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FORMATION OF CRYSTALLINE COMPLEXES OF AMYLOSE WITH SOME SELECTED FLAVOUR COMPOUNDS

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Crystalline precipitates of pea amylose with selected food flavour compounds: hexanal, *trans*-2-nonenal and *trans*-2, 4-decadienal, were obtained through a controlled method of heating at 80°C and slowly cooling. Amylose from pea starch was prepared by precipitation with 1-butanol after aqueous leaching at 70°C. The amylose-flavour precipitates were examined by light microscopy, differential scanning calorimetry and X-ray diffraction. The crystallinity of the complexes followed the order: amylose-hexanal > amylose-*trans*-2-nonenal > amylose-*trans*-2,4-decadienal, demonstrated by: the dimension of the crystals, the melting temperatures and enthalpies, the Bragg angles (2θ) and distances *d* (nm). The crystalline structure of the amylose-hexanal complex, determined by X-ray diffraction, was assigned to be V_{6I} type. The amylose- *trans*-2-nonenal showed intermediate characteristics with a tendency to the V_{6II} form and the conformation of the *trans-trans*-2,4-decadienal chain seems to preclude full inclusion.

INTRODUCTION

Amylose, the linear constituent of starch, which consists of α (1→4)-linked glucose monomers, is unstable in aqueous solutions at room temperature and readily forms poorly crystalline precipitates and gels. This process is known as retrogradation. Depending on the chain length, concentration and temperature, gels, spherulites or lamellar crystals can be formed with A or B allomorphic type. Other ligand--dependent allomorphs (the various V-types) are obtained when amylose is complexed with molecules such as alcohols, lipids or flavours [Buléon *et al*., 2007]. The presence of other solutes including the polyiodide ion, linear alcohols [Whittam *et al.*, 1989; Helbert & Chanzy, 1994; Rondeau-Mouro *et al*., 2004], fatty acids [Tufvesson *et al.,* 2003a,b; Godet *et al*., 1995] and other small organic species, including aroma molecules [Jouquand *et al*., 2006; Kawada & Marchessault, 2004; Nuessli *et al*., 2003], can induce the formation of single helical conformations of amylose which can form different crystalline arrays, known as the V type. The interaction of amylose from native potato starch with decanal, carvone, geraniol, campher, thymol, 1-naphtol, menthone, limonene and monostearate was studied [Rutschmann & Solms, 1990a,b; Nuessli *et al.,* 2003] through X-ray diffraction. The binding of d-limonene, ethyl hexanoate, octanal and 1-hexanol with different types of starches (standard corn, waxy corn, high amylose corn starch, pregelatinised standard corn starch, pregelatinised waxy corn starch, acetylated standard corn starch and maltodextrin) and their retention were studied by inverse gas chromatography [Boutboul *et al.,* 2002]. The interaction between potato amylose (1%w/w) and three aroma compounds: isoamylacetate, ethylhexanoate and linalool (20%v/v), was studied through DSC and X-ray diffraction [Arvisenet *et al.,* 2002]. The complexes of amylose with lactones [Heinemann *et al.,* 2003] were obtained from pregelatinised potato amylopectin dispersions and flavour compounds, followed by the stabilisation of complexes at 25°C. Tapioca starch inclusion complexes with primary and secondary alcohols having various lengths as well as ketone compounds were confirmed by DSC and the formation of complexes was confirmed by X-ray diffraction [Itthisoponkul *et al*., 2007].

The formation of highly crystalline forms of amylose is generally difficult. Crystallization is generally initiated by cooling below the melting temperature of the crystalline form. Complex formation in solution precedes subsequent crystallization. Through rapid quenching it is possible to prepare amorphous precipitates, as judged by X-ray diffraction of amylose/linear alcohol complexes, while with a slower cooling it is possible to prepare single crystals [Biliaderis & Galloway, 1989; Whittam *et al*., 1990]. For water-soluble linear alcohols such as 1-butanol, the formation of complexed precipitates and crystalline materials on cooling amylose solutions is preferred over the retrogradation process.

The formation of crystalline complexes of amylose with linear alcohols is relatively easy through the saturation of the amylose solution with pure alcohol and a controlled cooling. In the case of flavour compounds, with different chemical structures, different polarities, a special orientation of the molecules which influence the entrapment of the flavour compounds inside the amylose helix, the experiments for complexation are not easy.

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The flavour retention in starch from food matrices is a mechanism frequently induced during processing and the knowledge of interactions between aroma and starch is essential to optimize the food processing [Le Bail *et al*., 2005; Condet-Petit *et al.,* 2006].

The aim of the following experiments was to analyse the complexation phenomena between amylose and selected flavour compounds: hexanal, *trans*-2-nonenal and *trans*-*trans*-2,4-decadienal, considering the conditions of complexation as: the ratio flavour:amylose (the quantity of organic species-flavour compounds added to amylose), the complexation temperature and the cooling procedure. The differential scanning calorimetry (DSC) was used to determine the thermal properties and the parameters of the transformation processes of the ordered structure and X-ray diffraction was used to determine the diffraction form of the crystals.

MATERIALS AND METHODS

Materials

Flavour compounds, hexanal, *trans*-2-nonenal and *trans*-*trans*-2,4-decadienal were obtained from Sigma-Aldrich UK. Amylose from pea starch was prepared by precipitation with 1-butanol after aqueous leaching at 70°C. Amylose solutions were regenerated by heating to 95°C followed by removal of the 1-butanol in a heated nitrogen stream [Ring *et al*., 1985; Kim & Whillett, 2004].

Characterization of amylose

The estimation of the molecular weight of pure amylose was done by intrinsic viscosity method, measured using a glass capillary tube. The intrinsic viscosity of a dilute polymer solution can be correlated with the molecular weight of polymer with the empirical formula: $[\eta] = K * M_w^a$ (Mark-Houwink relation), where: K and a are constants which depend on the solvent, solvate and temperature. K value is 0.113 [Ring, 1983]. The purified amylose had an intrinsic viscosity of 94 mL/g, in dilute aqueous solution at 20°C, indicating a M_w of ~700,000 g/mol [Miles *et al*., 1985]. The dry weight of 0.5% w/w was analysed by vacuum drying method (60°C, 12 h, under P_2O_5). Pea amylose was free of lipids and amylopectine. The microscopic examination of amylose demonstrated its purity.

Preparation of complexes

Preliminary experiments were carried out to probe the conditions for obtaining amylose-flavour crystalline complexes: the ratio amylose:flavours, the proper temperature for complexation and the cooling rate. The established amylose:flavour compounds ratios for preliminary experiments were: 1:0.025; 1:0.050; and 1:0.5 (v/v). These ratios were chosen on the base of literature data which mention the importance of saturation of the amylose solutions in order to obtain a complexation. Also, data are available in the literature regarding the maximal quest molecule content of 5.7-10.4% for high amylose maize starch [Wulff *et al.,* 2005]. For 0.5% w/w amylose solutions and amylose:flavour ratios 1:0.025; 1:0.050; 1:0.5 (v/v), isothermal crystallization (5 h) at temperatures in the range of 20–60°C, followed by quench cooling to 20°C gave materials with weak endothermic transitions with a midpoint of ~76°C. Crystallization at 70-80°C gave a further weak transition with a midpoint at 85-90°C. The same amylose:flavour ratios 1:0.025; 1:0.050; 1:0.5 (v/v) were used, maintained to 80°C and cooled to room temperature without a special control of the cooling rate. The DSC curves were very large and broad and only small peaks could be visible. When a proper cooling rate was applied, 5°C/h, an improvement in the DSC curves was observed: more visible peaks and higher melting temperatures. However, the higher amount for the flavour compounds did not give better results. Further experiments were done with the smallest tested amount of flavour compounds.

The pure flavour compounds (275 μL from each sample: hexanal, *trans*-2-nonenal, *trans-trans*-2,4-decadienal) were heated at the complexation temperature in sealed glass tubes. Pure hot amylose solutions (10 mL) freshly prepared (0.5% w/w) were added to the flavour compounds in the tubes, flushed with nitrogen, and sealed into a screw-capped test tube. The tubes were placed in a programmable water bath and the temperature monitored with a quartz crystal thermometer (HP2804A) and the effect of thermal history on the complexation/precipitation examined. After cooling, the complexes precipitate and the precipitates were collected through centrifugation at 12,500 rpm for 15 min using a Sigma Howe 3-10 bench centrifuge.

Light microscopy

One drop of each precipitate was transferred onto the microscopic glass and covered with the lamella and immediately examined by light and polarized light microscopy using an Olympus BHS/BHT polarizing microscope with a 40x objective.

The photomicrographs were registered using a Nikon Coolpix 4500 digital camera. The dimension of the crystals was estimated comparing the image of the crystals with the distances between the lines of a graded standardized lamella.

Differential scanning calorimetry

Amylose precipitates were analysed by differential scanning calorimetry using a Perkin-Elmer DSC7 calorimeter at a scanning rate of 10°C/min. Aqueous precipitates (~20 mg) were sealed in 50 μL aluminium pans. An empty pan was used as a reference. The pans were equilibrated for 1 h and then scanned over the temperature range of 17-127 °C. The initial temperature (T_i, °C), the maximum temperature (T_m, °C) and the final temperature (T_f, °C) of the thermal transitions, and the dissociation enthalpy ΔH of the complexes was calculated using Perkin-Elmer software. The DSC was calibrated from the melting transitions of indium and octadecane. The samples were rescanned immediately after scanning without moving them from the carousel of the DSC equipment.

X-ray diffraction

Moist precipitates, obtained after centrifugation, were removed from the plastic centrifugation tubes and spread on a sample cell of the diffractometer as a uniform layer

and examined at a wavelength of λ=0.154 nm, and an intensity ratio of 0.5, over the angular range (2θ) from 5° to 30°, at a temperature of 25°C as described by Moates *et al.* [1997]. The instrument used was PW1710, with anodic tube CuKα1. The values obtained with the Philips PC-APD software were transferred into Excel and calculated. The distance *d* between the crystals layers was calculated in nm, using the Bragg relation: $d = \lambda / (2x\sin\theta)$. The diffraction model obtained was compared with the models already established for starches and crystalline complexes (A, B, C and V models). The Bragg angles (2θ) and the distances d (nm) of the main peaks from the literature were correlated with the diffraction models.

RESULTS AND DISCUSSION

Light microscopy

Under light microscopy, the amylose-hexanal precipitates consisted of lamellar crystals, with a major axis of ~ 6 μm, which were clearly visible when oriented edge on (Figure 1a). The crystals formed radial aggregates, as in the initial stages of spherulitic crystallization and were birefringent when viewed between crossed polars (Figure 1b). The amylose-*trans*-2-nonenal precipitates had a similar crystal habit but with smaller crystals with an estimated major dimension of ~ 4 μm (Figure 1c). The amylose-*trans*-2,4-decadienal precipitates were only weakly birefringent and formed spherical agglomerates

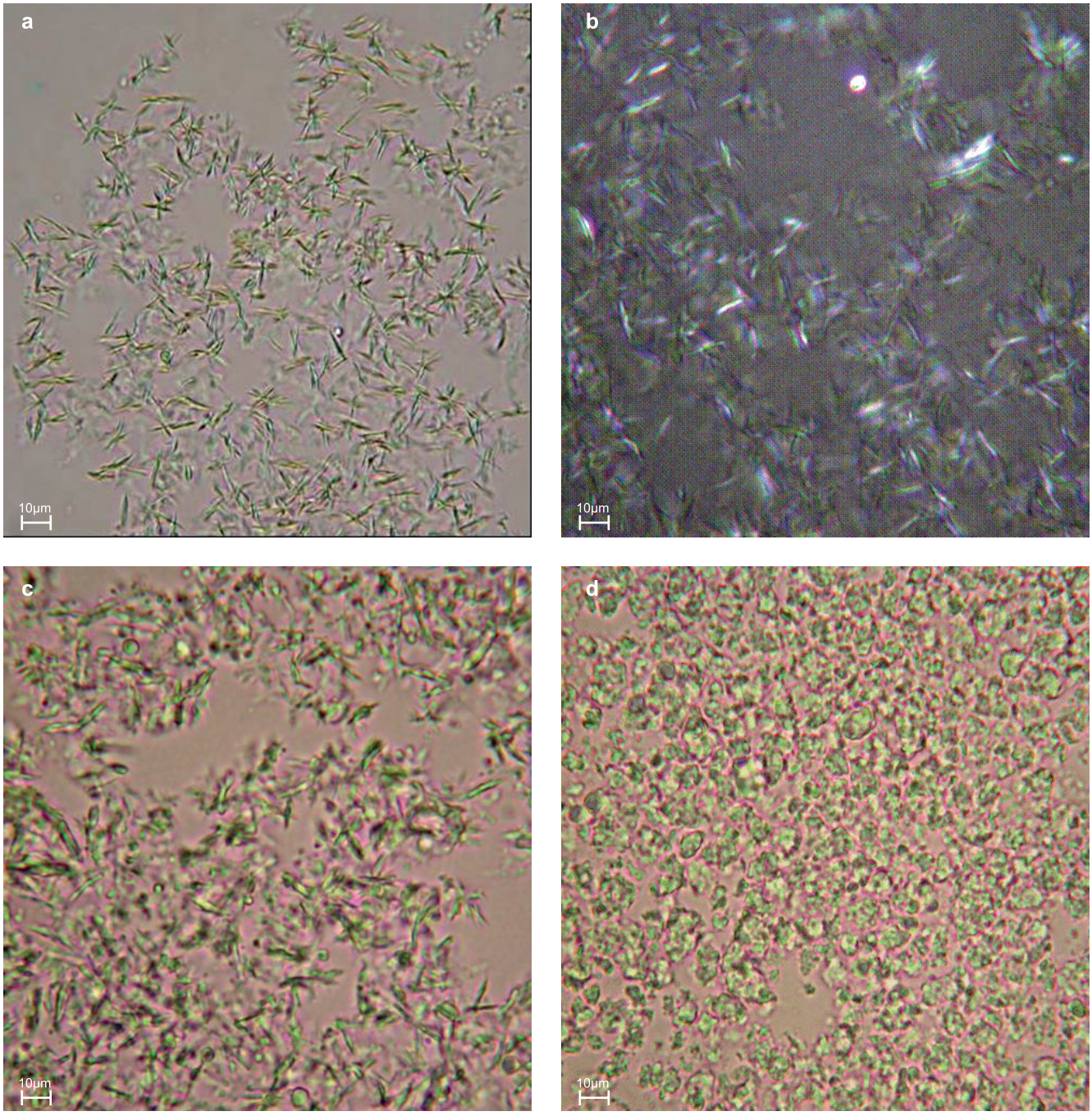

FIGURE 1. Light microscopy of amylose/hexanal (a, b); amylase-*trans*-2-nonenal (c); amylase- *trans-trans*-2,4-decadienal amylose complexes (d).

(Figure 1d). The obtained form of crystals is in accordance with the results obtained by Whittam *et al.* [1989] for alcohol-amylose complexes, where the form of the amylose-hexanol crystals were hexagonal, with a diameter of 12 μm and a perpendicular arrangement of the crystals was obtained. The amylose-butanol crystals had a rectangular shape.

Differential scanning calorimetry

The extent of association of amylose chains was examined by differential scanning calorimetry. All the precipitates gave relatively broad transitions with two main transitions with midpoints in the range of 77.8-79.2°C and 87.4-90°C (Figure 2).

At the scanning of the first complex: amylose-hexanal, more than 2 peaks are visible on the scanning graphic, with values for the melting temperature reaching: Tm_1=77°C for the first peak, Tm_2=88°C for the second peak and Tm_3=96°C for the third peak. The value of enthalpy ΔH was around 30 J/g d.w. During rescan, 2 peaks were obtained, at 69°C and at 82°C. The appearance of 2 peaks with a lower intensity during rescan suggests that the complex could not recover its initial crystalinity, so more time could be necessary for recrystalization of this complex.

Amylose-*trans*-2-nonenal complex had a similar behaviour during the calorimetric scanning as the previous complex. The obtained peaks at scan had the following melting temperatures: Tm_1=78°C, Tm_2=90°C and Tm_3=93°C and the dissociation enthalpy ΔH was 64% lower (ΔH=11 J/g d.w.) than for the amylose-hexanal complex. During rescan, no peak was observed.

The complex amylose-*trans*-2, 4-decadienal had a similar behaviour with the complex amylose-*trans*-2-nonenal with 3 visible peaks during scan (Tm_1=72°C, Tm_2=78°C and Tm_3=84°C) and no peak during rescan. The melting temperature and the dissociation enthalpy ΔH (8 J/g d.w.) were lower that those for the first 2 complexes.

The combined size of the endothermic transitions were in the order amylose-hexanal > amylose-*trans*-2-nonenal > amylose-*trans-trans*-2,4-decadienal. Multiple transitions for amylose-linear alcohol complexes were associated with the presence of both amorphous and crystalline complexes [Biliaderis & Galloway 1989; Whittam *et al.*, 1990]. So, the existence of multiple transitions for amylose-selected flavour compounds indicates the presence of different solid forms of the complex: amorphous and crystalline. Quench cooling of the melted complexes to 20°C followed by rescanning revealed no detectable endothermic transitions for the *trans*-2--nonenal and *trans-trans*-2,4-decadienal complexes and a very weak transition of the amylose-hexanal mixture. These observations confirm the difficulty of obtaining complexed material and the need to control the crystallization conditions.

X-ray diffraction

The crystallization of the complexes was further examined by X-ray diffraction. X-ray diffractograms of intensity as a function of angle of the different complexes are shown in Figure 3. The obtained diffractograms were compared with the literature results regarding the classification of the crystalline amylose complexes into families according to the constitutive helix (V_6 and V_8) and to the space available between helices in the unit cell: V_{6I}, V_{6II} and V_{6III} for V_h(h-hydrated), $V_{butanol}$ and $V_{isopropanol}$, respectively. The amylose-hexanal complex has a well-resolved pattern characteristic of the V_{6II} complex [Le Bail *et al.*, 2005] with reflections at d spacings of 0.745; 0.662; 0.54; 0.50; 0.447; 0.43; 0.42 at an angle (2θ) of 11.9; 13.4; 16.4; 17.9; 19.9; 20.7; 21.1, respectively. The reflection at a d-spacing of 0.70 (12.6) is usually associated with the presence of the V_{6I} complex. The diffraction pattern obtained from the amylose-*trans-trans*--2,4-decadienal complex was less well resolved, with reflections at a d-spacing of 0.70 and 0.447 characteristic of the V_{6I} form with a weaker reflection in the region of 0.42 showing some V_{6II} character [Le Bail *et al.*, 2005]. While linear aliphatic chains may be accommodated within the amylose helix, the conformation of the *trans-trans*-2,4-decadienal chain would seem to preclude full inclusion. The amylose-*trans*-2-nonenal complex showed intermediate characteristics with a tendency to the V_{6II} form. The difference in the forms is a result of differences in the spacing of the 6 fold helices. The diffraction data indicate that part of the complexity observed in the calorimetric experiment may have its origin in the presence of different crystalline forms in the same preparation of amylose complex (V_{6I} and V_{6II}).

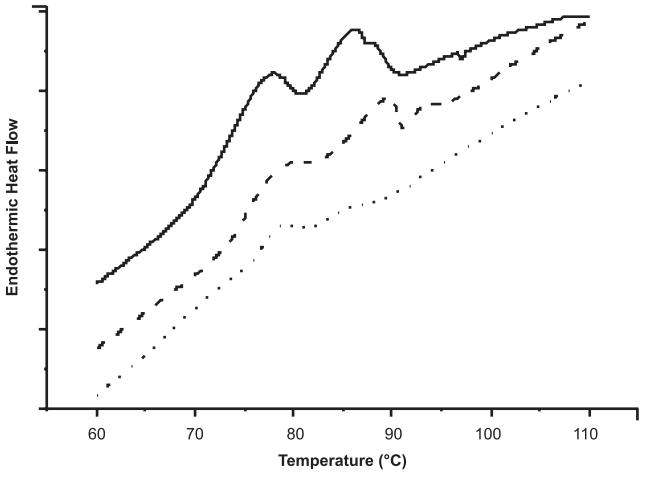

FIGURE 2. Melting of amylose crystallites as observed by differential scanning calorimetry for complexes with hexanal (——); *trans*-2-nonenal (-----); and *trans-trans*-2,4,-decadienal (·····).

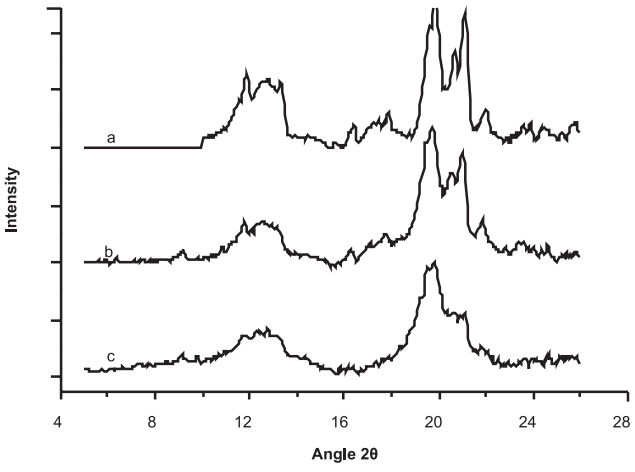

FIGURE 3. X-ray diffractograms of complexes of amylose with hexanal (a); *trans*-2-nonenal (b); and *trans-trans*-2,4-decadienal (c).

CONCLUSIONS

Amylose has the ability to complex or partially complex the flavours: hexanal, *trans*-2-nonenal and *trans-trans*-2,4--decadienal. The extent of complex formation is very sensitive to thermal history. A combination of isothermal crystallization at elevated temperatures followed by slow cooling to room temperature was necessary to obtain sufficient crystallinity of the complexed product for analysis by X-ray diffraction and differential scanning calorimetry.

The crystallinity was firstly demonstrated by the microscopic aspect through the evidence of the birefringence crystals. The presence of multiple peaks in the thermograms can be observed in the thermal scanning of semicrystalline complexes and these peaks represent a melting followed by a recristallisation and the final melting. Also, the multiple peaks can be obtained if the samples contain crystals with different thermal stabilities. The appearance of an endothermic peak on the first scan of the samples shows the presence of a complex (crystalline or amorphous). The strongest crystals obtained were amylose-hexanal crystals. The crystals obtained have distinct melting temperatures, influenced by their dimensions; the small crystals melt at lower temperature. Although the dissociation temperatures of the complexes were similar, the melting enthalpies followed the order: amylose-hexanal > amylose-*trans*-2--nonenal > amylose-*trans-trans*-2, 4-decadienal.

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