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Evaluation of biological value of sprouts I. Fat content, fatty acid composition

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Abstract. During our research work fatty acid content of the most important sprouts, wheat, lentil, alfalfa, radish and sunflower seed was investigated during the germination. It was found that both the saturated and unsaturated fatty acids hardly changed during the germination. The most important saturated fatty acid of the sprouts investigated by us is palmitic acid, amount of which hardly changed or even increased in the case of alfalfa sprout during germination. Oleic acid and linoleic acid were present in the highest concentration among the unsaturated fatty acids in the sprouts investigated. The concentration of oleic acid remained unchanged during the germination period, and the same applies, except lentil, to linoleic acid in case of every sprouts examined. In the case of lentil sprout the concentration of oleic acid decreased while linoleic acid content increased significantly. Based on our investigation it can be stated that most of the fatty acids hardly changed during the germination, and there was no verifiable tendency.

Key words and phrases: sprouts, chemical changes during germination, fat content, fatty acid composition

1 Introduction

In the recent decades more and more attention is paid to the healthy nutrition, to its role in health-maintaining and in the prevention of certain diseases. Changes in the nutritional habits, in foods consumed and in the food preparation methods have contributed to the decrease of the nutritional value. Various pathological researches came to the conclusion that consumption of higher amount of foods of vegetable origin could be effective in the prevention against some chronic diseases. These beneficial effects have been attributed partly to the high antioxidant activity of the vegetables. Most important antioxidants found in the plants are vitamin C, carotenoids and phenolic derivatives especially flavonoids.

Sprouting is a natural biological process that every higher plants exhibit, during which the seed at rest starts to grow under favourable environmental conditions (appropriate moisture content, temperature, oxygen) and a new plant develops. During the germination the polysaccharides degrade into oligo- and monosaccharides, the fats into free fatty acids, whereas the proteins into oligopeptides and free amino acids, which processes support the biochemical mechanisms in our organism. They improve the efficiency of both the protein-decomposing and the carbohydrate- and fatty acid-decomposing enzymes therefore germination can be considered as one kind of predigestion that helps in breaking down the high-molecular complex materials into their building blocks. After the germination also compounds with health-maintaining effects and phytochemical properties (glucosinolates, natural antioxidants) could be detected that can have a considerable role among others also in the prevention of cancer. (*Sangronis and Machado, 2007*). Thus, germination can lead to the development of such functional foods that have a positive effect on the human organism and that help in maintaining the health (*Sangronis and Machado, 2007*).

The sprouts fulfill the requirements of the modern nutritional science for whole-food. Compared to seeds, the sprouts have a higher nutritional value: higher quality of protein, more favourable amino acid composition, higher polyunsaturated fatty acid content, better bioavailability of trace elements and essential minerals and higher vitamin content. During sprouting the amount of such antinutritive materials as haemagglutinins, trypsin inhibitor activity, tannins, pentosans, phytic acid, decreases. Researches found that the sprouts are a good source of ascorbic acid, riboflavin, choline, thiamin, tocopherol and pantothenic acid (*Lintschinger et al., 1997*).

Urbano et al. (2005) examined the protein digestibility of various sprouts and bioavailability of the minerals, *Gill et al.* (2004) the relationship between the consumption of vegetables and the prevention of cancer, and *Clarke et al.* (2008) the efficiency of the sulforaphane content of different sprouts in cancer prevention. *Kim et al.* (2004) examined the change in fatty acid composition due to sprouting. It was established that in most of the sprouts among the fatty acids linolenic acid was present in the highest concentration, its concentration increased during seven days up to 52.1% and the total amount of the fatty acids was higher than 83%, that is, the unsaturated ones dominated over the saturated ones. The amount of oleic acid was 36.8%, that of linoleic acid was 38.1%, and that of linolenic acid was 2.7% in the original seed. During sprouting the concentration of the saturated fatty acids rapidly decreased, and myristic acid and stearic acid disappeared from the sample during one day of germination. Out of the unsaturated fatty acids oleic acid decreased to a greater extent, whereas linoleic acid and linolenic acid increased during the germination. This is very important since linoleic acid, linolenic acid and arachidonic acid are essential for the human organism. Linoleic acid is capable of transporting bioactive compounds and can transform into arachidonic acid from which hormone-like compounds are forming. Summarized, it was established that majority of fatty acids of buckwheat is unsaturated ones, out of which linoleic acid can be found in the highest amount.

Tokiko and Koji (2006) examining fat content and fatty acid composition of various sprouts established that the fat content ranged between 0.4 and 1.6%. In the course of fatty acid content analysis it was found that linolenic acid was present in the highest concentration, 23% in case of buckwheat, 48% in the soybean, 47.7% in the clover and 40.6% in the pea.

Studying the literature, we found no more data on the change in fat content and fatty acid composition during sprouting. Furthermore, we find some of the findings of the above two cited articles available to be hard to imagine, the mechanisms transforming the saturated fatty acids into unsaturated ones, and the monounsaturated oleic acid into polyunsaturated fatty acids, are unknown. Because of the above we started our investigation relating to fatty acid composition of nutritional sprouts and the changes in the fatty acid composition due to germination. During our work we determined the fatty acid composition of wheat, lentil, sunflower, alfalfa and radish seed sprouts and its change in the function of germination time. In this paper we wish to report our results.

2 Materials and methods

The examined samples, germination

Commercially obtainable organic wheat, lentil, sunflower, alfalfa and radish seeds were obtained. The seeds were washed in 0.1% H₂O₂ for 1 min then soaked in distilled water for 24 hrs. After the 24 hrs elapsed, the seeds were placed into germination bowls, and germinated at 20 °C in a Memmert 200 incubator. They were rinsed twice a day with distilled water and samples were taken in every 24 hrs. According to the domestic practice and international recommendations wheat and lentil were germinated for 3 days, radish for 7 days, alfalfa for 8 days, sunflower for 5 days. After germination the sprouts were washed with distilled water, dried at 60 °C, then stored frozen at -10 °C until the analyses. Crude fat content of the sprouts were determined in a Soxhlet extractor after extraction with diethylether according to the Hungarian Standard.

Crude fat content was given with 0.1% accuracy as the mean value of two repetitions. The allowed maximal difference between the two repetitions was 0.3%.

During the sample preparation for the fatty acid analysis a sample quantity containing 1 g fat was destructed with 8-20 cm³ of concentrated hydrochloric acid (37%) for 1 hour on hot water bath. After having cooled down, 7 cm³ of ethanol was added. Lipids were extracted with 15 cm³ diethylether then with 15 cm³ benzine (b.p.<60 °C), and the organic layers were combined. The solvents were removed under reduced pressure. To the residue 4 cm³ of 0.5 M sodium hydroxide methanol solution was added and under a reflux cooler it was boiled until all the fat drops disappeared (approx. 5 min), then 4 cm³ of 14% boron trifluoride methanol solution was added, boiled for 3 min, finally 4 cm³ of hexane dried on water-free sodium sulphate was added and boiled for 1 min, and the mixture was allowed to cool down. The reflux cooler was removed and saturated aqueous sodium chloride solution was added and after having separated the organic layer was collected into a 4 cm³ vial containing water-free sodium sulphate and was directly examined by gas chromatography.

Determination of the fatty acid composition was performed using a Varian 3800 gas chromatograph. The chromatographic column was a fused silica capillary column (100 m, 0.25 mm id) with a CP-Sil 88 (FAME) stationary phase (film thickness: 0.2 µm). FID detector was used. Detector gas flow rates were as follows: hydrogen 30 ml/min, air 200 ml/min, make up gas 30 ml/min. Detector temperature was 270 °C. The carrier gas was high-purity

hydrogen, column head pressure was 235 kPa. Temperature program: 140 °C for 10 min; at 5 °C/min up to 235 °C; isotherm for 30 min. Injected volume was 1 μ l. The fatty acid methyl esters were identified using the “37 component FAME Mix” standard from Supelco. Results were given as fatty acid methyl ester relative weight%.

3 Results and discussion

Table 1 shows crude fat content of the starting seeds and the sprouts. Results were given in weight% on air dry matter basis.

Table 1: Crude fat content of the seeds and sprouts

Number	Description	Crude fat content (%) (on air dry-matter basis)
1.	Wheat seed	1.7
2.	Wheat sprout, day 3	1.7
3.	Lentil seed	1.4
4.	Lentil sprout, day 3	1.4
5.	Alfalfa seed	10.3
6.	Alfalfa sprout, day 3	9.8
7.	Alfalfa sprout, day 7	4.5
8.	Radish seed	39.0
9.	Radish sprout, day 2	39.2
10.	Radish sprout, day 6	20.2
11.	Sunflower seed	60.3
12.	Sunflower sprout, day 3	57.7
13.	Sunflower sprout, day 5	43.4

In case of wheat and lentil sprouts no change was experienced, in case of the alfalfa sprout the fat content decreased around to its half value, and the same applies for the six-day radish sprout. In case of sunflower sprout the decrease is around 30%.

Table 2 shows fatty acid composition of wheat seed and wheat sprout.

In the wheat sprout the fatty acids being present in the highest concentration are palmitic acid, linoleic acid and oleic acid. Out of the saturated fatty acids palmitic acid is present in 33.5% in the wheat sprout which value is higher than that for the wheat seed (31.2%), that is, due to the germination the

concentration of palmitic acid increased.

Table 2: Fatty acid composition of wheat seed and wheat sprout

Fatty acid		Wheat	Wheat sprout
		seed	Day 3
		Fatty acid methyl ester %	
Undecanoic acid	11:0	1.7	1.7
Lauric acid	12:0	0.1	0.1
Tridecanoic acid	13:0	0.7	0.7
Myristic acid	14:0	0.8	0.5
Pentadecanoic acid	15:0	0.3	0.3
Palmitic acid	16:0	31.2	33.5
Stearic acid	18:0	1.9	1.2
Oleic acid	18:1	10.7	7.8
Linoleic acid	18:2	25.6	27.3
Arachidic acid	20:0	0.3	0.2
Eicosenoic acid	20:1	0.9	0.4
α -Linolenic acid	18:3n3	2.0	2.5
Behenic acid	22:0	1.2	1.2
Eicosatrienoic acid	20:3n6	1.4	1.5
Eicosatrienoic acid	20:3n3	2.3	0.2

The value for stearic acid decreased from 1.9% in the seed to 1.2% in the wheat sprout. Out of the saturated fatty acids beyond the mentioned ones also undecanoic acid (1.7% in the wheat seed and also in the sprout), lauric acid (0.1% in both of the samples), tridecanoic acid (0.7% in the seed and the sprout), myristic acid (0.8% in the wheat seed and 0.5% in the wheat sprout), pentadecanoic acid (0.3% in both samples), arachidic acid (0.3% in the seed and 0.2% in the sprout) and behenic acid (1.2% in both samples) could be detected in the samples.

Out of the monounsaturated fatty acids oleic acid is present in the highest concentration, its value decreased from 10.7% in the wheat seed to 7.8% due to germination. In the samples also eicosenoic acid could be detected: 0.9% in the wheat seed and 0.4% in the wheat sprout.

Out of the polyunsaturated fatty acids linoleic acid was present in the highest concentration, with 25.6% in the starting wheat seed and increasing to 27.3% in the sprout. In the samples also α -linolenic acid (2.0% in the wheat seed and 2.5% in the sprout) and eicosatrienoic acid (C20:3n6): (1.4% in the

wheat seed and 1.5% in the wheat sprout), (20:3n3): (2.3% in the wheat seed, 0.2% in the sprout) were detectable.

Table 3 contains fatty acid composition of lentil and lentil sprout.

Table 3: Fatty acid composition of lentil seed and lentil sprout

Fatty acid		Lentil seed	Lentil sprout, Day 3
		Fatty acid methyl ester %	
Undecanoic acid	11:0	0.4	0.6
Lauric acid	12:0	0.2	0.2
Tridecanoic acid	13:0	0.4	0.4
Myristic acid	14:0	1.1	1.1
Pentadecanoic acid	15:0	0.4	0.7
Palmitic acid	16:0	26.2	27.0
Stearic acid	18:0	1.6	2.2
Oleic acid	18:1	14.0	9.3
Linoleic acid	18:2	19.4	27.4
Arachidic acid	20:0	0.3	0.5
Eicosenoic acid	20:1	0.3	0.4
α -Linolenic acid	18:3n3	3.3	4.7
Behenic acid	22:0	1.2	1.6
Eicosatrienoic acid	20:3n6	1.4	1.4
Eicosatrienoic acid	20:3n3	0.1	0.1

In the lentil sprouts palmitic acid, linoleic acid and oleic acid can be found in the highest concentration. Out of the saturated fatty acids palmitic acid can be found in the lentil seed in 26.2%, this value is higher in the sprout (27.0%). Out of the saturated fatty acids also undecanoic acid (0.4% in the lentil seed, 0.6% in the lentil sprout), lauric acid (0.2% in both samples), tridecanoic acid (0.4% in both samples), myristic acid (1.1% in both samples), pentadecanoic acid (0.4% in the lentil seed, 0.7% in the sprout), stearic acid (the value of 1.6% in the starting seed increased to 2.2% in the sprout), arachidic acid (0.3% in the lentil seed, 0.5% in the sprout) and behenic acid (1.2% in the lentil seed, 1.6% in the sprout) could be detected.

Out of the unsaturated fatty acids linoleic acid and oleic acid can be found in the highest amount. Concentration of linoleic acid in the lentil seed was 19.4%; due to the germination this value increased up to 27.4% in the sprout. Oleic acid is present in the seed in 14.0% which decreases to 9.3% in the sprout.

Out of the unsaturated fatty acids also eicosenoic acid was detectable (0.3% in the lentil seed and 0.4% in the sprout). Out of the polyunsaturated fatty acids concentration of α -linolenic acid increased from 3.3% in the starting lentil seed up to 4.7% in the sprout due to the germination, concentration of eicosatrienoic acid hardly changed, however (20:3n6: 1.4% in the seed and 1.5% in the sprout, 20:3n3: 0,1% in both samples).

Table 4 contains fatty acid composition of alfalfa seed and alfalfa sprout.

Table 4: Fatty acid composition of alfalfa seed and alfalfa sprout

Fatty acid		Alfalfa	Alfalfa sprout,	Alfalfa sprout,
		seed	Day 3	Day 7
		Fatty acid methyl ester %		
Lauric acid	12:0	0.1	0.2	0.1
Myristic acid	14:0	0.5	0.4	0.6
Pentadecanoic acid	15:0	0.2	0.3	0.6
Palmitic acid	16:0	15.9	15.9	22.4
Palmitoleic acid	16:1	0.1	0.1	0.3
Margaric acid	17:0	0.1	0.2	0.3
Stearic acid	18:0	3.2	2.9	4.4
Oleic acid	18:1	10.4	9.1	9.8
Linoleic acid	18:2	34.3	34.7	29.1
Arachidic acid	20:0	0.7	0.8	1.0
γ -Linolenic acid	18:3n6	0.2	0.2	0.2
Eicosenoic acid	20:1	0.3	0.2	0.3
α -Linolenic acid	18:3n3	24.9	24.9	15.8
Eicosadienoic acid	20:2	0.1	< 0.1	0.1
Behenic acid	22:0	0.8	1.0	1.7
Eicosatrienoic acid	20:3n6	0.4	0.7	1.3
Eicosatrienoic acid	20:3n3	0.4	0.2	0.3
Arachidonic acid	20:4n6	0.1	0.1	0.7
Docosapentaenoic acid	22:5n3	0.9	1.2	1.6

In the sprout the fatty acids being present in the highest concentration are linoleic acid, α -linolenic acid, palmitic acid, and oleic acid. Out of the saturated fatty acids in the highest concentration palmitic acid could be detected. Its concentration increased due to the germination: 15.9% in the seed, the same value in the three days old sprout, and 22.4% in the seven days old sprout. Concentration of stearic acid increased during the germination (from 3.2% to 4.4%). Out of the saturated fatty acids under the given chromatographic conditions lauric acid, myristic acid, pentadecanoic acid, margaric

acid, arachidic acid and behenic acid were detectable, their amount remained below 1%, however.

Out of the unsaturated fatty acids linoleic acid, α -linolenic acid and oleic acid were present in the highest concentration. Concentration of linoleic acid in the original seed was 34.3%, which decreased during the germination to 29.1%. Concentration of α -linolenic acid also decreased during the germination: from 24.9% in the starting alfalfa seed to 15.8% in the sprout. The amount of oleic acid also decreased during the germination: from 10.4% in the seed to 9.4% in the sprout. Out of the unsaturated fatty acids also palmitoleic acid, α -linolenic acid, eicosenoic acid, eicosadienoic acid, eicosatrienoic acid, arachidonic acid and docosapentaenoic acid could be detected, their amount remained below 1%, however.

Table 5 contains fatty acid composition of radish seed and radish sprout.

Table 5: Fatty acid composition of radish seed and radish sprout

Fatty acid		Radish seed	Radish sprout, Day 2	Radish sprout, Day 6
		Fatty acid methyl ester %		
Lauric acid	12:0	<0.1	<0.1	<0.1
Myristic acid	14:0	0.1	0.1	0.1
Pentadecanoic acid	15:0	<0.1	<0.1	<0.1
Palmitic acid	16:0	8.2	6.5	8.0
Palmitoleic acid	16:1	0.2	0.2	0.2
Stearic acid	18:0	3.2	2.7	3.0
Oleic acid	18:1	35.1	27.4	34.6
Linoleic acid	18:2	15.5	12.6	15.9
Arachidic acid	20:0	2.0	1.6	2.0
α -Linolenic acid	18:3n6	0.1	0.1	0.1
Eicosenoic acid	20:1	14.8	11.5	15.1
α -Linolenic acid	18:3n3	13.6	11.3	14.0
Eicosadienoic acid	20:2	0.6	0.5	0.7
Behenic acid	22:0	1.9	1.4	1.9
Arachidonic acid	20:4n6	0.1	<0.1	0.1
Docosadienoic acid	22:2	0.4	0.3	0.4
Lignoceric acid	24:0	1.2	0.9	1.2

In the radish sprout in the highest concentration oleic acid, linoleic acid, eicosenoic acid, α -linolenic acid and palmitic acid were present. Out of the saturated fatty acids concentration of palmitic acid in the radish seed was 8.2%, during the germination this reduced to 6.5% in the two days old radish

sprout, whereas in the six days old sprout it increased to 8.0%. Concentration of stearic acid decreased from 3.2% in the starting radish seed to 2.7 and 3.0% in the sprout. Out of the saturated fatty acids also lauric acid, myristic acid, pentadecanoic acid, arachidic acid, behenic acid and lignoceric acid were detectable, their concentration was below 2%, however.

Out of the unsaturated fatty acids oleic acid was present in 35.1% in the radish seed, in 27.4% in the two days old sprout, in 34.6% in the six days old sprout. Concentration of linoleic acid increased due to the germination from the starting 15.5% to 15.9% in the six days old sprouts. Concentration of eicosenoic acid also increased due to the germination from the starting 14.8% to 15.1%, whereas that of α -linolenic acid from 13.6% to 14.0%. Under the given chromatographic conditions also palmitoleic acid, α -linolenic acid, eicosadienoic acid, arachidonic acid, docosadienoic acid, however, their concentration was less than 0.5%.

Table 6 contains fatty acid composition of sunflower seed and sunflower sprout.

Table 6: Fatty acid composition of sunflower seed and sunflower sprout

Fatty acid		Sunflower seed	Sunflower sprout, day 3	Sunflower sprout, day 5
Fatty acid methyl ester %				
Myristic acid	14:0	0.1	0.1	0.2
Pentadecanoic acid	15:0	<0.1	<0.1	<0.1
Palmitic acid	16:0	5.8	5.7	5.8
Palmitoleic acid	16:1	0.1	0.1	0.1
Margaric acid	17:0	0.1	0.1	0.1
Stearic acid	18:0	5.4	5.4	5.6
Oleic acid	18:1	21.7	21.0	20.9
Linoleic acid	18:2	65.0	65.6	64.8
Arachidic acid	20:0	0.4	0.4	0.4
Eicosenoic acid	20:1	0.2	0.1	0.2
α -Linolenic acid	18:3n3	0.1	0.4	1.0
Behenic acid	22:0	0.9	0.9	1.0
Arachidonic acid	20:4n6	0.2	<0.1	<0.1
Lignoceric acid	24:0	0.2	0.3	0.3

In the sunflower sprout out of the saturated fatty acids palmitic acid and stearic acid are present in the highest concentration. Concentration of palmitic acid hardly changes due to the germination: it is 5.8% in the seed, in the three

days old sprout is 5.7% , and in the five days old sprout 5.8%. Also the value of stearic acid changes to a very little extent during the germination, the starting value of 5.4% in the seed remains unchanged in the three days old sprout, it increases in the five days old sprout to 5.6%, however. Out of the saturated fatty acids also myristic acid, pentadecanoic acid, margaric acid, arachidic acid, behenic acid and lignoceric acid were detectable, their concentration was negligible, however.

In the sunflower sprout linoleic acid is the highest concentration unsaturated fatty acid which increased from the starting 65.0% by day 3 to 65.6%, then reduced by day 5 to 64.8%. Oleic acid is present similarly in a high concentration, the starting value of 21.7% reduced to 21.0% in the three days old sprout and to 20.9% in the five days old sprout. Out of the unsaturated fatty acids also palmitoleic acid, eicosenoic acid, α -linolenic acid, arachidonic acid could be detected, their concentration was below 1%, however.

By the analysis of the fatty acid composition of wheat, lentil, sunflower, alfalfa and radish seed sprouts we established that by far no such radical changes occurred during the germination as reported by *Kim et al.* (2004), and *Tokiko and Koji* (2006). Calculating on dry-matter basis the crude fat content of the sprouted plant either did not change or decreased during the sprouting.

Regarding the fatty acid composition, concentration of palmitic acid, the saturated fatty acid being present in the highest concentration, increased in case of wheat, lentil and alfalfa sprout, in the case of radish sprout decreased somewhat, and in case of sunflower sprout practically did not change during the germination. Similar change can be reported in case of stearic acid, and we cannot give a definite answer to how the stearic acid content changed during the germination. It is very probable that either the stearic acid content or palmitic acid content does not suffer a substantial change due to the germination.

We can formulate almost similar tendencies in case of unsaturated fatty acids. In case of wheat, lentil and alfalfa sprout the amount of oleic acid decreased somewhat, in case of radish and sunflower sprout the change is minimal. The increase of linoleic acid is considerable only in case of the lentil sprout, whereas for all the other sprouts its amount remains practically unchanged in the germination period. The other polyunsaturated fatty acids occur in such a small concentration, that even the tendency of the changes is difficult to follow.

Summarized, it can be established that some of the saturated fatty acids decrease minimally, others remain unchange. Out of the unsaturated fatty

acids oleic acid practically hardly changes, and also the amount of linoleic acid shows a considerable increase only in case of the lentil sprout. From hardly affected the fatty acid composition of the fat of the sprouting plant, consequently the biological value of the fat. We cannot confirm the results found in the literature reporting that due to the germination the amount of the saturated fatty acids considerably decreases, and the amount of the polyunsaturated essential fatty acids considerably increases.

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