WARSAW UNIVERSITY
OF LIFE SCIENCES - SGGW

INSTITUTE OF FOOD SCIENCES

THE USE OF CHROMATOGRAPHIC AND THERMAL TECHNIQUES TO ASSESS THE STABILITY OF FAT ISOLATED FROM CHICKPEA PROTEIN CONCENTRATE DURING ITS STORAGE

Diana Mańko-Jurkowska, Agnieszka Górska, Angelika Kosowska

CHICKPEA PROTEIN CONCENTRATE

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 [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 [3,4].

Chickpea proteins are available in the form of chickpea flour, **chickpea protein concentrates (CPC)**, and chickpea protein isolates for use as functional ingredients in food products [5].

CPC is an excellent substitute for meat in vegetarian and vegan diets, which due to its very good gelling strength can be used, among others, in meat products, dairy analogues, nutritional products, puddings, mayonnaises.

CPC enables the creation of nutritious, clean-label and sustainable food and beverage products [6].

Apart from protein, the main ingredient of chickpea protein concentrates is **fat**, the amount of which can reach over **20%**.

OXIDATIVE STABILITY OF EDIBLE FATS AND OILS

Oxidation is one of the most important processes to take place in food lipids during storage. **Lipid oxidation** reduces the nutritional value of food and limits its shelf-life with the development of unpleasant rancid odor and taste, consequently making food unacceptable for consumption. Moreover, lipid oxidation products can cause various biological reactions negatively affecting human health [7].

Oxidation of lipids is affected by a number of factors including:

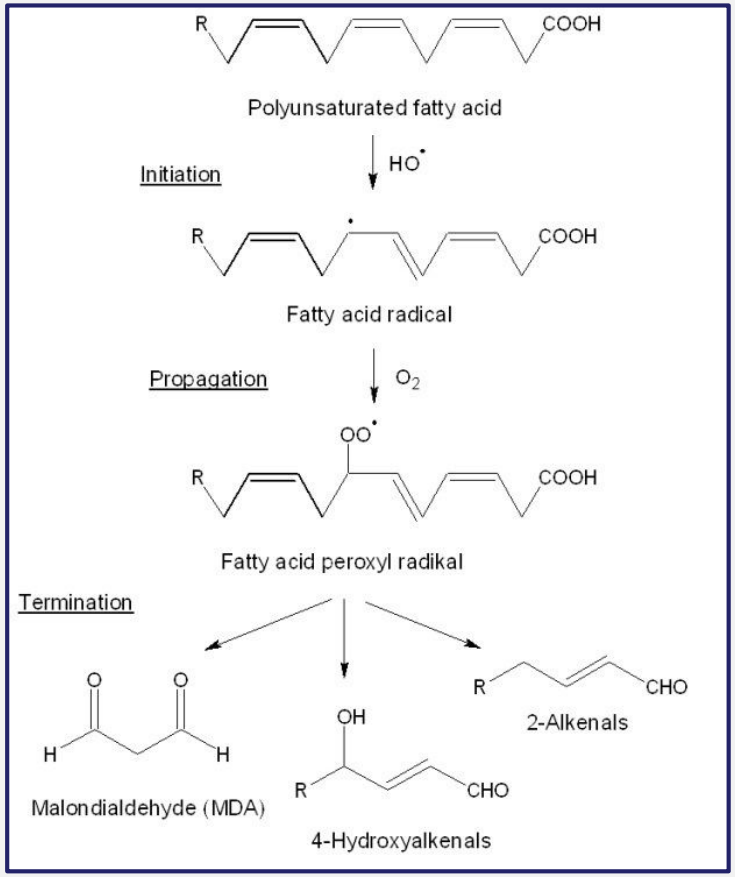
(1) processing and storage conditions (temperature, light, oxygen);

(2) content of unsaturated fatty acids and their distribution in TAG molecule;

(3) the presence of antioxidants (inhibitors) or prooxidants (catalysts) [8].

The assessment of edible oils and fats quality can be conducted using the **oxidative stability** determination. Due to time factors, which are very important for the industry, primarily **accelerated tests** are currently used to determined the oxidative stability of oil, like **pressure differential scanning calorimetry (PDSC)** [9].

In a PDSC experiment, the isothermal mode oil and the reference sample are confined under a defined atmosphere in the reaction chamber. As a result of oil oxidation the generated heat is recorded constantly with respect to a reference material in which there are no thermal events. The result of the test is a graph from which time for the **maximum of oxidation** can be determined directly [9].



https://alchetron.com/Lipid-peroxidation

MATRIALS AND METHODS

The aim of the study: assess the stability of fat isolated from chickpea protein concentrate during its 28 days of storage under different conditions (freezing, CPC_{-20}, and cooling conditions, CPC_4, as well as at room temperature with, CPC_{20}, and without access to light, CPC_{20}) using chromatographic and thermal techniques.

The scope of the study: (1) the extraction of fat from commercially available chickpea protein concentrates with 67% protein content in dry matter (**the Folch method** [10]) (2) determination of **fatty acid composition** of the isolated fat fraction by **gas chromatography (GC)** (3) analysis of **oxidative stability** of CPC fat by **pressure differential scanning calorimetry (PDSC)** technique.

CPC

YL6100 GC chromatograph

TA instrument DSC Q-20

FATTY ACID COMPOSITION OF OIL ISOLATED FROM CPC

The profile of fatty acids (FA) and their distribution in triacylglycerols (TAGs) are important factors affecting the stability of fat as well as the physical and sensory properties of products containing it in its composition.

Table 1. Fatty acid composition of oil a) immediately after its isolation from 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}
C 14:0	0.30 ±0.04	0.25 ±0.05	0.30 ±0.04	0.40 ±0.05	0.50 ±0.05
C 16:0	**11.45 ±0.13**	**11.80 ±0.27**	**12.20 ±0.18**	**13.60 ±0.22**	**14.70 ±0.55**
C 16:1	0.30 ±0.01	0.30 ±0.02	0.30 ±0.00	0.30 ±0.01	0.30 ±0.00
C 18:0	2.30 ±0.05	2.30 ±0.08	2.30 ±0.10	2.20 ±0.05	2.20 ±0.05
C 18:1 n-9c	**37.35 ±0.21**	**37.35 ±0.35**	**37.30 ±0.55**	**36.60 ±0.55**	**36.30 ±0.50**
C 18:2 n-6c	**44.20 ±1.37**	**43.80 ±1.29**	**43.40 ±1.36**	**42.90 ±0.95**	**42.10 ±0.36**
C 18:3 n-3	2.10 ±0.02	2.10 ±0.03	2.10 ±0.02	2.10 ±0.01	2.05 ±0.03
C 20:0	0.90 ±0.05	0.90 ±0.08	0.90 ±0.10	0.70 ±0.05	0.70 ±0.02
C 20:1	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

* presented values are means (±SD) of at least 2 replicate experiments

The main FA in CPC are **palmitic (~11.45%), oleic (~37.35%) and linoleic (~44.20%)** acids, and in total composed around **93.0%** of the total FA content of the sample studied immediately after oil extraction (Table 1).

The obtained data are consistent with those obtained by Marioli Nobile et al. [11] for kabuli type chickpea seed oils.

GC analyzes performed after 28 days showed only slight changes in the FA profile of CPC. The share of most MUFA and PUFA decreased, primarily oleic and linoleic acids, while the share of palmitic one increased.

FATTY ACID COMPOSITION OF OIL ISOLATED FROM CPC

The total content of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids for the tested oil immediately after its isolation from CPC and b) after 28 days of storage in various conditions is shown in Figure 1.

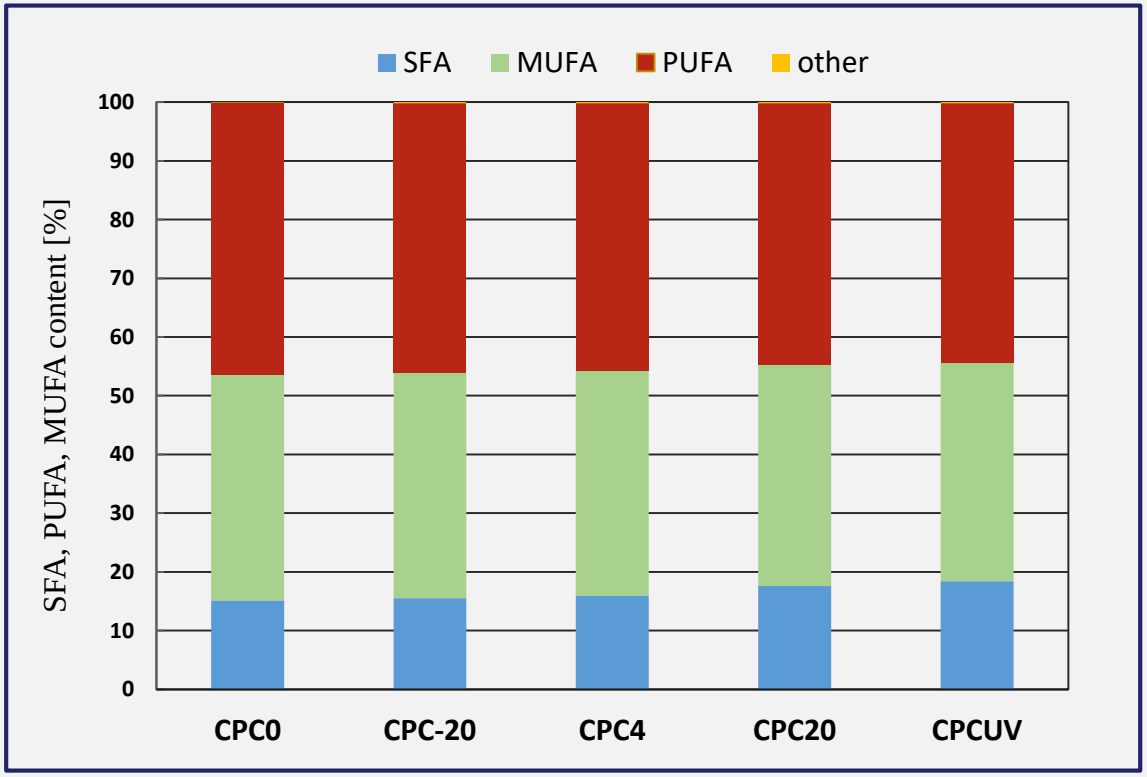


Fig. 1. The percentage of SFA, MUFA, PUFA (%) in the CPC samples.

The FA profile of oil isolated from CPC is definitely dominated by **unsaturated fatty acids**, constituting approx. **85%** of all FA, with more **PUFA (46.3%)** than **MUFA (38.45%)**.

Unsaturated fatty acids dominate in the FA profile of all legumes [12].

GC analyzes performed after 28 days showed only slight changes in the FA profile of CPC oil. The share of most MUFA and PUFA decreased.

OXIDATIVE STABILITY OF OIL ISOLATED FROM CPC

The **PDSC method** was used to monitor **the oxidative stability** of oil isolated from CPC. Analyzes were performed immediately after oil 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).

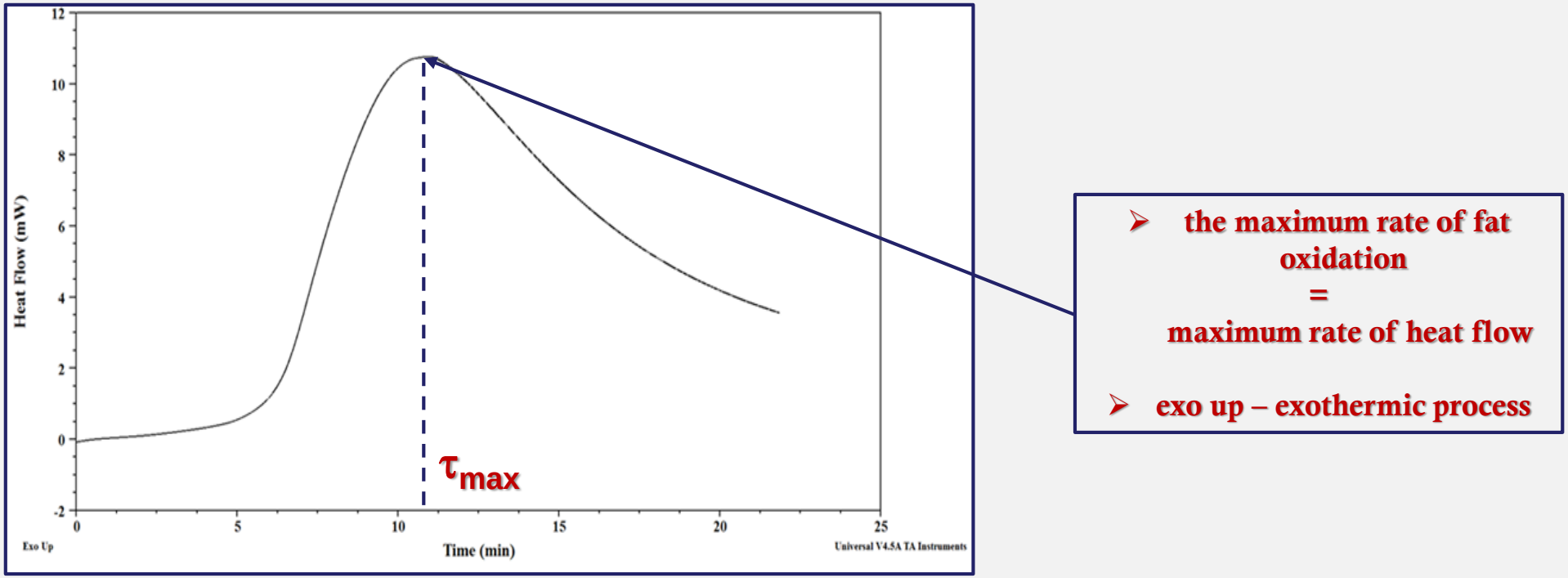


Fig. 2. The representative PDSC diagram of oil extracted from CPC.

OXIDATIVE STABILITY OF OIL ISOLATED FROM CPC

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

Table 2. The PDSC oxidation time (τ_{max}) of oil a) immediately after isolation from 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

* presented values are means (±SD) of at least 2 replicate experiments

The PDSC oxidation time of CPC oil 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 oil. The shortest τ_{max} after 28 days of storage was obtained for CPC_{-20}, and the longest for CPC_{UV}.

Despite the high content of unsaturated fatty acids, CPC oil retained oxidative stability after 28 days of storage in various conditions, which may be related to the presence of **antioxidants** that effectively inhibit the oil oxidation process.

CONCLUSIONS

1. The composition of fatty acids in CPC fat is dominated by unsaturated fatty acids, the content of which is about 85%.

2. The main fatty acids of CPC fat are oleic (~37%) and linoleic (~44%) ones.

3. The studies showed only slight changes in the fatty acids composition of CPC fat during 28 days of storage under various conditions (freezing, cooling, room temperature with and without access to the light) - decrease in the percentage of unsaturated fatty acids and an accompanying increase in the percentage of saturated ones.

4. Despite the high content of unsaturated fatty acids, the oxidation time in the PDSC tests changed only slightly, which proved the oxidative stability of fat isolated from CPC during 28 days of storage under various conditions.

5. 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].

LITERATURE

[Photos 1-5] https://foodproteins.globalfoodforums.com/product-profiles/chickpea-isolate-90-percent-protein/; https://innovopro.com/cp-pro70-chickpea-protein-concentrate/; https://www.bakingbusiness.com/articles/52215-chickpea-protein-startup-raises-18-million-in-funding; https://www.pinterest.co.uk/pin/11470174030915477/

[1] Jukanti, A.K.; Gaur, P.M.; Gowda, L.L.C.; Chibbar, R.N. Nutritional quality and health benefits of chickpea (*Cicer arietinum* L.): a review. Brit. J. Nutr. 2012, 108, S11–S26. http://dx.doi.org/10.1017/S0007114512000797

[2] Goldstein, N.; Reifen, R. The potential of legume-derived proteins in the food industry. Grain Oil Sci. Technol. 2022, 5, 167–178, https://doi.org/10.1016/j.gaost.2022.06.002

[3] Karaca, A.C.; Low, N.; Nickerson, M. Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. Food Res. Int. 2011, 44, 2742–2750. https://doi.org/10.1016/j.foodres.2011.06.012

[4] Johnston, S.P.; Nickerson, M.T.; Low, N.H. The physicochemical properties of legume protein isolates and their ability to stabilize oil-in-water emulsions with and without genipin. J. Food Sci. Technol. 2015, 52, 4135–4145. https://doi.org/10.1007/s13197-014-1523-3

[5] Grasso, N.; Lynch, N.L.; Arendt, E.K.; O'Mahony, J.A. Chickpea protein ingredients: A review of composition, functionality, and applications. Compr. Rev. Food Sci. Food Saf. 2022, 21, 435–452. https://doi.org/10.1111/1541-4337.12878

[6] https://innovopro.com/cp-pro70-chickpea-protein-concentrate/

[7] Wang, D.; Xiao, H.; Lyu, X.; Chen, H.; Wei F. Lipid oxidation in food science and nutritional health: A comprehensive re-view. Oil Crop Sci. 2023, 8, 35-44. https://doi.org/10.1016/j.ocsci.2023.02.002.

[8] Wąsowicz E.; Gramza A.; Hęś M.; Jeleń H.H.; Korczak J.; Małecka M.; Mildner-Szkudlarz S.; Rudzińska M.; Samotyja U.; Zawirska-Wojtasiak R. Oxidation of lipids in food. Pol. J. Food Nutr. Sci. 2004, 54, 87-100.

[9] Symoniuk, E.; Ratusz, K.; Krygier, K. Comparison of the oxidative stability of linseed (*Linum usitatissimum* L.) oil by pressure differential scanning calorimetry and Rancimat measurements. J. Food Sci. Techno. 2016, 53, 3986–3995. https://doi.org/10.1007/s13197-016-2398-2

[10] Boselli, E.; Velazco, V., Fiorenza Caboni, M.; Lercker, G. Pressurized liquid extraction of lipids for the determination of oxysterols in egg-containing food. J. Chromatogr. A. 2001, 917, 239-244. https://doi.org/10.1016/s0021-9673(01)00688-4

[11] Marioli Nobile, C.G.; Carreras, J.; Grosso, R.; Inga, M.; Silva, M.; Aguilar, R.; Allende, M.J.; Badini, R.; Martinez, M.J. Proxi-mate composition and seed lipid components of "kabuli"-type chickpea (Cicer arietinum L.) from Argentina. Agric. Sci. 2013, 4, 729-737. http://dx.doi.org/10.4236/as.2013.412099

[12] Sipeniece, E.; Mišina, I.; Qian, Y.; Grygier, A.; Sobieszczańska, N.; Sahu, P.K.; Rudzińska, M.; Patel, K.S.; Górnaś P. Fatty Acid Profile and Squalene, Tocopherol, Carotenoid, Sterol Content of Seven Selected Consumed Legumes. Plant Foods Hum. Nutr. 2021, 76, 53–59. https://doi.org/10.1007/s11130-020-00875-3