The taste of future foods: molecular insight into plant protein and flavour binding

CRISTINA BARALLAT[1], Teresa Oliviero[1], Hans-Gerd Janssen[1,2], Sara I.F.S. Martins[1,2], Vincenzo Fogliano[1]

[1]Wageningen University & Research, Food Quality and Design Group, Wageningen, The Netherlands
[2]Unilever Research & Development, Wageningen, The Netherlands
cristina1.barallatperez@wur.nl

Abstract

Plant proteins have gained attention to be used as healthier ingredients in new food applications. However, indigenous off-flavours, such as bitterness and astringency, reduce consumer liking and acceptability of plant-based food products. To tackle this concern often flavour addition is seen as a solution. Nevertheless, proteins can interact extensively with flavour molecules that can bind the protein´s binding sites, thus affecting their release. As a result, overall flavour perception is disrupted. Therefore, this study aims to unravel the drivers of the binding mechanism at the molecular level, and determine how the chemical structure of both aroma molecules and proteins has an impact on the interactions with plant proteins and thus, on aroma release and retention. Hence, we hypothesize that size and shape of aroma molecules may influence the strength, nature and behaviour of these interactions. In the current preliminary study, binding to PPI (1%) capacity increased by enlarging the chain length of ketones, which is related to hydrophobic interactions. Exponential growth by 1.5% is observed when adding an extra carbon atom to the ketone molecules. A flavour chemical structure with an extensive number of carbon atoms and thus, long carbon chains, will lead to the existence of more binding sites and lastly, to a higher binding tendency. Besides, binding to PPI (1%) decreased in the following order: *trans*-2-nonenal (95%)>nonanal (85%)>2-nonanone (52%). The location of the functional group at the end of nonanal resulted in a higher binding as compared to the functional group located more in the middle of the structure (2-nonanone). This is partly explained by an occurring reaction of the alkenal double bond with lysine and histidine residues. Sensory studies will be carried out to investigate the impact of aroma retention on the dynamics of *in-vivo* aroma release, thus to acquire a complete picture of the flavour molecules engaged in the binding mechanism.

Keywords: flavour, protein, binding, chemical structure, retention, interactions, release.

Introduction

Aroma compounds are known to interact with other food nutrients of the food matrix such as lipids, carbohydrates and proteins by molecular interactions [1, 2]. Hydrophobic interactions, hydrogen bonds, van der Waals, ionic and electrostatic linkages and covalent bonds are the major categories reported [3-5]. Proteins can interact with flavour molecules by reversible or irreversible binding [6]. When binding mechanism occurs, the flavour is retained since the available binding pockets of the protein are occupied by flavour molecules, hindering its release while food consumption. This issue reduces the consumer acceptability of such products. Therefore, this preliminary study aims to determine if the ability of a flavour compound to bind to the protein is influenced by aroma molecule´s structure, but also to confirm, the fact that retention of flavours to protein increases with increasing carbon chain length of the flavour molecules [7, 8]. As plant food ingredients, in particular pulses proteins, are one of the most promising food components regarding the development of novel high protein food products, pea protein was selected.

Experimental

To assess the binding behaviour between plant protein and aroma compounds, headspace analysis was carried out through GC-MS/MS (AGILENT- 7890A GC coupled with an AGILENT 5975C with triple-axis detector MS). It operated in split mode 1:10 and 8 mL/min split flow. Samples were incubated and shaken for 14 min at 40°C. 1 mL of sample headspace was injected into the GC injector. A DB-WAX 121-7023 column (20 m*180 µm* 0.3 µm) run at 0.8 mL/min constant flow. The temperature was programed by heating the GC column at a rate of 40°C/min to 240°C.

Plant protein stock solutions

Plant protein solutions were prepared using Pea Protein Isolate (PPI) EMSLAND E86 F30 (Emlichheim, Germany) at an initial concentration of 2% (w/v) in sodium phosphate as buffer solution 0.01 M (pH 7.85). Subsequently, samples were placed into a multipoint stirrer (Variomag Multipoint magnetic stirrer, Sigma-Aldrich, St. Louis, MO, USA) for 1.5 h to ensure complete dispersion of the protein isolates.

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Flavour stock solutions

Each volatile flavour compound was prepared in sodium phosphate buffer solution at an initial concentration of 10.0 mg/kg. Aroma stock solutions were placed in an ultrasonic water bath (Elma, Elma Schmidbauer GmbH, Singen, Germany) to ensure a proper mixture of the flavour. Opaque 100 mL vessels (Pyrex, Sigma-Aldrich, St. Louis, MO, USA) were used to protect them from the light. Stock solutions were kept in the fridge after each use.

GC-MS/MS samples

In order to determine the binding, each aroma and PPI stock solutions were mixed in a specific ratio. 1 mL of 2% (w/v) PPI stock solution was loaded into a 20 mL GC-MS/MS vial followed by the addition of 1mL of flavour stock solution. Thus, a final protein solution of 1% (w/v) and 5.0 mg/kg flavour concentration was produced. The reference sample was 1 mL of PPI stock solution in 1 mL of buffer, without the addition of flavour. The vials were then closed and introduced in a water bath shaker (SW22, Julabo GmbH, Seelbach, Germany) at 30°C and 125 rpm for 3 h before headspace sampling.

Binding calculation

To determine the binding percentage of flavours to PPI, retention was calculated as followed (adapted from [8])

$$\text{Binding (\%)} = 1 - \frac{Peak\ area\ (protein\ solution + aroma) - Peak\ area\ (protein\ solution + buffer)}{Peak\ area\ (aroma\ solution + buffer)} x100$$

Results and discussion

Several authors have noted that by increasing the carbon chain length within the same chemical family, the binding effect becomes stronger regardless of the chemical class or type of protein [8-12]. To confirm this fact, a homologous series of ketones at 5.0 mg/kg was investigated (Figure 1). Overall, it was demonstrated that lengthy flavour molecules are retained to a greater extent than when compared to shorter chain flavour molecules. Binding increased from 9.6% for the 2-hexanone to 73.94% for 2-decanone, showing an exponential growth of 1.5% when adding an extra carbon atom to the flavour structure. These results are in line with previous research works [10, 13, 14].

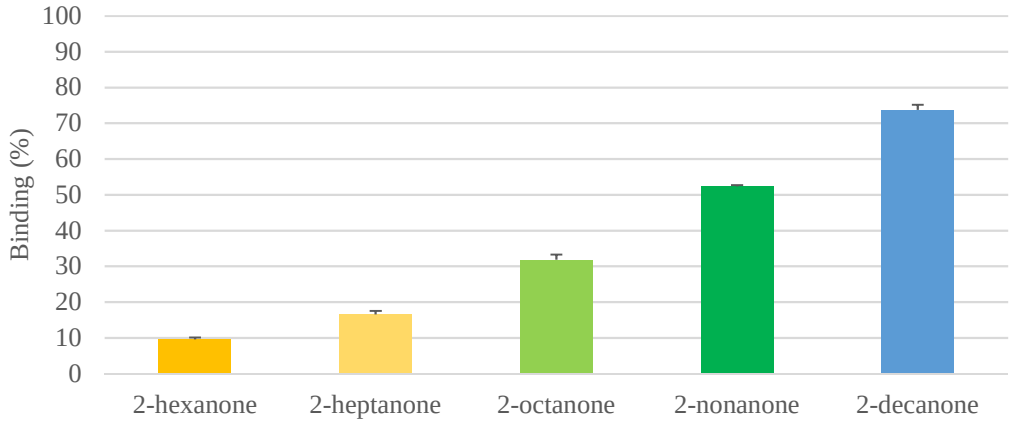

Figure 1. Binding of a homologous series of ketones (5.0 mg/kg) to PPI (1%).

Binding capacity increased by enlarging chain length. The affinity of the longer carbon chain structures of the ketones to proteins is linked to hydrophobic interactions [4]. A chemical structure with an extensive number of carbon atoms and hence, long structures, leads to the existence of more binding sites and thus, a higher adsorption ability and binding tendency [15].

To evaluate the effect of both the location of the radical group and the unsaturation on the retention mechanism with PPI, flavour compounds built up of nine carbon atoms were used. They differed on the number of double bonds and the position of the functional group. At the flavour concentration of 5.0 mg/kg, binding to PPI (1%) decreased in the following order: trans-2-nonenal (95%)>nonanal (85%)>2-nonanone (52%) (Figure 2). Even though, trans-2-nonenal is less hydrophobic than nonanal (logP values 3.1 and 3.3, respectively) bound more to the PPI. However, when compared to 2-nonanone with the same logP of 3.1, trans-2-nonenal showed a

considerable higher binding. The presence of more double bonds seems to influence and enhance the binding to the protein [9].

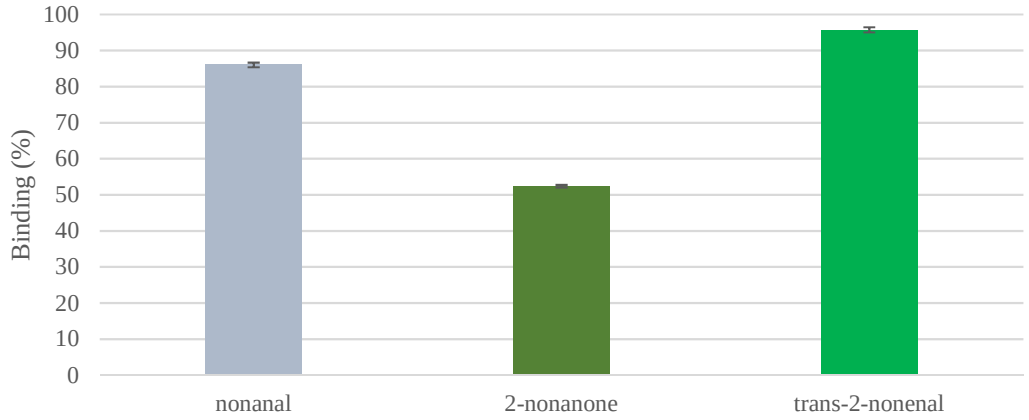

Figure 2. Binding of C9 flavour compounds (5.0 mg/kg) to PPI (1%).

The presence of the keto group (-CHO) located at the end of nonanal derived in a greater binding (85%) as compared to the keto group positioned more into the middle of the structure (2-nonanone) (52%). This is partly explained by an occurring reaction of the alkenal double bond with lysine and histidine residues. There is less binding when incorporating unsaturation more into the middle of the chain. The keto group at the 2-position in ketones may limit hydrophobic interactions and thus, intercept these hydrophobic flavours from binding to the protein. This effect was already explained and observed by Kühn et al., 2008 [9] when studying Whey Protein Isolate (WPI) at 0.5% and C9 flavour compounds at 1.0 mg/kg.

Conclusion and future research

The molecular behaviour of protein-flavour interactions is a complex mechanism where several parameters are involved. From the current preliminary results, it can be concluded that the location of the radical group, number of double bonds and chain length affects the retention phenomena to industrialized PPI.

To further unravel the drivers of the molecular retention phenomena between protein and flavour molecules, headspace measurements will be conducted using advanced methods such as Proton-Transfer-Reaction Mass-Spectrometry (PTR-MS, Ionicon, Austria). Different plant protein will be assessed.

In addition, a flow of sensory studies will be foreseen to study the repercussion of flavour retention on the dynamics on in-vivo aroma release. By combining analytical and sensory methodologies, would be possible to acquire a complete picture of the flavour molecules involved in the overall binding mechanism.

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