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INSTITUTE OF FOOD SCIENCES

ASSESSMENT OF THE POSSIBILITY OF USING DIFFERENTIAL SCANNING CALORIMETRY IN THERMAL ANALYSIS OF FAT ISOLATED FROM WHEY PROTEIN CONCENTRATE

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**Foods
2022**

**The 3rd International Electronic Conference
on Foods: Food, Microbiome, and Health**

01-15 OCTOBER 2022 | ONLINE

WHEY PROTEIN CONCENTRATE

Whey proteins are soluble milk proteins representing about **20% of total milk proteins**.

They are characterized by a very high nutritional and biological value, contain all the essential amino acids and are easily digestible, which is especially important in the nutrition of children and the elderly.



Whey proteins are available as an ingredient in the form of whey powder, **whey protein concentrates (WPCs)**, and whey protein isolates, mainly manufactured from whey - a by-product of cheese industry.



WPCs of varying protein concentrations (**35–85% protein**) are manufactured under mild conditions of pH and temperature mainly by ultrafiltration.

They are used in confectionery products, cereal and nutrition bars, processed cheeses, baked goods, sports beverages and muscle gain formulations.

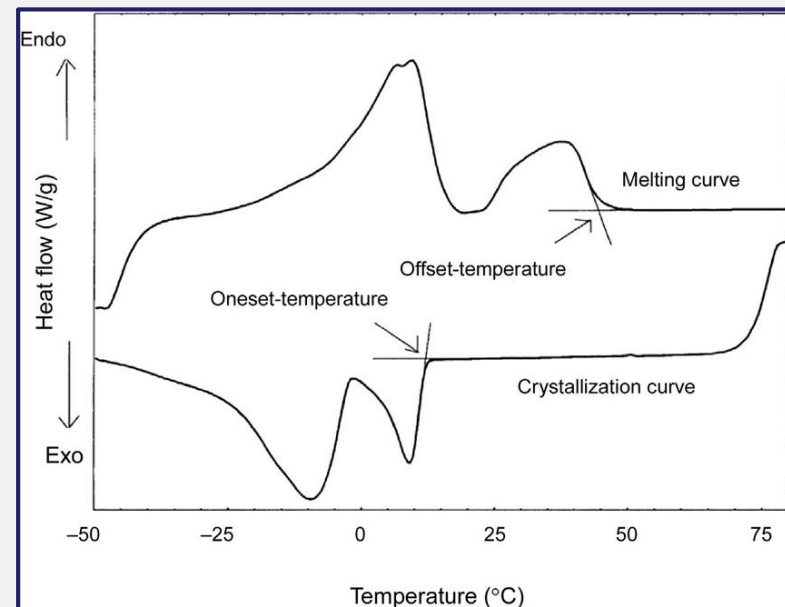
WPCs are also considered as **fat mimetics** and they have found extensive use in reduced-fat foods. Their multifunctional characteristics provide several fat-like attributes. Their major functions are gelling, water binding, emulsification, viscosification and adhesion [3].

DIFFERENTIAL SCANNING CALORIMETRY

Differential scanning calorimetry (DSC) is a sensitive, rapid and reproducible fingerprint **thermoanalytical technique**, in which a small amount of sample (a few mg) allows the determination of the conditions of phase transformations and the accompanying changes in physicochemical properties with given changes in heat flow. This method involves heating (or cooling) a sample simultaneously with a reference sample with a known heat flow rate. The result is obtained by calculating the heat flow difference between the samples. Energy changes are presented as a function of time or temperature as the so-called **DSC curves**.

In the case of lipids, the DSC technique is used to study **polymorphic transformations, oxidation, crystallization / melting**, or to determine **the thermal stability of edible oils and fats**.

Melting and crystallisation behaviours of edible oils and fats are two of the important properties for functionality in many prepared food products. These thermal properties are counterparts of the triacylglycerol profile in edible oils and fats and can both be measured by DSC [4].

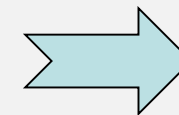
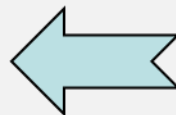
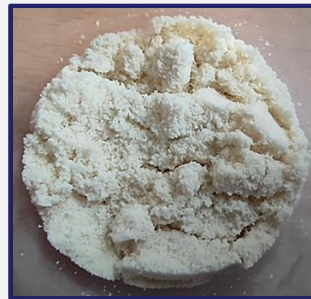


DSC melting and crystallisation curves [4]

The aim of the study: assess the possibility of using **differential scanning calorimetry (DSC)** in the thermal analysis of **fat extracted from commercial whey protein concentrates**

The scope of the study: (1) the extraction of fat from three commercially available whey protein concentrates with 80% protein content in dry matter (WPC80) (**the Folch method**) (2) determination of **fatty acid composition** of the isolated fat fraction by gas chromatography (GC) (3) analysis of thermal properties of WPC fat by differential scanning calorimetry (DSC) technique

WPC80



YL6100 GC chromatograph



TA instrument DSC Q-200

FATTY ACID COMPOSITION OF WPC FAT

The profile of fatty acids 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.

Tab. 1. The percentage of fatty acids (%) in the WPC fat samples.

Fatty acids	WPC1	WPC2	WPC3
C 10:0	2.05 ±0.21	1.58 ±0.05	1.63 ±0.06
C 12:0	2.75 ±0.21	2.28 ±0.05	2.33 ±0.10
C 14:0	9.90 ±0.28	9.00 ±0.14	9.08 ±0.13
C 15:0	1.35±0.07	1.30 ±0.00	1.30 ±0.00
C 16:0	29.95 ±0.21	33.20 ±0.34	33.08 ±0.15
C 16:1	2.00 ±0.00	1.98 ±0.05	1.95 ±0.05
C 18:0	12.00 ±0.28	11.58 ±0.19	11.75 ±0.13
C 18:1 n-9t	0.60 ±0.42	1.15 ±0.26	1.10 ±0.31
C 18:1 n-9c	23.60 ±0.28	27.08 ±0.15	27.10 ±0.28
C 18:2 n-6c	8.85 ±0.21	4.43 ±0.49	4.33 ±0.13
Other	7.00 ±0.07	6.49 ±0.06	6.40 ±0.05

The obtained values for the two main fatty acids are similar to the data available in the literature for milk and milk powders [5,6,7], where for palmitic acid they range from **20 to 38%**, and for cis-oleic acid from **17 to 39%**. The fatty acid profile obtained for WPC fat is the closest to the fatty acid profile of cow's milk fat presented in Bryś et al. [5].

* presented values are means (±SD) of 3 replicate experiments

FATTY ACID COMPOSITION OF WPC FAT

The total content of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids for the tested WPC fat is shown in Figure 1.

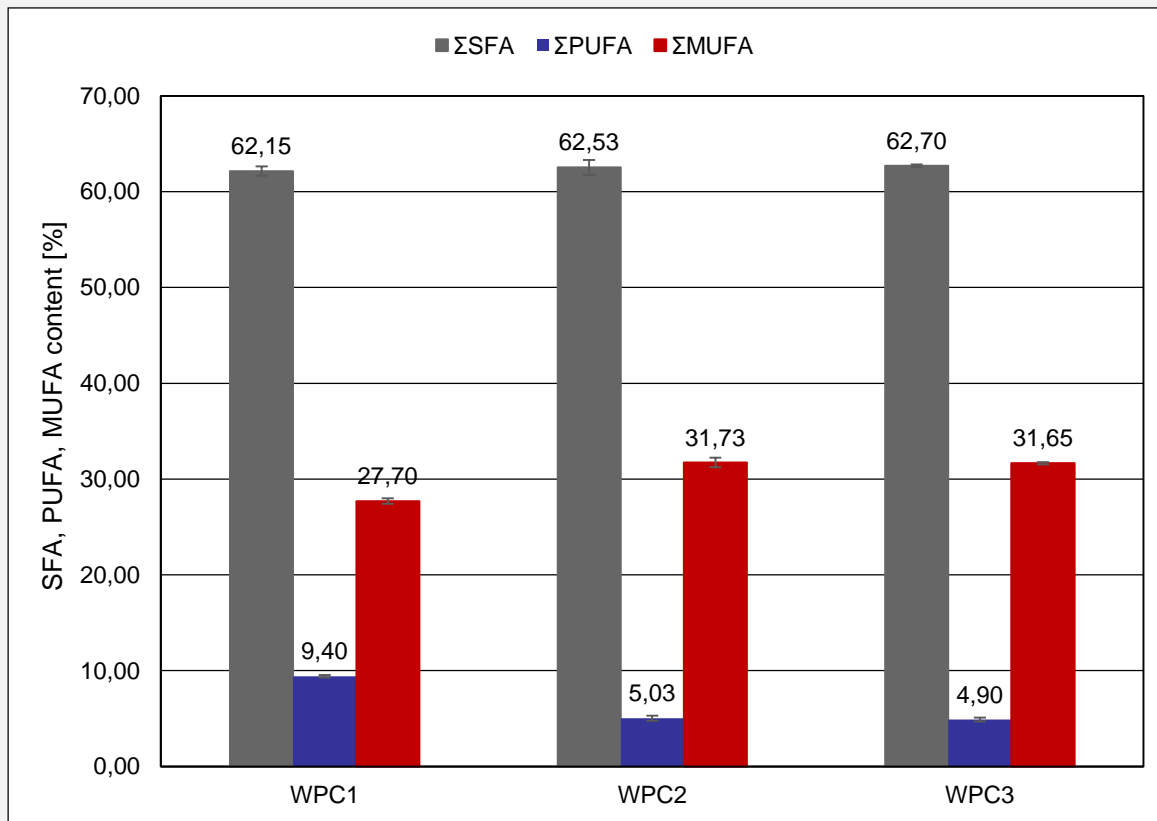


Fig. 1. The percentage of SFA, MUFA, PUFA (%) in the WPC fat samples.

The total content of **SFA** for all WPC fats is about **62%** and is close to the SFA value in cow's milk fat presented by Bryś et al. [5] (**63.3%**) and Karrar et al. [6] (**60.46%**).

The tested WPC fats differ significantly in the content of PUFA, which is similar for WPC2 and WPC3, but twice as high for WPC1 (**9.40%**). This is due to the high content of **linoleic acid** in WPC1 (**8,85%**).

MELTING PROFILE OF WPC FAT

Literature data indicate the presence of **three endothermic peaks** in the melting curves of milk fat [8]. Lopez et al. [9], referring to their own research and analysis of literature data, suggest, however, that these peaks may **overlap**, which is related to the wide distribution of the TAGs composition and their polymorphism.

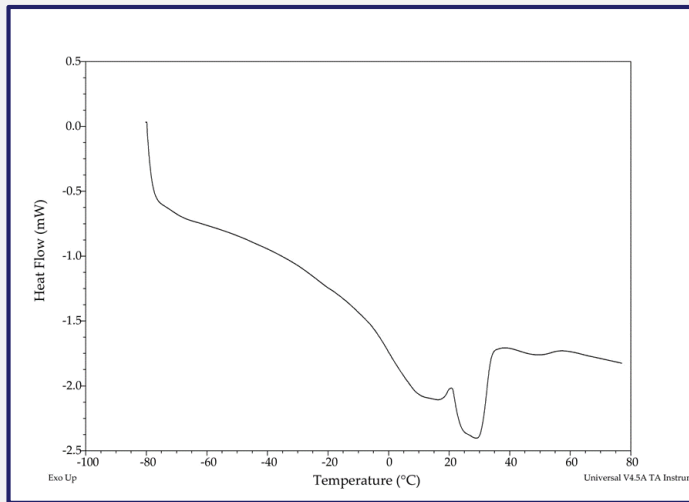


Fig 2. DSC curve of melting of WPC1 fat.

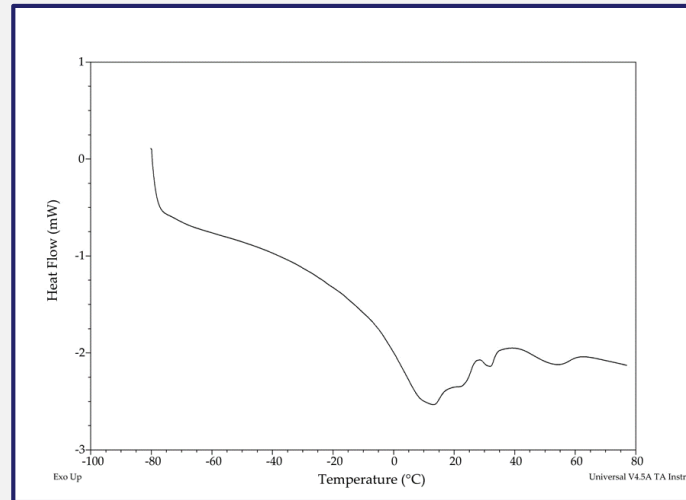


Fig 3. DSC curve of melting of WPC2 fat.

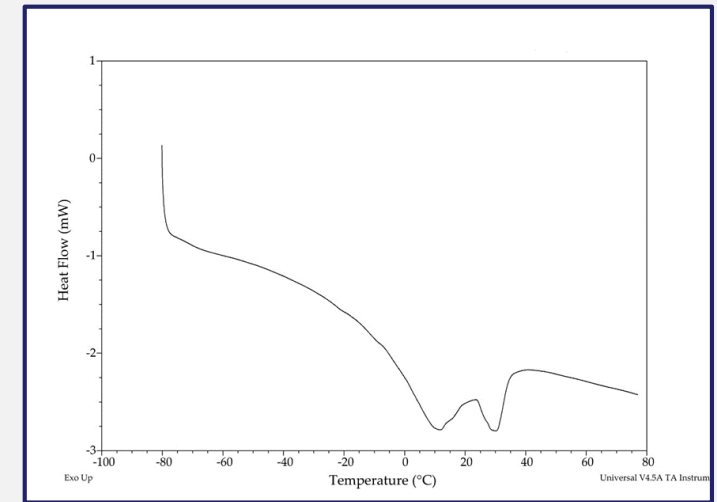


Fig 4. DSC curve of melting of WPC3 fat.

On the obtained curves, **two or three endothermic peaks** recorded on heating and corresponding to separate groups of TGs that melt separately can be observed in the temperature range from **-5.5°C to 35°C**.

MELTING TEMPERATURES OF WPC FRACTIONS

The obtained DSC curves were used to determine the characteristic temperatures of phase changes of individual TAG fractions taking place during the heating of the fat. The temperatures of the phase transitions were taken as the temperatures corresponding to the maximum endotherms (Tab. 2, Figs 2-4), where: t_1 is the melting point for the first peak (melting of the **low melting fraction. LMF**), t_2 are the temperature of the second melting peak of the **medium melting fraction (MMF)**, t_3 is the temperature of the third melting peak of the **high melting fraction (HMF)**.

Tab. 2. The temperature of melting peaks of WPC fat fractions.

Fat sample	Temperature [°C]		
	t_1	t_2	t_3
WPC 1	15.83 ± 0.40	-	29.24 ± 0.25
WPC 2	13.15 ± 0.17	22.73 ± 0.32	31.26 ± 0.92
WPC 3	11.53 ± 0.49	-	30.72 ± 0.33

* presented values are means (\pm SD) of 3 replicate experiments

Ostrowska-Ligeza et al. [10] studied the thermal properties of whole and skimmed milk powder, obtaining two endothermic peaks in the DSC diagrams in the range from **7.2°C to 52.8°C**.

Wang et al. [11] obtained diagrams of phase transitions for samples of anhydrous milk fat (AMF) in the temperature range from **-5 °C to ~ 37°C**.

The similar content of saturated fatty acids in all tested WPC fats results in the presence of **the endothermic peak of the HMF** with a maximum in a narrow temperature range (from **29 to 31.5°C**).

1. The composition of WPC fatty acids is dominated by saturated fatty acids, the content of which is about 62%.
2. The main fatty acids of WPC fat are: palmitic (~ 30%) and oleic in the *cis* conformation (> 23%).
3. In the WPC fat melting DSC curves there are endothermic peaks corresponding to the phase changes of the low and / or medium and high melting TAG fractions, which take place in the same temperature range as the TAG transformations of anhydrous milk fat. The DSC curves of fat melting show three endothermic peaks characteristic of milk fat only in the case of WPC2.
4. With a steady heat flow rate in the DSC, the peak of the second phase transition (for the medium melting TAG fraction) of WPC1 and WPC3 is superimposed on the peak of the first phase transition (low melting fraction). This effect may be related to the wide distribution of the TAG composition and their polymorphism.
5. Positive values of enthalpy change confirm the endothermic nature of the fat melting process.

- [1] https://th.bing.com/th/id/R.6d8d6da58835cea6628d3f40997afeee?rik=gyUyNBAJDfDyqw&riu=http%3a%2f%2fcdn14.beszamel.smcloud.net%2f%2fthumbs%2f660%2f441%2f1%2fuser_photos%2fserwatka-co-to-jest-wlasciwosci-serwatki-wartosci-odzywcz.jpg&ehk=91r%2fZ%2fO2XDqQ3GMge7mADiWtWp66FZ7H46BW%2bjiUxFg%3d&risl=&pid=ImgRaw&r=0&sres=1&sresct=1
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