or already in its soybeans) than in TSP 2–4.

Except for tofu 3 and 4, these results indicated a trend towards lower D-/M-FuFA concentrations with increased degree of processing of soy products (Fig. 1). Hence, processing and storage seem to play an important role in the fate of FuFAs in soy products. Except for TSP, loss of FuFAs was accompanied with an increase of the share of 9M5. Since the antioxidant capacity of D-FuFAs is higher than of M-FuFAs [11], loss of D-FuFAs will lead to a product less protected from lipid oxidation. This in turn could lead to the formation of N-FuFAs.

Table 3 FuFA concentrations [mg/100 g FAMES], mean values and shares [%] of 9M5, 9D5 and 11D5 in processed soy products

	D-/M-FuFAs							N-FuFA	
	[mg/100 g FAMES]				Share [%]			[mg/100 g FAMES]	
	9M5-ME	9D5-ME	11D5-ME	Sum	9M5	9D5	11D5	8F6	7F7
<i>Flour</i> ^a	2.2	14	9.0	25	9	54	37	3.1	
Flour 1	1.9	13	8.2	23	9	55	36	1.7	n.d. ^b
Flour 2	2.0	17	10	29	7	58	35	2.6	n.d. ^b
Flour 3	3.1	16	11	30	11	53	36	3.6	n.d. ^b
Flour 4	2.1	12	8.9	23	9	53	39	2.3	n.d. ^b
Flour 5	1.5	9.1	6.2	17	9	54	37	3.8	n.d. ^b
<i>Flakes</i> ^a	1.6	9.1	6.2	17	8	54	37	1.7	
Flakes 1	1.6	9.3	6.2	17	9	54	36	0.7	n.d. ^b
Flakes 2	1.3	5.9	3.8	11	12	54	34	2.6	n.d. ^b
Flakes 3	1.8	9.5	6.4	18	10	54	36	1.6	n.d. ^b
Flakes 4	1.6	12	8.6	22	7	54	39	2.1	n.d. ^b
<i>Drink</i> ^a	2.2	1.7	1.2	5.2	61	23	16	1.4	
Drink 1	0.9	n.d. ^b	n.d. ^b	0.9	100	0	0	tr ^c	n.d. ^b
Drink 2	4.6	n.d. ^b	n.d. ^b	4.6	100	0	0	0.8	n.d. ^b
Drink 3	1.7	1.4	1.0	4.0	41	34	25	0.8	n.d. ^b
Drink 4	1.3	2.4	1.8	5.5	24	44	36	2.0	n.d. ^b
Drink 5	2.0	4.6	3.1	9.7	20	47	33	tr ^c	n.d. ^b
<i>Tofu</i> ^a	0.9	5.2	3.3	9.3	24	44	32	1.6	
Tofu 1	0.7	0.3	0.3	1.3	56	23	21	1.1	n.d. ^b
Tofu 2	0.9	1.2	1.1	3.2	29	37	34	1.4	n.d. ^b
Tofu 3	1.0	9.9	5.7	17	6	60	34	2.6	tr ^c
Tofu 4	0.8	9.4	6.0	16	5	58	37	1.3	n.d. ^b
<i>TSP</i> ^a	0.4	1.4	0.9	2.7	14	50	35	0.9	
TSP 1	0.8	1.6	0.9	3.3	25	48	27	1.6	0.7
TSP 2	0.2	1.3	0.9	2.4	10	53	37	0.7	tr ^c
TSP 3	0.3	1.2	0.9	2.4	11	49	39	0.8	n.d. ^b
TSP 4	0.3	1.4	1.1	2.8	11	51	39	0.7	n.d. ^b

^aMean values of the product group^bNot detected^cTraces

N-FuFAs in soybeans and soy products

Freshly harvested soybeans did not contain any N-FuFAs (Fig. 3a). In contrast, GC/MS chromatograms of processed soy products indicated the presence of two N-FuFAs by virtue of the characteristic fragment ion at m/z 95.0. Both N-FuFAs, which eluted prior to 9M5-ME (Fig. 3b), showed the molecular ion at m/z 308.2 and thus had 18 carbon atoms (plus one for the methyl ester moiety). Peak 2 (base peak at m/z 165.2) was identified as 8F6-ME by means of an authentic standard. The base peak at m/z 179.2 (along with M^+ at m/z 308.2 and McLafferty ion at m/z 95.0) in the GC/MS spectrum of peak 1 was in agreement with a shift of furan moiety by one methylene unit toward the carboxyl group. Hence, this N-FuFA was found to be 7F7-ME. As expected [15], the retention time of FuFA methyl esters with both,

the same methylation degree and carbon atom number, increased with increasing length of the carboxyl chain (t_R 7F7 < t_R 8F6). The relative abundances of the three GC/MS-SIM ions of 7F7-ME (m/z 179.2/95.0/308.2, Table 1) were also similar to those of 8F6-ME (m/z 165.2/95.0/308.2, Table 1). Concentrations of 8F6 in the processed samples ($n=22$) were up to 3.9 mg/100 g FAMES (Fig. 4, Table 2). The presence of 8F6 in processed soybean products and its absence in fresh soybeans showed that N-FuFAs were no native substances of soybeans. Surprisingly, full-fat soy flour was the product group with highest concentrations of 8F6 (1.7–3.8 mg/100 g FAMES) followed by tofu (1.1–2.6 mg/100 g FAMES) and full-fat soy flakes (0.7–2.6 mg/100 g FAMES). TSP and soy drink samples showed the lowest concentrations of N-FuFAs (Fig. 4, Table 2). In addition, 7F7 was detected in one tofu and two

Fig. 3 GC/MS-SIM chromatogram (excerpt, 22–30 min) of the FuFA fraction obtained from directly transesterified samples after silver ion chromatography (fraction II) of **a** a freshly harvested soybean and **b** a TSP sample

Fig. 4 Concentration range and median value of the predominant N-FuFA 8F6 (determined as methyl ester in mg/100 g FAMES) of soy products of different processing stages

TSP samples. Total N-FuFA concentrations in processed soy product samples were up to 3.8 mg/100 g FAMES which is comparable with the range of 9M5 in soybean samples

(Fig. 3b). Depending on the presence of D-/M-FuFAs, N-FuFA contributed up to 45% (mean 17%; median 15%) to the total D-/M-/N-FuFA content.

N-FuFAs are known as minor secondary oxidation products of CLAs [24, 25]. With linoleic acid (18:2(Δ 9,12)) being the predominant fatty acid in soybeans [33], isomerisation of the double bonds in 18:2(Δ 9,12) could be the initial step of N-FuFA formation, e.g., conjugation of the double bonds to 18:2(Δ 9,11) has already the correct positions as in 8F6, while the direct insertion of oxygen in the case of 7F7 would require the rather unusual 18:2(Δ 8,10) as precursor.

Lipid peroxidation is strongest in dry state [29]. This could explain the comparatively low N-FuFA content in soy drink but not the low N-FuFA content in TSP or the high N-FuFA values in tofu. Furthermore, the storage time as well as temperature or availability of oxygen are important influence factors for lipid peroxidation. These facts were unknown for the analysed samples from the retail market.

Conclusions

Although not all relations could be clarified, the combined determination of D-/M-FuFAs and N-FuFAs was found to be suited to get insights into the processing of soy products. High levels of total D-/M-FuFAs along with a low share of 9M5 and no or low formation of N-FuFAs were found to be good indicators for a gentle processing and adequate storage. This is remarkable, because the soybean samples and soy products in this study were not from the same production line. In the future, more details should be elaborated inspecting the fate of the same soybean charge in different processed products of a manufacturer. This may also allow to study further intermediate stages of the production process to gain further insights into the conditions leading to the degradation of D-/M-FuFAs and the formation of N-FuFAs.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human or animal subjects.

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