



Amadori products formation in emulsified systems



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ARTICLE INFO

Article history:

Received 24 July 2015

Received in revised form 17 November 2015

Accepted 24 November 2015

Available online 2 December 2015

List of compounds:

L-Leucine (PubChem CID: 6106)

L-Phenylalanine (PubChem CID: 6140)

D-Glucose (PubChem CID: 5793)

N-(1-Deoxy-D-fructos-1-yl)-L-leucine

(PubChem CID: 71316981)

N-(1-Deoxy-D-fructos-1-yl)-L-phenylalanine

(PubChem CID: 71316982)

Tricaprylin (PubChem CID:10850)

Tween 20 (PubChem CID: 443314).

Keywords:

Amadori products

Maillard reaction

Amino acids

Emulsions

Partition coefficient

High resolution mass spectrometry

ABSTRACT

The formation of Amadori products (APs) is the key step determining the development of the Maillard reaction (MR). The information on the chemical behaviour of the reaction between amino acids and reducing sugars in emulsions during thermal treatments is scanty and mainly focused on volatile compounds. The aim of this work was to investigate the formation of APs from glucose and two amino acids with different partition coefficients (phenylalanine and leucine) in emulsions. Two submicron oil-in-water (O/W) emulsions consisting of water, tricaprylin and Tween 20 were prepared, thermally treated and the formation of fructose-phenylalanine (Fru-Phe) and fructose-leucine (Fru-Leu) was monitored by mass spectrometry. The concentration of Fru-Phe in submicron emulsions was similar to that in water, while Fru-Leu was reduced up to 47% in the emulsions. These data indicated that partition coefficient of amino acids, determining the reactants location, can substantially influence the MR and the final quality of foods.

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1. Introduction

The Maillard reaction (MR) largely determines the final quality of foods by controlling the development of attributes such as colour, flavour and nutritional values (Nursten, 2005). The complexity of the MR chemical networks leads to the following dichotomy: on the one hand it promotes the formation of desired aroma and colour, on the other hand it causes the formation of potentially toxic molecules which can affect the safety of the foods. Thereby, the MR directly influences two quality pillars of food products: the ability

to satisfy sensory expectations of the consumers and the absence of compounds with potential negative effects on health (van Boekel et al., 2010).

The reaction between reducing sugars and amino groups results in the formation of the Amadori products, the central hub from which a myriad of reactions starts off (Yaylayan & Huyghuesdespointes, 1994). It has been established that the initial amino-carbonyl condensation forms a labile N-substituted D-glycosylamine, which undergoes the Amadori rearrangement to form the respective N-substituted D-fructosamine. Specifically, after the nucleophilic addition, the Schiff base can rearrange via 1,2-eneaminals which lead to an aminoketose, or Amadori rearrangement product (1-amino-1-deoxyketose, AP). If fructose reacts in a similar way with an amine or an amino acid, an aminoaldose (Heyns rearrangement compound, 2-amino-2-deoxyaldose, HP) can be formed (Mossine & Mawhinney, 2010). Since the

Abbreviations: APs, Amadori products; MR, Maillard reaction; O/W, oil-in-water emulsion; HRMS, high resolution mass spectrometry; Leu, leucine; Phe, phenylalanine; Fru-Leu, N-(1-deoxy-D-fructos-1-yl)-L-leucine; Fru-Phe, N-(1-deoxy-D-fructos-1-yl)-L-phenylalanine.

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publication of the pivotal paper by Hodge about the APs role (Hodge, 1953), a huge variety of papers have dealt with the significance of the molecules formed by their degradation (Bastos & Gugliucci, 2015; Hellwig & Henle, 2014; Ren et al., 2015). Up to now, the MR has been mainly studied in aqueous media and less frequently in dry systems (Capuano, Ferrigno, Acampa, Ait-Ameur, & Fogliano, 2008), while the information available regarding the development of MR in complex colloidal systems, such as sub-micron emulsion systems, has been primarily focused on flavour compounds formation (Garti, 2003). In emulsion systems, environments with various properties and polarities are available, promoting the partitioning and segregation of the reactants (in the case of the MR, amino acids and sugars) and reaction products, according to their molecular structures and partition coefficients (Sangster, 1997).

An emulsion consists of two immiscible liquids, with one of the liquids dispersed as small spherical droplets in the other. When oil is the dispersed phase, oil-in-water (O/W) emulsions are formed, which represent a variety of lipid-containing foods, such as milk, dairy products, salad dressings, mayonnaise, sauces, soups, beverages, and cream (McClements, 2004). Emulsions can be classified according to their long term storage, sample history, particle size distribution, particle shape and thermodynamic stability (McClements, 2011, 2012).

Food emulsions may not only be subjected to physical destabilisation, but their major components (i.e., proteins, lipids and polysaccharides) and their inner structures with microscale and nanoscale compartments may also undergo chemical degradation because of thermal treatments, shelf-life and end use conditions (Aguilera, 2014). In an emulsion environment, the conventional relationship between reactants and products should also consider the partition coefficients of the reactants between the available phases. One of the most common examples is how the antioxidants polarity determines their partitioning between oil, water, interfacial regions, and as a consequence their specific reactivity (McClements & Decker, 2000). Lipid oxidation is a well-studied case in this respect: the large surface area of dispersed droplets facilitates interactions between the lipids and water-soluble pro-oxidants and the total extent of the reaction is mediated by several properties related not only to the ingredients or target compounds but also to pH, particle size, concentration, physical state, oxygen and light (Berton-Carabin, Coupland, & Elias, 2013; Berton-Carabin, Ropers, & Genot, 2014; Waraho, McClements, & Decker, 2011).

A field of specific interest for investigating the MR in emulsions has been flavor development. Several authors have used structured self-assembled liquids as efficient microreactors for flavour development. They studied the development of Maillard volatile products in binary structured fluids composed of mono-glycerides of fatty acids and water forming microemulsions and lyotropic liquid crystalline structures; pseudoternary and pseudo-quaternary W/O microemulsions; U-type microemulsions (water-in-oil W/O, O/W and bicontinuous microemulsions). In such systems, the combination of water activity at the interface of lipid droplets and the presence of surfactants revealed the formation of unique aroma compounds and in the presence of polyol the reaction rates can be buffered, resulting as the major “activity controller” (Garti, 2003; Lutz, Aserin, & Garti, 2005; Vauthey et al., 2000). The impact of emulsions combined with the thermal treatment has been evaluated also in ghee production: Newton and co-workers found that the ratio and the type of volatile compounds produced in cooked samples can be altered by switching the dispersed phase from oil to water (Newton, Fairbanks, Golding, Andrewes, & Gerrard, 2015).

Tailored emulsified systems can be proposed to control the MR development, not only for flavour, but also for non-volatile

compounds responsible for colour and for the generation of potentially harmful products. The possibility to control the MR by encapsulation or spray-drying was previously proposed segregating sodium chloride into lipid capsules to delay its catalytic action (Fiore et al., 2012) and including functional polyphenols inside maltodextrins and acacia fibre coating (Troise et al., 2014). The research hypothesis is that also in the emulsion system it is possible to modify reactant locations, modulating the MR accordingly (Troise & Fogliano, 2013). In fact, the presence of oil could favour the location of some reactants at the oil–water interface, thus modulating the final extent of the Maillard reaction products (MRPs) formation, compared to a pure aqueous system.

In the present paper, an experimental design using two amino acids with different side chains and partition coefficients was developed to investigate the formation of *N*-(1-deoxy-D-fructos-1-yl)-L-phenylalanine (Fru-Phe) and *N*-(1-deoxy-D-fructos-1-yl)-L-leucine (Fru-Leu) in O/W sub-micron emulsion systems obtained by using high pressure homogenisation. High resolution mass spectrometry was used to verify the influence of this system on the formation of APs during thermal treatment. Based on these findings, an overview of the reaction mechanisms leading to MRPs formation in emulsion systems is proposed.

2. Material and methods

2.1. Chemicals and reagents

Mass spectrometry grade acetonitrile and water were obtained from Merck (Darmstadt, Germany). The chemicals for the preparation of the model systems, L-leucine (Leu), L-phenylalanine (Phe), glyceryl trioctanoate (tricaprylin), poly(oxy-1,2-ethanediyl)-sorbitan monododecanoate (Tween 20), benzoic acid, sulfuric acid, glucose oxidase/peroxidase reagents kit (GAGO-20) and *o*-dianisidine were all obtained from Sigma–Aldrich (St. Louis, MO). *N*-(1-Deoxy-D-fructos-1-yl)-L-phenylalanine and *N*-(1-deoxy-D-fructos-1-yl)-L-leucine were obtained from Toronto Research Chemicals (Toronto, Canada). The MS calibration solution was obtained from Thermo Fisher (Bremen, Germany).

2.2. Model systems preparation

Aqueous control solution of D-glucose (25 mM) was mixed with Leu (4.5 mM, partition coefficient $\log P$: 0.799 ± 0.275) and another aqueous solution of D-glucose (25 mM) was mixed with Phe (6.6 mM, partition coefficient $\log P$: 0.235 ± 0.277) (SciFinder, 2015). The ratios between glucose and leucine, and glucose and phenylalanine were 4.5:1 and 6.5:1, respectively. The two resulting solutions (Leu/glucose and Phe/glucose) were separately stirred for 30 min at room temperature. For the preparation of O/W sub-micron emulsions, an aliquot of the two control solutions was mixed with Tween 20 (final concentration of Tween 20 in the emulsion system, 1% w/w). A coarse emulsion was prepared by mixing 10% (w/w) oil phase and 90% (w/w) aqueous phase, then the solution was sonicated (1 min) by using a water bath, finally it was blended by a high-shear mixer at 10,800 g for 40 s, three times. Fine emulsions containing glyceryl trioctanoate were obtained by passing the coarse emulsion three times through a high pressure homogeniser (Microfluidizer M110Y; Microfluidics, Westwood, MA, configured with 75- μ m and 200- μ m interaction chambers in series) working at 30,000 psi. The same process was also applied for Leu and Phe in the control aqueous solutions, in order to take into account any interference due to the homogenisation process, as highlighted in Fig. 1.

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