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Study on the thermal behavior of casein in air

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Abstract: The present paper is focused on the study of the thermal behavior of casein, the main protein in milk, by applying the TG-FTIR technique and calorimetric measurements. The research is of a great interest due to the large application areas of casein in cosmetic, food and pharmaceutical industries. The analysis of the thermal behavior by the TG-DTG-DTA methods allwos the finding of the temperature range where the casein is thermally stable and the casein-containing cosmetics, food and pharmaceutical products proper for using. The identification of the gaseous species released allwos the TG-FTIR coupled technique to give useful information on the possible environmental impact when the casein-containing products are used at temperatures above the casein initial degradation temperature. The combustion heat of casein estimated by means of the

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Berthelot calorimeter made evident this value to be close to those of the most important and used fuels which would recommend the waste casein-containing products to be used as fuels.

Keywords: Casein; TG-FTIR; DSC; Berthelot calorimeter; Combustion heat; Degradation mechanism.

Introduction

In the present paper our researches on the thermal behavior of micro- and macromolecular compounds by thermal non-isothermal , TG-FTIR, and calorimetric methods[1-8] are extended over the casein.

The casein is the main protein in the cow milk (94%)[9] being thoroughly studied due to its numerous uses in cosmetic,[10,11] food[12,13] and drug[14,15] industries and also to the possibility of its use as a study material for elucidating some main aspects of the protein chemistry.[9]

The four casein types are essentially different in their molecular weights, as follows:

- αs_1 - casein: MW = 23.000 (~ 38.49%)
- αs_2 - casein: MW = 25.000 (~ 10.06%)
- β - casein: MW = 24.000 (~ 38.74%)
- k - casein: MW = 19.000 (~ 12.57%)

and they are to be found in the αs_1: αs_2 : β : k ratio of 4:1:4:1.[9,10] Their poor solubility is minimal at pH values between 4.6-4.8 referred to as the isoelectric point (pI).[10,16]

The casein chemical structure is that of a block bio-copolymer[9,10] sparingly soluble in water and very soluble when a base is added[17,9].

The most proteins in casein make up a colloid formation, a biocolloid, referred to as the casein micelle. These formations are in fact aggregates of diameters between 9-150 nm. By means of different methods, the electronic microscopy among them, smaller entities of 10-20 nm diameters named submicelles, have been made evident[16,18] (Figure 1).

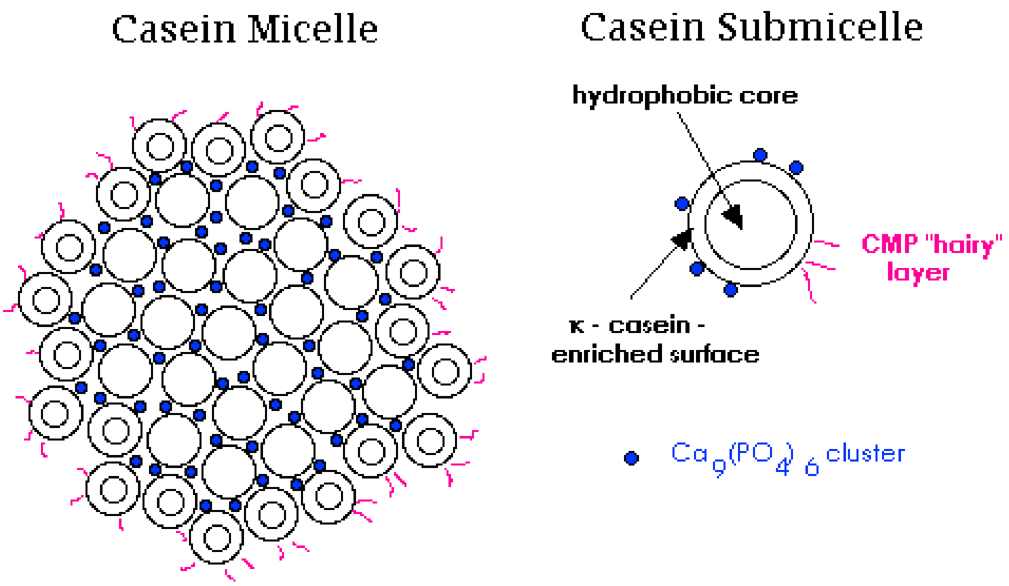


Figure 1. The structure of casein micelle and submicelle.

Unlike the most proteins, showing different structures of the secondary and tertiary conformations, the caseins have flexible external structures characterized as being random. The casein "molecules" do not form crystals, which has been taken as a proof of the absence of a well-defined tridimensional structure and of an unstable secondary structure.[2,9]

The casein is obtained from milk by precipitation in the presence of an acid at pH 4.6. The pure casein is a white tasteless and odorless powder. It can be also coagulated by some enzymes (pepside, rennet).[2,9]

The study on the thermal behavior of casein was performed in air by the TG-FTIR method and calorimetric measurements. The temperature influence on casein was thus followed by the TG-DTG-DTA thermal method under non-isothermal conditions in correlation with the qualitative analysis of the data for the gaseous species (FTIR) released by thermal degradation and also with the calorimetric analysis results (DSC, Berthelot calorimeter)[6].

The TG-DTG-DTA analysis indicated the thermal degradation of casein in air within the 30-900°C range to show two domains in function of temperature (time) where the resulting gaseous species are also grouped: an endothermic one between 30-350 °C and an exothermal one within the 350-900 °C range. The FTIR spectra (absorbance vs.temperature) of the gaseous species resulting by casein degradation revealed the gaseous species H_2O, CO, CH_4, HNCO, CO_2, NH_3 to be released within the endothermic domain and H_2O and CO_2 within the exothermal one.

The gaseous species made evident by the TG-FTIR analysis were identified by means of their specific absorbance.[19]

The calorimetric results obtained by means of DSC and Berthelot calorimeter are in agreement with those given by the TG-FTIR method and bring useful information for developing the thermal degradation mechanism.

The present study is particularly important since the casein is largely used in cosmetic, food and drug industries, and the influence of temperature on its behavior is an essential feature.

Results and discussions

TG-FTIR analysis

The TG-DTG and DTA curves registered for casein degradation in air are drawn in Figure 2.

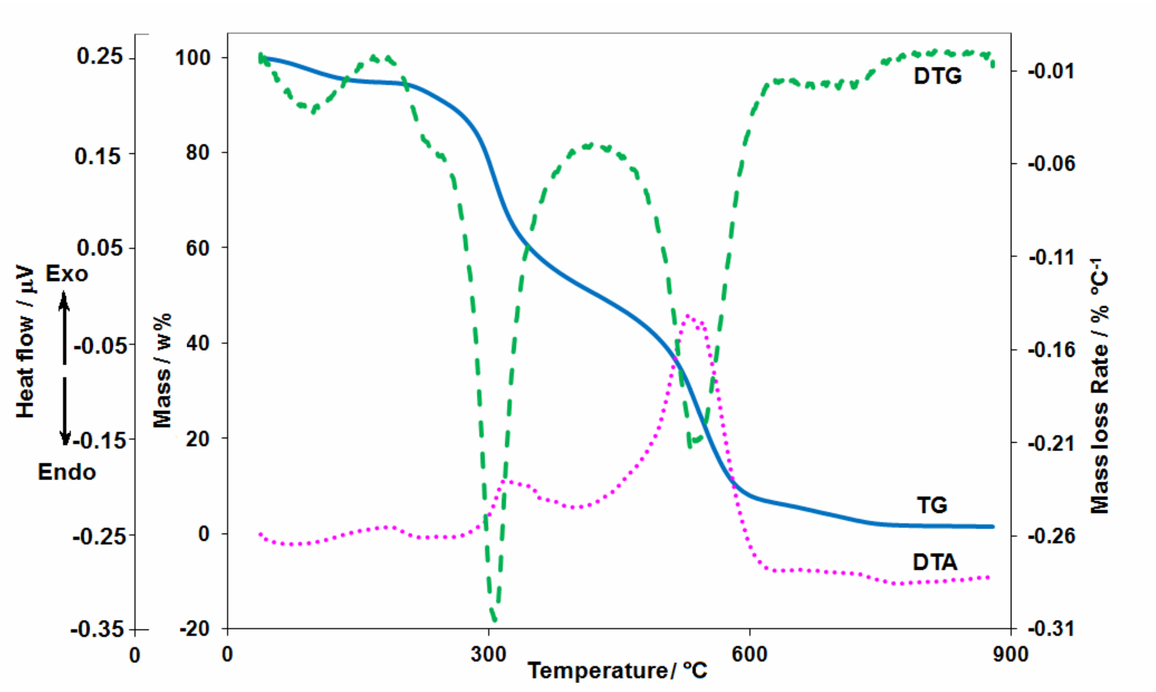


Figure 2. TG, DTG, DTA curves registered on thermal degradation of casein in air.

The shapes of the TG,DTG,DTA curves are indicative of a complex mechanism of casein degradation in air under the recording conditions when two domains are to be found, namely an endothermic one between 30-350°C followed by a strongly exothermal one between 350-900°C[5].

The casein is thermally degraded in air into four well separated stages visible in both TG-DTG and DTA curves.

In Table I the characteristic amounts from TG-DTG and DTA are given, namely the characteristic temperatures, mass losses in every stage and the thermal nature of the degradation stages.

The data in Table 1 indicate the first degradation stage to develop between 41.9°C - 172.4°C. These low temperatures are indicative of the elimination of the physically retained water in casein (4.60%). The thermal stability of casein is expressed by the initial degradation temperature of the second stage, T_i = 172.4°C. It follows that casein is thermally stable between 0-172.4 °C, a temperature range where the casein containing cosmetics, food and drug products are proper for being used.

Table 1. Characteristic amounts from TG-DTG-DTA

Stage	Characteristic temperatures	TG-DTG	DTA	Nature of thermal stage
I	T_i°C T_m°C T_f°C ΔT°C w_∞%	46.3 99.5 172.4 126.1 4.6	42.1 97.2 180.7 138.6 -	Endo
II	T_i°C T_m°C T_f°C ΔT°C w_∞%	172.4 - 237.8 65.4 3.73	180.7 228.9 270.8 90.1 -	Endo
III	T_i°C T_m°C T_f°C ΔT°C w_∞%	237.8 314.0 425.8 188.0 4.18	270.8 314.0 352.9 79.1 -	Exo
IV	T_i°C T_m°C T_f°C ΔT°C w_∞%	425.8 533.3 642.0 216.2 43.99	352.9 533.3 634.2 281.3 -	Exo
V	T_i°C T_m°C T_f°C ΔT°C w_∞%	642.0 700.2 770.6 128.6 5.88	634.2 682.6 782.3 148.1 -	Exo

T_i°C = initial temperature of degradation, T_m°C = temperature corresponding to the maximum of the DTG peak, T_f°C = final thermal degradation temperature; $\Delta T = T_f - T_i$ temperature range of the degradation

The characteristic temperatures of the degradation stages from TG-DTG and DTA are close. By means of the TG-FTIR analysis of the gaseous species resulting by casein thermal degradation in air the absorbance vs. temperature spectrum in Figure 3 was obtained.

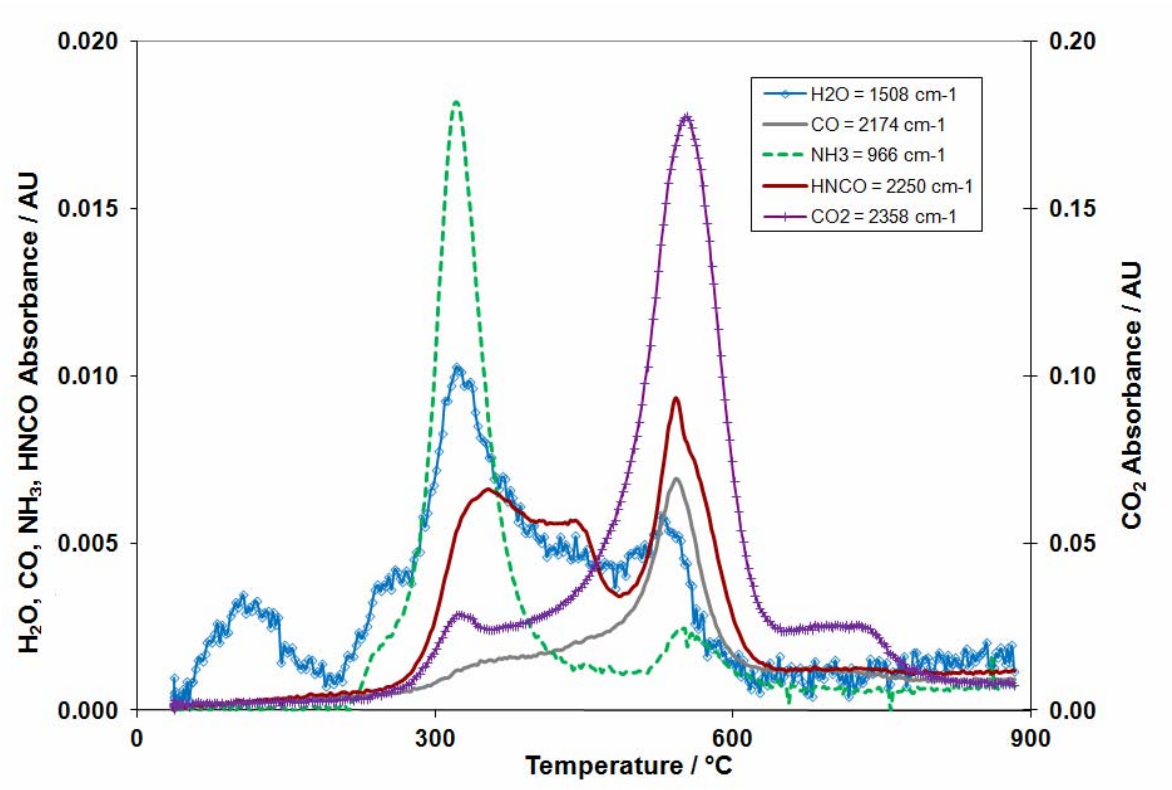


Figure 3. Temperature dependence of the IR absorbances of the identified gaseous species (CO_2, H_2O, NH_3, HNCO, CO) released from casein thermal degradation in air.

Identification of the gaseous species from TG-FTIR analysis was performed by means of the IR standard spectra[19] according to their specific absorbance. As an example, the FTIR spectrum obtained for the casein degradation in air, at 38.6 min (409 °C), is depicted in Figure 4.

As made evident by Figure 3 and 4, the order of gaseous species release is CO_2, H_2O, NH_3, HNCO and CO within the endothermic domain and CO_2, H_2O and traces of CO, NH_3, HNCO within the exothermal one (248-770 °C).

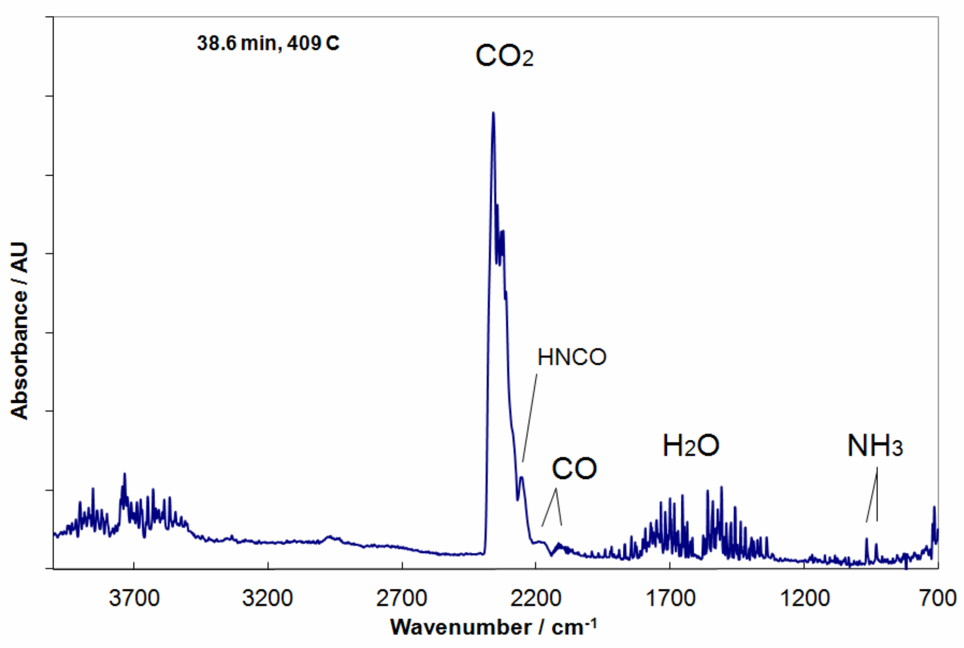


Figure 4. FTIR spectrum for casein thermal degradation at 38.6 min in air.

At the beginning, the CO_2 is noticed to be eliminated simultaneously with NH_3 which would suggest the end bonds of casein [HOOC-R-NH_2] to split firstly and then the internal bonds in the molecule when H_2O, HNCO, CO are formed resulting an intermediate stable at 640°C that suffers subsequently an intense burning to CO_2 and H_2O as main products.

The above conclusions are supported by the 3D spectrum of casein thermal degradation in air depicted in Figure 5.

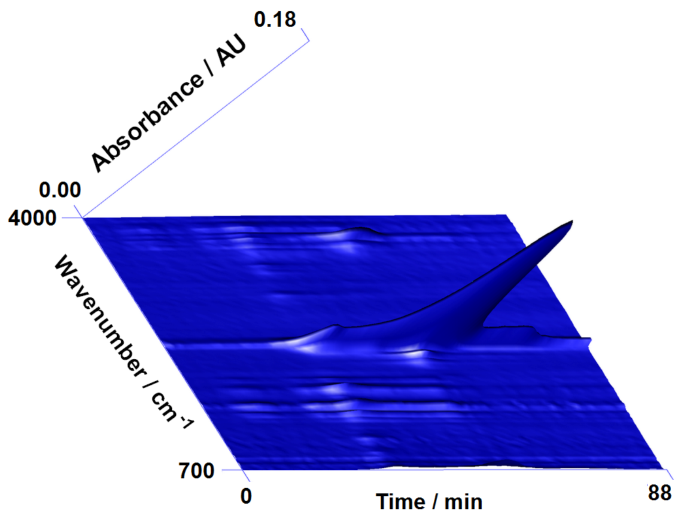


Figure 5. 3D FTIR spectrum obtained for the casein thermal degradation in air.

DSC analysis

The DSC curve of casein is drawn in Figure 6.

The DSC analysis of casein indicates an endothermic process developing within the 25-165 °C temperature range with an enthalpy of 154.34 J/g. Other physical transition are not noticed. The temperature range of the endothermic process is noticed to be close to that of the first stage resulting from TG-DTG (46.3-172.4 °C) and DTA (42.1-180.7 °C). It means that the water retained physically in casein (4.6%) is eliminated during this process since the low enthalpy is characteristic for physical bonds.

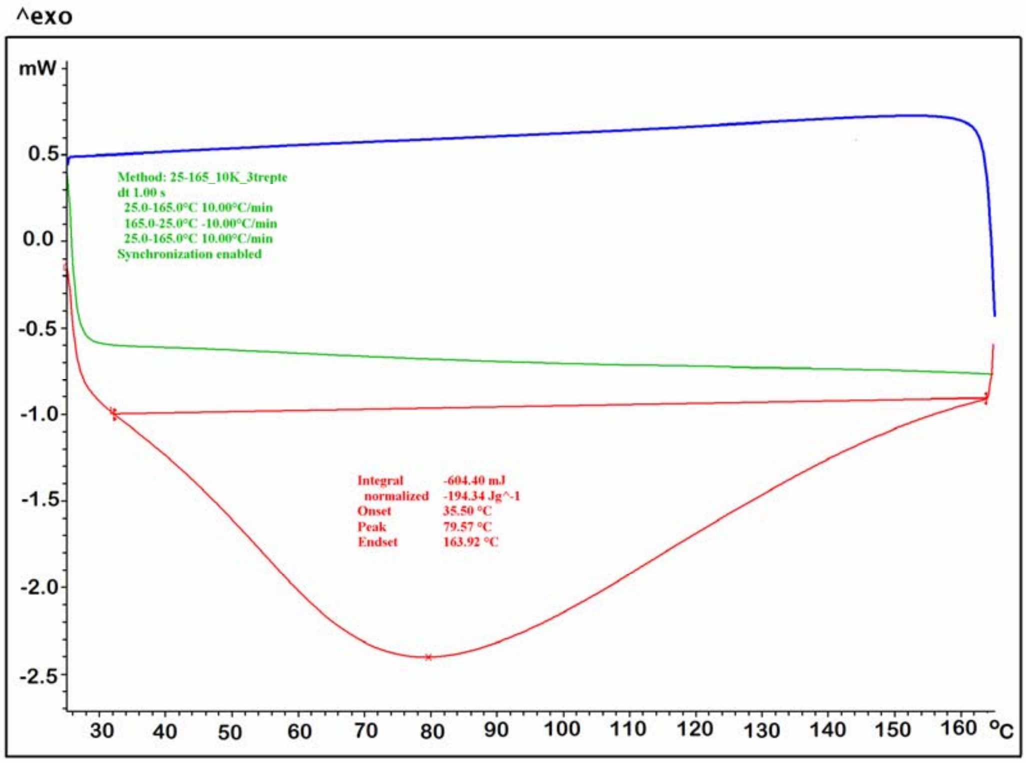


Figure 6. The DSC curve of casein.

The DSC analysis confirms the results obtained from TG-DTG-DTA for the 0-170° C temperature range.

Berthelot calorimeter analysis

The combustion enthalpy (combustion heat) of casein was estimated by the relationship (1).[20,21]

$$\Delta H^0_{c_{298}} = \frac{-c\Delta T - m_{Fe}\Delta H^0_{c(Fe)}}{m_p} \quad (1)$$

where:

c – heat capacity of the calorimeter ($1.04 \cdot 10^4$ J/K)

ΔT – temperature difference accompanying the combustion (°C)

m_{Fe} – mass of the burned Fe thread (g)

$\Delta H^0_{c(Fe)}$ – iron standard enthalpy of combustion ($-6.658\cdot10^3$ J/g)

m_p – mass of the sample submitted to combustion (g)

$\Delta H^0_{c_{298}}$ – standard enthalpy of combustion of the sample submitted to degradation

The experimental data for the combustion heat estimation by means of the relationship (1) are given in Table 2.

Table 2. The experimental data for the combustion heat estimation by means of the relationship (1).

Sample	**m_p(g)**	**m_{Fe}(g)**	**ΔT(°C)**	**$\Delta H^0_{c_{298}}$ (J/g)**	**$\overline{\Delta H}^0_{c_{298}}$ (J/g)**
Casein	0.4993 0.4950	0.0188 0.0179	1.09 1.06	20190.066 22270.460	21230.263

The data in Table 3 present comparatively the composition and combustion heat of some common solid fuels and of the casein.

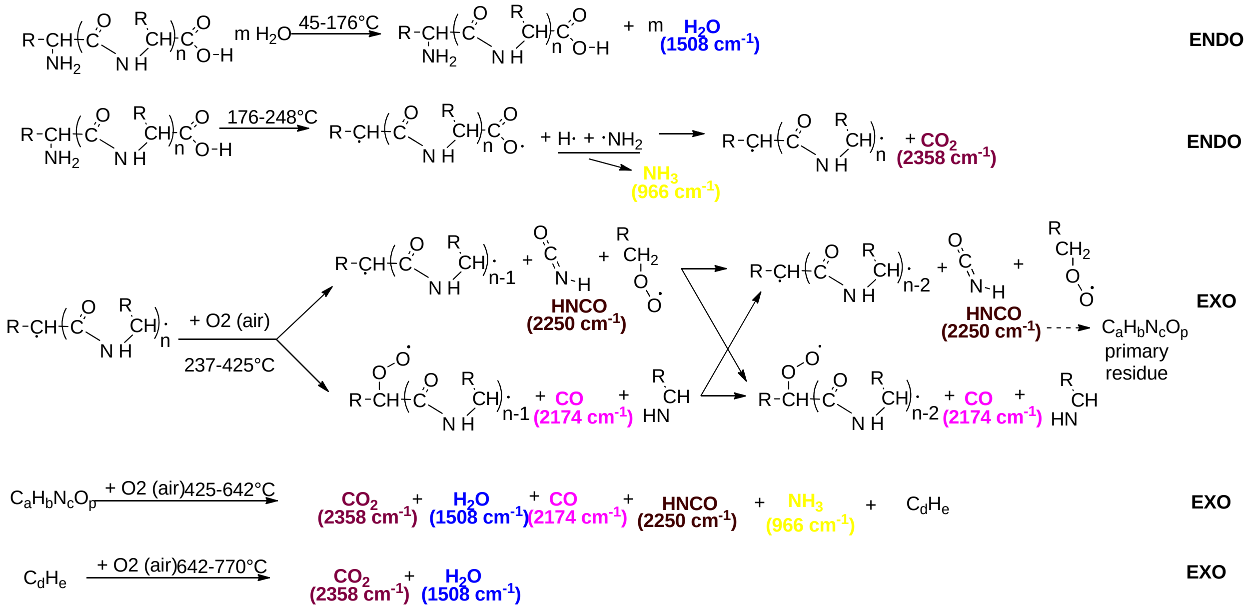
Table 3. The composition and combustion heat of some common solid fuels and of the casein.

Fuels	**C%**	**H%**	**O%**	**N%**	**S%**	**Combustion heat (J/g)**
Wood Peat coal	48-52 49-60	5,8-6,2 5-8	43-45 28-45	0,05-1 1-4	- 0,1-1	18800-20100 21000-24000
Casein	47.82	6.92	-	13.86	-	21230.263

The study by means of the Berthelot calorimeter confirms the findings of the TG-DTG-DTA analysis that the thermal degradation of casein in air is complete with no residue remaining after combustion.

The casein combustion heat of 21230 J/g lies within the variation domain of the combustion heat of the known solid fuels having the carbon and hydrogen contents close to those of casein.[22] This result recommend the use of the products of a high casein content as a source of caloric energy if the gases released from burning are prevented from reaching the environment.

Based on the TG-FTIR analysis and calorimetric data the possible mechanism of casein degradation in air was proposed as described in Scheme 1.



Scheme 1

Experimental

Materials

The casein under study, 99% purity (C- 47.82%; N_2- 13.86%, H_2- 6.96 %) and bovine nature, was supplied by Lancaster company (England).

TG-FTIR technique

The TG-FTIR analyzer consists of a TG/DTA Diamond (Perkin Elmer) thermo-balance and a FTIR spectrometer, Spectrum 100 (Perkin Elmer), provided with a TG-FTIR (Perkin Elmer) gas transfer accessory with a gas cell of 100 mm length and KBr windows, heated at 150 °C. The FTIR spectra were recorded within the 700-4000 cm^{-1} range at a resolution of 4 cm^{-1} and scanning rate of 200 $cm^{-1}s^{-1}$, a single spectrum being recorded every 15 seconds by means of the Spectrum Time Bose Perkin Elmer program. A G7 gas analyzer (Dominic Hunter) supplies the dry air (pearl point: -50 °C) entering the TG/DTA analyzer at a flow rate of 100 mL min^{-1} as well as the nitrogen for purging the analysis room of the FTIR spectrophotometer. The analysis was run with 10 mg sample placed into a platinum crucible, at a heating rate of 10K min^{-1} within the 30-900 °C temperature range.

The qualitative identification of the gaseous species resulting by thermal degradation was made with the IR standard spectra.[19]

Differential scanning calorimeter (DSC)

The differential scanning calorimeter DSC Metler Toledo allows a high accuracy estimation of the heat amount either released or absorbed in a physical-chemical process. The DSC method is applied to the characterization of the materials, qualitative control, identification of substances or substance mixtures, stability investigations, evaluation of the phase diagrams, purity estimations, kinetic studies, etc. The apparatus operates within the 100-550 °C range with an accuracy of temperature adjustment of + 0.02 °C at a heating rate from 0.02 to 300 °C min^{-1} and a cooling rate from 0.02 to 50 °C min^{-1}. The cooling from 100 to 0 °C is made by the InterCooler system within 5 min. The apparatus also allows the utilization of the temperature modulated DSC technique TOPEM. This temperature-modulated DSC (TMDSC) method allows both temperature-dependent and time-dependent processes to be separated.

Berthelot calorimeter (calorimetric bomb)

The combustion enthalpy (combustion heat) of casein was estimated by means of a Berthelot calorimeter of the M-K type (Germany). The variation of temperature for casein burning (combustion) was measured by a Beckman thermometer (differential thermometer) with an accuracy of 0.001 °C. The calorimeter calibration was made by knowing the combustion heat of benzoic acid ($\Delta H^0_{c_{C6H5COOH}}$ = -2.642·10^4 J/g). The heat capacity of the calorimeter is c = 1.04 10^4 J/K.

Conclusions

The TG-DTG-DTA analysis of casein thermal degradation in air is indicative of a complex degradation mechanism.

Two degradation domains are noticed, namely an endothermic one (two stages) and the other one – a strongly exothermal (three stages).

The casein is thermally stable between 0-172 °C.

The thermal degradation of casein in air is complete.

The TG-FTIR analysis allows the conclusion that the gaseous species released by degradation are grouped into the same domains resulting from TG-DTG-DTA analysis.

The gaseous species eliminated in the endothermic domain are CO_2,

H_2O, NH_3, HNCO and CO while in the exothermal one the CO_2 and H_2O prevail, as also supported by the 3D-FTIR spectrum.

The combustion heat of casein, of 21230 J/g, is close to those of the most common solid fuels which would suggest the using of the casein-containing products as fuels.

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