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The use of chromatographic and thermal techniques to assess the stability of fat isolated from chickpea protein concentrate during its storage

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Abstract: The aim of the study was to assess the stability of fat isolated from chickpea protein concentrate (CPC) during its storage using chromatographic and thermal techniques. Fat was extracted from CPC using the Folch method, and the fat fraction was analyzed for the fatty acid (FA) composition by gas chromatography (GC) and for the oxidative stability by pressure differential scanning calorimetry (PDSC). The isolated fat was stored in freezing and cooling conditions, as well as at room temperature with and without access to light. The GC analysis of the stored fat was repeated 28 days after extraction, and PDSC tests were done also 7, 14, 21 and 28 days after extraction.

Keywords: chickpea protein concentrate; fatty acid composition; oxidative stability; pressure differential scanning calorimetry

1. Introduction

Chickpea (*Cicer arietinum* L.) is a highly nutritious pulse crop grown and consumed all over the world [1]. It is a valuable source of good quality protein rich in a number of essential amino acids (e.g., lysine, leucine, and arginine) including branched chain ones [2]. Chickpea proteins have been reported to be highly digestible ingredients with a wide range of useful techno-functional properties such as foaming, emulsification and gelling [3-5]. Therefore, chickpea is a promising protein source, both for new products such as meat analogues, and for supplementation of traditional foods [6].

Chickpea proteins can be found in high content in chickpea flour, which can be further processed into chickpea protein concentrates (CPC) and isolates (CPI) for use as functional ingredients in food products [5]. The second ingredient found in the highest amount in such powders, after protein, is fat. In concentrates, its amount can reach up to several dozen percent and can significantly affect both the taste and stability of food products containing CPC.

Lipids are important components of food, in addition to providing energy, texture, and mouthfeel, are a source of essential fatty acids and provide a heat transfer medium for food processing [7]. Unfortunately, they are also components that are very susceptible to oxidation processes. Oxidation of lipids is affected by a number of factors including: (1) processing and storage conditions (temperature, light, oxygen); (2) content of unsaturated fatty acids and their distribution in triacylglycerol molecule; and (3) the presence of antioxidants (inhibitors) or prooxidants (catalysts) [8]. Lipid oxidation reduces the nutritional value of food and limits its shelf-life with the development of unpleasant rancid

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odor and taste, consequently making food unacceptable for consumption. Moreover, lipid oxidation products can cause various biological reactions negatively affecting human health [7]. Therefore, in the food industry it is so important to study the process of lipid oxidation, especially edible oils with a high content of unsaturated fatty acids, which are particularly susceptible to oxidation.

The purpose of the present paper was to assess the oxidative stability of fat isolated from CPC during its storage under different conditions (in freezing and cooling conditions, as well as at room temperature with and without access to light) using chromatographic (gas chromatography, GC) and thermal (pressure differential scanning calorimetry, PDSC) techniques.

2. Materials and Methods

2.1. Materials

Commercially available chickpea protein concentrate, Cicerpro™ 70G (Exeller, Poland) were used in this study. The CPC composed of 67% protein, 20% fat, 5% fiber, max. 8% ash and <0.2% carbohydrates on a dry basis as stated by the manufacturer.

Fat from CPC was extracted using Folch's method according to the procedure described by Boselli et al. [9] immediately after opening the package. The isolated fat was divided into four vials, which were kept tightly closed:

- in freezing conditions (laboratory freezer - temp.-20°C) - sample designed as CPC_{-20};
- in cooling conditions (laboratory refrigerator - temp. 4°C) - sample designed as CPC_4;
- at room temperature without access to sunlight - sample designed as CPC_{20};
- at room temperature exposed to sunlight - sample designed as CPC_{UV}.

2.2. Gas chromatography (GC)

The determination of fatty acid (FA) composition in examined samples was carried out by GC analysis of fatty acid methyl esters prepared through esterification with KOH/methanol (1M) according to ISO 5509:2001 [10].

An YL6100 GC chromatograph equipped with a flame ionization detector and BPX70 capillary column of 60 m x 0.25 mm x 0.25 μm was used. The oven temperature was programmed as follows: initial temperature 70˚C (for 0.5 min) was increased by 15˚C min^{-1} to 160˚C next from 160 to 200˚C it was increased by 1.1˚C min^{-1}; and then kept at 200˚C for 6 min, in the next step from 200 to 225˚C it was increased by 30˚C min^{-1} and kept at 225˚C another 1 min.

The temperature of the injector was 225˚C (with a split ratio of 1:50) and the detector temperature was 250˚C. The carrier gas was nitrogen at a flow rate of 1.2 mL min^{-1}. For each sample measurements were carried out at least two times. The results were expressed as relative percentages of each FA. Reference fatty acid methyl esters from Sigma-Aldrich were used as standards for identification and quantitation purpose.

2.3. Pressure differential scanning calorimetry (PDSC)

The oxidative stability of fat extracted from CPC was determined using differential scanning calorimeter (DSC Q20, TA Instruments) coupled with a high-pressure cell (Q20P). Weighed fat samples (3–4 mg) were placed on an open aluminum pan in the heating sample chamber of the PDSC cell. Experiments were performed under oxygen atmosphere with an initial pressure of 1 380 kPa and with the 100 mL min^{-1} gas flow rate. The isothermal temperature for each sample was programmed at 120˚C. Obtained PDSC diagrams were analyzed using TA Universal Analysis 2000 software.

For each sample, measurements were carried out at least two times, and the PDSC oxidation time (τ_{max}) was determined on the basis of the maximum rate of heat flow with an accuracy of 0.005.

3. Results

3.1. Fatty acid compostion

Fatty acid (FA) composition of the studied fat isolated from CPC determined immediately after the extraction process (CPC_0) and after 28 days of storage in room conditions (with, CPC_{UV}, and without access to light, CPC_{20}) as well as in cooling (CPC_4) and freezing conditions (CPC_{-20}) is presented in Table 1 along with the total percentage of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA).

Table 1. Fatty acid composition of oil a) immediately after its isolation from chickpea protein concentrate (CPC) and b) after 28 days of storage in various conditions.

Fatty acid	Percentage of fatty acid [%]				
	immediately after isolation	after 28 days of storage in various conditions			
	CPC_0	**CPC_{-20}**	**CPC_4**	**CPC_{20}**	**CPC_{UV}**
C14:0	0.30 ±0.04	0.25 ±0.05	0.30 ±0.04	0.40 ±0.05	0.50 ±0.05
C16:0	11.45 ±0.13	11.80 ±0.27	12.20 ±0.18	13.60 ±0.22	14.70 ±0.55
C16:1	0.30 ±0.01	0.30 ±0.02	0.30 ±0.00	0.30 ±0.01	0.30 ±0.00
C18:0	2.30 ±0.05	2.30 ±0.08	2.30 ±0.10	2.20 ±0.05	2.20 ±0.05
C18:1 n-9c	37.35 ±0.21	37.35 ±0.35	37.30 ±0.55	36.60 ±0.55	36.30 ±0.50
C18:2 n-6c	44.20 ±1.37	43.80 ±1.29	43.40 ±1.36	42.90 ±0.95	42.10 ±0.36
C18:3 n-3	2.10 ±0.02	2.10 ±0.03	2.10 ±0.02	2.10 ±0.01	2.05 ±0.03
C20:0	0.90 ±0.05	0.90 ±0.08	0.90 ±0.10	0.70 ±0.05	0.70 ±0.02
C20:1	0.70 ±0.02	0.70 ±0.04	0.70 ±0.04	0.60 ±0.05	0.55 ±0.03
Σother	0.40 ±0.05	0.50 ±0.02	0.50 ±0.02	0.60 ±0.08	0.60 ±0.05
ΣSFA	14.95	15.25	15.70	16.90	18.10
ΣMUFA	38.35	38.35	38.30	37.50	37.15
ΣPUFA	46.30	45.90	45.50	45.00	44.15

The percentage of individual FA in the sample was presented as the mean ± SD (standard deviation)
CPC_0 – oil immediately after isolation with CPC (day 1); CPC_{-20} – oil stored in freezing conditions; CPC_4 – oil stored in cooling conditions; CPC_{20} – oil stored at room temperature without access to light; CPC_{UV} – oil stored at room temperature and exposed to sunlight
SFA, MUFA, PUFA – saturated, monounsaturated and polyunsaturated fatty acids, respectively.

The fat isolated from CPC contains only long-chain fatty acids. The main FA are palmitic (PA, C16:0) (~11.45%), oleic (OA, C18:1) (~37.35%) and linoleic (LA, C18:2) (~44.20%) acids, and in total composed around 93.0% of the total FA content of the sample studied immediately after extraction (Table 1).

GC analyzes performed after 28 days of storage showed only slight changes in the FA profile of CPC oil. The share of most MUFA and PUFA decreased, primarily LA and OA, while the share of PA increased (Table 1). These changes depended on the oil storage conditions and were greater the higher the storage temperature. Taking into account the fact that the greatest changes were observed in the conditions of fat storage at room temperature (with and without access to light) - probably some of the OA and LA were oxidized, and the reduced amount of these acids in the sample could have resulted in an increase in the percentage of SFA.

After 28 days, the FA profile for the fat sample stored in freezing conditions (CPC_{-20}) was most similar to the one obtained immediately after isolation from CPC (CPC_0).

3.2. Oxidative stability

The PDSC method was used to monitor the oxidative stability of fat isolated from CPC. Analyzes were performed immediately after fat extraction and after 7, 14, 21 and 28 days of storage in freezing and cooling conditions as well as at room temperature (without and with access to sunlight).

As a result of the analyses, a PDSC diagram of fat oxidation with single exothermic signal was obtained for each tested sample (Figure 1 as representative).

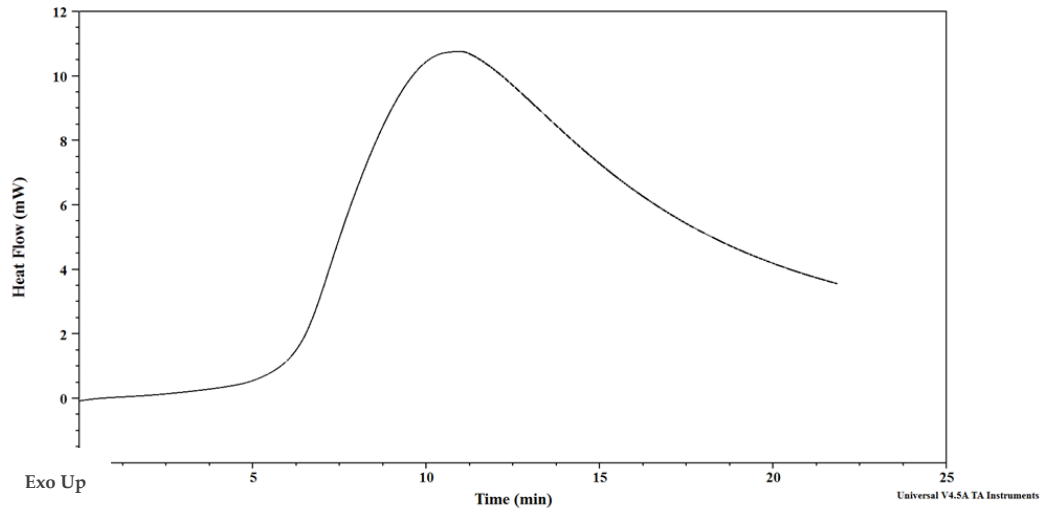

Figure 1. The representative PDSC diagram of fat isolated from chickpea protein concentrate (CPC).

Based on the obtained PDSC curves, the PDSC oxidation time (τ_{max}), the time corresponding to the maximum value of heat flow/the maximum rate of fat oxidation, was determined (Table 2).

Table 2. The PDSC oxidation time (τ_{max}) of fat a) immediately after isolation from chickpea protein concentrate (CPC) and b) 7, 14, 21 and 28 days after isolation.

Oil sample	τ_{max} [min]				
	immediately after isolation	7 day	14 day	21 day	28 day
CPC_{-20}	11.32 ±0.21	11.26 ±0.15	11.16 ±0.11	10.42 ±0.11	10.34 ±0.01
CPC_{4}	11.32 ±0.21	11.21 ±0.40	11.52 ±0.06	11.34 ±0.15	10.49 ±0.34
CPC_{20}	11.32 ±0.21	11.08 ±0.09	11.56 ±0.08	11.42 ±0.05	11.44 ±0.04
CPC_{UV}	11.32 ±0.21	11.17 ±0.32	11.60 ±0.34	11.53 ±0.23	11.86 ±0.10

* Footnotes as in Table 1.

The PDSC oxidation time of fat stored in various conditions for 28 days ranged from 10.34 to 11.86 min. The higher the τ_{max} value, the greater the oxidative stability of the tested fat. The shortest τ_{max} after 28 days of storage was obtained for CPC_{-20}, and the longest for CPC_{UV}. All samples showed a decrease in τ_{max} on day 7 of the study compared to the initial sample. However, only for CPC_{-20} there was a further gradual decline in τ_{max} with the storage time, and these changes were abrupt between the 14th and 21st day of the study.

3. Discussion

The FA profile of fat isolated from CPC is definitely dominated by unsaturated fatty acids, constituting approx. 85% of all FA, with more PUFA (46.30%) than MUFA (38.35%). Unsaturated fatty acids dominate in the FA profile of other legumes [11]. The main difference between the FA composition of various legumes is the content and proportion of oleic and linoleic acid. Chickpea has higher amounts of LA and OA compared with other edible pulses such as lentils, peas and beans [1].

The FA composition of fat isolated from CPC is consistent with those obtained by Marioli Nobile et al. [12] for kabuli type chickpea seed oils. They examined 14 genotypes, emphasizing that in each case the highest content of FA was found for LA (42.1 - 58.7%), OA (21.7 - 43%) and PA (9.0 - 10.7%). The authors emphasized that consuming chickpeas

makes it possible to increase the amount of PUFA and the PUFA:SFA ratio in the diet, which affects, among others, to lower total cholesterol levels in the blood.

The FA profiles of chickpea seed oil were also presented by Jukanti et al. [1] in a review paper. The authors reported the ranges of individual FA contents for oils from kabuli and desi chickpea seeds presented in the *Canadian Grain Commission Report*. These ranges for LA, OA and PA of the kabuli type were: 42.25 - 56.59%, 27.70 - 42.46% and 8.52 - 10.30%, respectively, and for the desi type: 53.10 - 65.25%, 18.44 - 28.5% and 8.56 - 11%. The FA composition of the tested CPC is therefore more similar to the composition presented for the kabuli variety.

It is known that edible oils with higher contents of unsaturated fatty acids are more susceptible to oxidation [13]. Despite the high content of unsaturated fatty acids, CPC fat retained oxidative stability and was characterized by slight changes in both, the FA profile and the PDSC oxidation time after 28 days of storage in various conditions, which may be related to the presence of antioxidants that effectively inhibit the oil oxidation process.

Literature data indicated that chickpea oil is a good source of tocopherols, sterols and tocotrienols. The α-tocopherol content in chickpea is reported to be relatively higher than for other pulse. Moreover, coupled with the concentration of δ-tocopherol makes chickpea oil oxidatively stable and contributes to a better shelf life during storage [1].

4. Conclusions

The fatty acid profile of fat isolated from chickpea protein concentrate is dominated by unsaturated fatty acids (~85%), with the highest content of oleic (~37%) and linoleic (~44%) ones. The studies showed only slight changes in the fatty acid composition of the chickpea protein concentrate fat after storage. Moreover, despite the high content of unsaturated fatty acids, also, the oxidation time in the PDSC tests changed only slightly, which proved the oxidative stability of the chickpea protein concentrate fat during 28 days of storage under various conditions (freezing, cooling, room temperature with and without access to the light).

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