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CALORIMETRY OF THE REACTIONS OF HYDROLYSATES OF CHROMED SHAVINGS WITH ALDEHYDES

F. Langmaier, M. Mládek, K. Kolomaznik, J. Šívarová and S. Sukop

Institute of Leather and Protein Technology, Faculty of Technology, Thomas Bata University, 762 72 Zlin, Czech Republic

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Abstract

Hydrolysates from chromed leather waste obtained in powdered form on an industrial scale by using biotechnical methods were analysed by TG an DSC techniques. Besides about 9% (mass/mass) of moisture, around 1% (mass/mass) of cyclohexylamine was found in the pulverized hydrolysates. Calorimetric measurement of the reaction heats of the reactions of the hydrolysates with commercially available aldehydes indicates that their reactivity decreases in the sequence

glutardialdehyde>>methylglyoxal≈acetaldehyde>>glyoxal>formaldehyde.

Keywords: calorimetry, chromed leather waste, collagen hydrolysate

Introduction

A major proportion of collagenous proteins, which are essentially by-products of the food-processing industry, are processed by the tanning industry, however, it also produces a considerable volume of proteinaceous waste. Various sources indicate that leathers constitute only 40 to 50 per cent of the original volume of raw hides [1, 3], the remainder being largely proteinaceous waste. The most problematic type of collagenous waste is chromed shavings, the amount of which (depending on the type of leathers produced) is approximately 21 per cent of the mass of the original raw hides. While non-tanned shavings are commonly used for the production of gelatine and glues, no industrial use has so far been found for collagenous chromed shavings and they are commonly disposed of by burning or dumping.

Both methods of disposal are objectionable, especially because of the easy transition of the inherent Cr^{3+} compound to Cr^{6+} compounds, which are generally considered to be highly toxic or cancerogenic. Following burning, Cr^{6+} compounds are present in the ash, and their isolation is very costly; after dumping, acid rain gradually washes out Cr^{3+} compounds, which then penetrate the ground-water. In the course of the conversion of ground-water into drinking-water, the oxidation procedures commonly used turn Cr^{3+} compounds into toxic Cr^{6+} compounds.

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Recently developed biotechnological techniques [4] provide a method of separation of Cr^{3+} compounds that is interesting from both economic and environmental aspects, and this method has been successfully applied in practice. The resulting water-soluble proteinic hydrolysates, which are practically free of Cr^{3+} compounds, can be used directly as agrotechnical fertilizers, or as feed additives for livestock. Some authors suggest the possibility of their use in the production of adhesives [5], or in the production of porous ceramics [6].

The properties of the enzymatic proteinic hydrolysates produced in this way are standardized [7]; their use as a secondary industrial raw material is now undergoing development. Their utilization in a number of reactions of industrial significance is being considered, the most likely possibility being reactions with various aldehydes.

Experimental

Original materials

A hydrolysate of chromed shavings was obtained by means of a standard technology [4, 5], in the form of a powder. Its basic analytical characteristics are summarized in Table 1.

Table 1 Characteristics of the enzymatic hydrolysate of chromed shavings

Dry matter content/%	9.04
Content of amidic nitrogen in dry matter/%	15.65
Ash in dry matter/%	3.09
Cr^{3+} in dry matter/ppm	13.60
Content of primary $-NH_2$ in dry matter/mmol	0.24
Mean molecular mass (arithmetic mean/kDa)	19.8

The pulverized hydrolysate was studied by TG and DSC techniques with the DuPont 990 (DuPont, Wilmington, USA) modular equipment for thermal analysis. Thermogravimetric analysis confirmed the content of moisture in the hydrolysate, determined by a standard method, and suggested the presence of another volatile substance, evolved at temperatures ranging from 131 to 147°C. The thermal degradation of the pulverized hydrolysate itself does not start until the temperature reaches approximately 168°C. The initial phase of its dissociation is characterized by a linear decrease in mass with rising temperature; this process, which is relatively slow, proceeds in the range 168–250°C. The second, much faster phase of its thermal degradation starts at temperatures above 250–255°C. A typical TG curve of the hydrolysate of chromed shavings is depicted in Fig. 1.

The DSC curves of the pulverized hydrolysate exhibit a distinct endothermic peak, caused by the moisture content, followed by a substantially lower endothermic peak (temperature interval 131–147°C), most probably caused by cyclohexamine

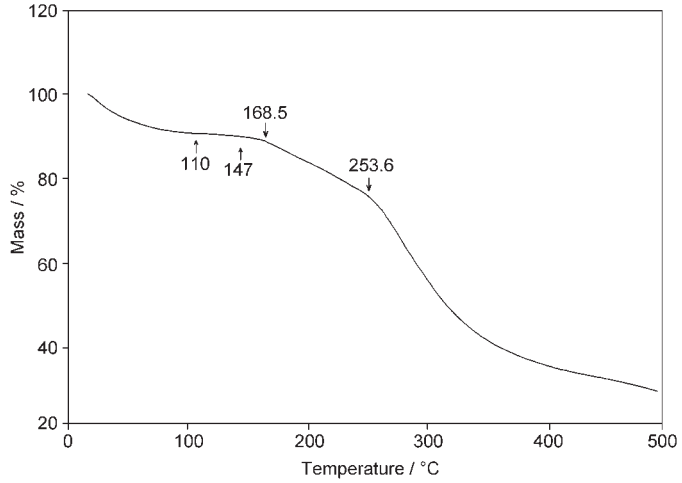

Fig. 1 TG curve of pulverized enzymatic hydrolysate of chromed shavings; Δm at 110°C=9.04%; Δm at 147°C=1.04%

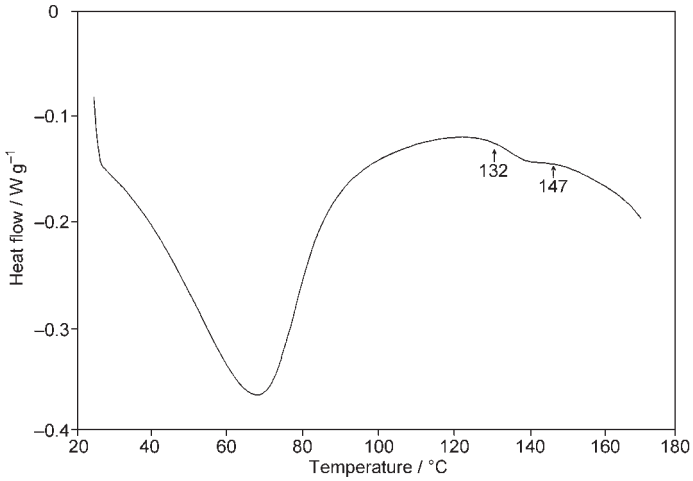

Fig. 2 DSC curve of pulverized enzymatic hydrolysate of chromed shavings

(boiling point 137°C), which is used during the enzymatic hydrolysis of the chromed shavings to create a slightly alkaline environment. The TG curve indicates that the pulverized hydrolysate contains (apart from 9.04% humidity) 1.04 mass per cent of cyclohexylamine. A typical DSC curve of the pulverized hydrolysate is presented in Fig. 2.

Of all the aldehydes whose reactions with the leather hydrolysate were monitored (formaldehyde, acetaldehyde, glyoxal, methylglyoxal and glutardialdehyde), only acetaldehyde was available as a pure substance (purity 99%); all the others were

available only in the form of aqueous solutions. Their concentrations were as follows: formaldehyde 32, glyoxal and methylglyoxal 40, and glutardialdehyde 25%.

Calorimetry

The reactivities of the hydrolysate of the chromed shavings with the tested aldehydes were evaluated by determining the enthalpy of reaction under constant conditions. The measurements were made with a Calvet C 80 calorimeter (Setaram, Lyon, France) with a divided calorimetric cell, the scheme of which is outlined in Fig. 3.

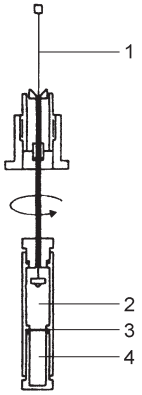

Fig. 3 Scheme of divided calorimetric 1234 cell of the Setaram Calvet C80 calorimeter. 1 – stirrer, capable of cutting the aluminium dividing partition after the upper part of the calorimeter cell has been tempered; 2 – upper section of the calorimetric cell; 3 – aluminium partition, dividing the two sections; 4 – lower section of the calorimetric cell

The lower section of the measurement cell contained 2 mL of a 10% aqueous solution of the hydrolysate of the chromed shavings (with a total dry matter content of 200 mg), while the upper section, divided by an aluminium foil, contained 5 mmol of the tested aldehyde. After the system had been heated to 40°C, a hole was cut in the dividing aluminium foil and a comparative measurement of the heat flow was made with a reference cell containing a corresponding amount of deionized water. Both the calorimeter and the overall measuring process were controlled with a computer providing graphical and numerical output of the experimental data (the time needed for the heating of the calorimeter, the beginning of the measurement, optional signal amplification, the measurement end-time, etc.). The sensitivity of the measuring system was 10 μW.

In calorimetric measurements conducted in this way, a certain role is played by the heat of dilution (dissolution) of the aldehyde. With the possible exception of formaldehyde [8, 9], the data provided in the literature cannot be relied on and are even difficult to find in some cases. We therefore chose to measure the energy effects in an analogous experimental set-up, in which the hydrolysate solution in the lower section was replaced by a standard volume of deionized water.

The heat effects caused by the dilution of 5 mmol of the tested aldehydes (apart from acetaldehyde, available in the form of aqueous solutions with different concentrations) with 2 mL of deionized water are listed in Table 2.

Table 2 Energy effects connected with the dilution of 5 mmol of tested aldehydes to a solution with a concentration of 2.5 (≈5 mmol/2 mL)

Aldehyde	Energy effect/J g^{-1}
Formaldehyde (32% aqueous solution)	–0.96
Acetaldehyde (99%)	17.4
Glyoxal (40% aqueous solution)	4.7
Methylglyoxal (40% aqueous solution)	–2.3
Glutardialdehyde (25% aqueous solution)	8.4

The reaction enthalpies for the reactions of 2 mL of a 10% solution of the hydrolysate of the chromed shavings with 5 mmol of the tested aldehydes, corrected for the enthalpy of dilution of the aldehydes under the given measuring conditions, are provided in Table 3.

Table 3 Thermal colouration of the reactions of hydrolysate of leather waste with tested aldehydes under standardized conditions*

Aldehyde	Energy colouring of reaction/J g^{-1}
Formaldehyde	1.9
Acetaldehyde	18.5
Glyoxal	2.43
Methylglyoxal	18.56
Glutardialdehyde	76.4

*Conditions: 2 mL of 10% solution of hydrolysate with a dry matter content of 200 mg; solution pH not adjusted 5 mmol of aldheyde, reaction temperature 40°C

Results and discussion

Apart from an endothermic peak caused by the inherent moisture content in the compound, the DSC curves of the pulverized hydrolysate of the chromed shavings display a distinct, but substantially lower endothermic peak, situated between approximately 131–147°C, which is almost definitely caused by the cyclohexylamine (boiling point 137°C) used during the enzymatic hydrolysis of the chromed shavings in order to create a slightly alkaline environment. A comparison of the DSC and TG curves of the pulverized hydrolysate clearly reveals that, besides 9.04 of moisture, the pulverized hydrolysate also contains 1.04% of cyclohexylamine. The results of routine analysis (Table 1) demonstrate that the content of Cr^{3+} in the hydrolysate solid matter does not exceed 13.6 ppm, with an inherent ash (neutral salts) content of

3.09%. The hydrolysate is therefore pure enough to be used in industrial applications. The analysed hydrolysate was obtained on an industrial scale and its parameters were not substantially different from those of the preceding and following batches.

The hydrolysate of the chromed shavings most probably reacts with aldehydes in the same way as simple nitrogen substances do, via its amino and imino groups, creating a complicated system of consecutive and competitive reactions; these have been studied in detail with urea. In addition to other factors, the course of such reactions is influenced by the pH of the reaction environment. To simplify the problem, the reactions of the leather hydrolysate with aldehydes were monitored here at the natural pH of its 10% solution (pH 6.0; not treated in any way), at a double molar excess of aldehyde (relative to the amidic hydrolysate nitrogen). For practical reasons (the time necessary for the tempering of the calorimeter, etc.), the reaction temperature was set at 40°C. When constant conditions of such reactions are maintained, their energy effects (reaction enthalpies) can be considered to reflect the reactivities of the tested aldehydes with the hydrolysate. The experimental results (corrected for the dilution enthalpies of the tested aldehydes, Table 3) allow the tested aldehydes to be arranged in sequence according to their increasing reactivity with the chromed shavings hydrolysate:

formaldehyde<glyoxal<<acetaldehyde≈methylglyoxal<<glutardialdehyde.

When the bifunctionality of some of the tested aldehydes (glyoxal and glutardialdehyde), is taken into account, the reactivity can be expressed numerically as follows:

formaldehyde:glyoxal:acetaldehyde:methylglyoxal:glutardialdehyde=

1.9:1.2:18.5:18.56:38.2.

The validity of such findings is limited to a certain degree by the selected reaction conditions (absence of acid-base catalysis, reaction temperature, etc.). It is known that a pH around the neutral point (pH 6.5–7.5) slows down both the addition and condensation phases of the reactions between formaldehyde and urea amino groups [10]. It cannot be ruled out, therefore, that a change in pH might to some extent influence the measured reactivities. On the other hand, the measured reactivities of these aldehydes with the chromed shavings hydrolysate correspond well with the reactivities of a series of aldehydes with collagenous fibres in an environment with pH 4.0 or 7.0, as described by Nimmi *et al*. [11].

The special ability of glutardialdehyde to react with polypeptide materials is confirmed by the findings [11] of an increased resistance of the cross-links created in the collagenous material by glutardialdehyde to biodeterioration and the hydrolytic activity of chemicals, and of the minimum immunity reactions of living organisms to collagen cross-linked with glutardialdehyde, as compared with the analogous cross-linking with other aldehydes. Further, glutardialdehyde is a highly valued tanning substance with specific features: high water sorption, high shrinkage temperature, etc. This all means that great attention should be paid to the reaction of

glutardialdehyde with the hydrolysate of chromed shavings when the possible industrial applications of hydrolysate are considered.

As far as the reaction mechanism of glutardialdehyde with polypeptidic substances is concerned, most authors tend to think that glutardialdehyde (similarly to other aldehydes) primarily reacts with their amino or imino groups [12, 13]. In the case of non-denaturated collagen, significant importance attached to the ε-amino groups of the lysine residues [14].

Studies involving the use of model amino compounds (6-aminohexanoic acid or glycine) [15–17] have revealed the significant complexity of such reactions. With simple model substances, the reactions of glutardialdehyde with amino groups lead to intermediates with appreciable spectral absorption in the range of 300 nm and a molecular mass of around 200 Da. With excess glutardialdehyde, such primary products undergo relatively fast transition to compounds which absorb strongly at around 265 nm and have significantly higher molecular masses. The final products are substances which absorb significantly at around 325 nm, with molecular masses similar to those of the preceding intermediates. The resulting structures range from Schiff's bases, via the α,β-unsaturated structures derived from them, to products of dihydropyridine and polymer types [18]. The reactions of glutardialdehyde with collagenous hydrolysates are therefore expected to be rather complicated. The techniques of differential scanning calorimetry will chiefly be used for further kinetic studies of these reactions.

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