The impact of plant proteins on vanilla flavour perception

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Abstract

Interactions between proteins and flavours have been reported to produce flavour retention and to decrease flavour perception in food products. Protein/flavour interactions, a type of flavour retention, can either be reversible such as hydrophobic, hydrogen, and electrostatic interactions or irreversible such as covalent binding. Proteins can also transmit undesirable off-flavours to food products affecting their organoleptic properties and thus also altering flavour perception. It has been previously confirmed that vanilla flavour intensity was reduced due to interactions between vanillin and milk proteins. However, less is known about plant protein/flavour interactions. Therefore, the aim of this study was to investigate interactions between vanillin and plant proteins (wheat, soy, lupin, pea, and potato) in aqueous systems and their impact on flavour perception. Results showed that interactions were dependant on the protein source. Vanillin was bound mainly by pea protein, followed by wheat protein. The final sensory profiles of model beverages were influenced by both, protein/vanillin interactions and off-flavour related to each protein.

Introduction

Multiple studies have shown that proteins can interact with various flavour components resulting in flavour retention and affecting flavour perception [1]–[3]. Protein/flavour interactions differ according to the amino acid composition of proteins and the chemical structure of flavour components. Retention of flavour by physico-chemical interactions can be either reversible such as hydrophobic, hydrogen, and electrostatic interactions or irreversible such as covalent binding. Protein/flavour interactions have been confirmed for vanillin (4-hydroxy-3-methoxybenzaldehyde), the main compound of vanilla flavour which is widely applied in food products [2], [4]–[9]. Vanillin binding affinity and flavour perception has been largely investigated for milk proteins [1], [2], [4]–[8]. Studies showed that sodium caseinate or whey proteins interact with vanillin, and that the binding affinity increases with protein concentration [2], [4]–[6]. Reversible interactions can even occur quickly and influence the flavour perception of food immediately [2], [9], [10]. On the other hand, fewer studies have focussed on interactions between plant proteins and flavours, although the plant protein usage is predicted to increase in the future [11]. Plant protein/flavour interactions have been previously investigated for soy protein [6], [12]–[15], in lesser extent for pea [16], [17] and wheat proteins [18], and no studies have focused on lupin or potato proteins. The usage of proteins may not only cause flavour retention but also transmit unwanted off-flavours, which represent the main limitation for their use in food [2], [19]. This sensory dimension is less taken into account in studies that focussed on protein/flavour interactions. Therefore, the aim of this study was to investigate both, flavour retention and flavour perception when vanillin is mixed with plant proteins (wheat, soy, pea, lupin, and potato), as well as the contribution of protein off-flavours in the final sensory profile of model beverages.

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Experimental

Vanillin (Mane, France), wheat protein concentrate (Tereos, France), soy protein concentrate (ADM, USA), pea protein isolate (Roquette, France), lupin protein-rich powder (Terrena, France), and potato protein isolate (Avebe, The Netherlands) were used to investigate protein/vanillin interactions and sensory flavour perception. The protein content in dry base were 80 %, 69 %, 83 %, 42 %, and 90 % for wheat, soy, pea, lupin, and potato proteins, respectively. Solutions were prepared in demineralised water by adding proteins and sugar at 3 % w/w and at 2.5 % w/w concentrations, respectively. The pH of wheat, soy, pea, lupin, and potato protein solutions was not adjusted and was around 5.8, 7.4, 7.2, 7.5, and 6.0, respectively. When vanillin was added to samples the final concentration was 100 ppm.

Sensory evaluation

Descriptive sensory analyses were performed by an internal panel composed between 10 and 15 panellists using the rank-rating evaluation method [20]. Per session, panellists tested protein and protein/vanillin solutions and evaluated the vanillin flavour and the off-flavours: cereal/wheat, herbal/vegetal, and bitterness, on a 0-10 scale. These three protein off-flavour descriptors were selected by their frequency from a separate sensory session testing protein solutions. Changes in the perceived intensity of each descriptor were determined by the difference between pure vanillin and protein/vanillin solutions. Data obtained was treated using an analysis of variance (ANOVA).

Determination of protein/vanillin interaction

Physico-chemical interactions between vanillin and plant proteins were determined by equilibrium dialysis experiments and High Performance Liquid Chromatography (HPLC) analysis for quantification of vanillin. In equilibrium dialysis experiments proteins were kept separated by using semi-permeable membranes (Spectra/Por1 MWMO: 6-8 kDa). Protein solutions were first dialysed overnight against demineralised water to purify samples prior to vanillin addition. After the equilibrium was reached (~72 h), samples were taken from the side of the membrane without proteins and centrifuged at 4500xg for 30 min. HPLC analysis was done using a UPLC HSS C18 column (150 mm x 2.1 mm with 1.8 µm particle size) (Waters, France) coupled to a UV spectrophotometric detector set at 280 nm. The mobile phase consisted of a mixture of demineralised water, acetic acid, and acetonitrile (83:2:15). 1 µl sample was injected at 0.4 $mL.min^{-1}$ of flow rate and 40°C of temperature. The loss of vanillin by interaction with proteins was calculated by the following relationship: *% Loss of vanillin = (concentration of vanillin in the control - concentration of vanillin in the sample)*100 /concentration of vanillin in the control*. Experiments were performed in triplicate and control samples did not contain proteins. Results were normalised by the protein content in solutions.

Results and discussion

To understand the impact of protein addition on flavour perception, the sensory profile of plant protein solutions containing vanillin or not were evaluated by a panel. The off-flavours of pure wheat, soy, pea, lupin, and potato protein solutions were mainly described as bitter, herbal, vegetal, cereal, wheat, astringent, flour, metallic, yeast, earthy, metallic, hay, fatty, soapy, and paper cardboard. Among these terms, the most frequents off-flavour descriptors generated for all proteins were: bitter, cereal/wheat, and herbal/vegetal which were later used for sensory evaluations. The off-flavour intensity scores in pure wheat, soy, pea, lupin, or potato protein solutions are presented in Table 1.

Table 1: Off-flavours intensity scores of wheat, soy, pea, lupin, and potato protein sweet solutions without vanillin. Analysis of differences between categories (a, ab, b) with a confidence level of 95%.

Protein	*Cereal/Wheat*	*Herbal/Vegetal*	*Bitter*
Wheat	5,6 [a]	3,8 [a]	2,2 [b]
Soy	6,3 [a]	3,5 [a]	4,1 [ab]
Lupin	5,8 [a]	4,9 [a]	6,0 [a]
Pea	6,6 [a]	3,4 [a]	4,2 [ab]

Results showed that the cereal/wheat flavour was characteristic for most of protein solutions, except for potato protein. Herbal/vegetal flavours were perceived at different degrees among all proteins. Bitterness was mainly pronounced in solutions containing lupin and potato proteins, while it was the least present in wheat protein solutions. Similarly, other studies on soy, pea, and lupin proteins described beany, green, bitter, grassy, metallic, and astringent off-flavours [16], [21], [22]. Especially, green and beany off-flavours in pulse and legume ingredients were explained by the presence of unsaturated lipids susceptible to oxidative deterioration by endogenous lipoxygenases [19], [22]. Changes on the perceived intensity of vanillin flavour and off-flavours (cereal/wheat, herbal/vegetal, and bitterness) of wheat, soy, pea, lupin, and potato protein solutions after addition of vanillin are shown in Figure 1. As expected, the perception of vanillin increased in most of protein solutions after addition of vanillin. However, the perceived intensity of vanillin was different for each protein. The vanillin flavour was best perceived in solutions containing lupin protein, producing an intensity increase of 2.4 significantly higher than the other proteins. In contrast, the vanillin flavour was least perceived in potato protein solutions. Off-flavours seemed to decrease after addition of vanillin in most of protein solutions, expect for potato protein.

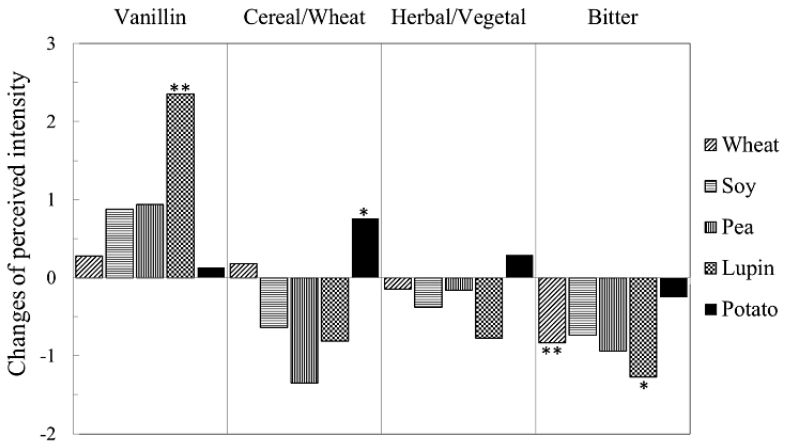

Figure 1: Changes in the perceived intensity of bitterness, herbal/vegetal, cereal/wheat, and vanillin flavours in wheat, soy, pea, lupin, and potato protein sweet solutions after addition of vanillin. Significant difference between categories with 90% (*) and 95% (**) of confidence level.

Protein/vanillin interactions were quantified in terms of vanillin loss for wheat, soy, pea, lupin, and potato protein solutions (figure 2). The loss of free vanillin varied

depending on the protein source. The strongest interaction with vanillin was observed for pea protein followed by wheat protein, with a vanillin loss of around 50 % and 22 %, respectively, compared to the control. In contrast, soy, lupin, and potato proteins slightly interacted with vanillin under the tested conditions. Different degrees of flavour retention by plant proteins were expected since there are many factors that can play a role on protein/flavour interactions, and there is no universal mechanism. Protein/flavours interactions have been reported to be mainly of reversible nature in aqueous system [2], [9], [14].

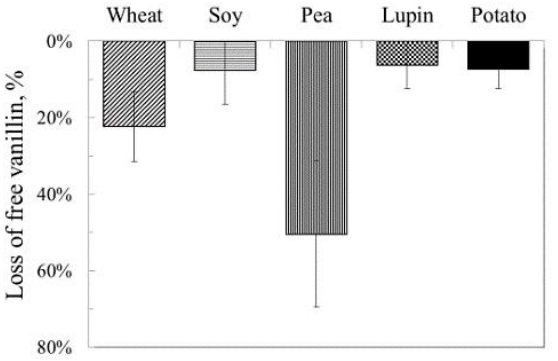

Figure 2: Loss of free vanillin (%) by interactions with wheat, soy, pea, lupin, or potato proteins in sweet aqueous systems with respect to the control without vanillin. Values were normalised by the protein content in dry base and error bars represent one standard deviation.

This study suggested that lupin was the most suitable source of plant protein to be used with vanillin, and thus vanilla flavour. Lupin protein had moderated off-flavours, and vanillin was almost not retained by the protein. Therefore, vanillin stayed free and enhanced the vanilla flavour profile of model beverages. In line with this statement, other studies showed that lupin ingredients had cheese-like, milky, fruity, and fatty off-flavours [21]. This creamy-like sensory profile certainly contributed to a better vanillin perception and, simultaneously, to the decrease of off-flavours such as bitterness in lupin protein solutions. On the other hand, potato protein also displayed low interaction with vanillin but did not produce an increase in vanillin perception after its addition. Contrary to lupin, solutions containing potato protein and vanillin displayed a slight increase of cereal/wheat and herbal/vegetal flavours. This was likely due to the strong and characteristic off-flavours related to this protein (i.e. earthy, paper cardboard, algae). So, for masking potato protein off-flavours, we may suggest to use other warm flavours rather than vanilla (e.g. chocolate). Controversially, soy protein did not have strong affinity for vanillin but displayed relatively low vanillin perception. Soy proteins are known to interact reversibly by hydrophobic binding with carbonyl compounds, such as vanillin [6], [13]. Soy protein/flavour interactions were mainly entropy driven, which means that conformational changes of soy protein may be important in binding of vanillin [6], [14], [23]. The traditional extraction of our commercial soy protein could tentatively explain the low interaction with vanillin. Due to thermal treatment and/or acid precipitation, the protein may have aggregated irreversibly and reduced its flavour binding capacity. Anyhow, further research is necessary to evaluate protein denaturation. Finally, in this study, pea and wheat proteins primarily interacted with vanillin. Similar to our findings, previous studies showed that pea globulins had more flavour binding capacity than wheat

gluten [18]. Pea protein/flavour interactions were mainly of hydrophobic nature [16], [17], while for wheat gluten also inter- and intra-molecular disulphide linkages can participate in flavour binding [18]. Interestingly, even if pea protein retained almost twice more vanillin than wheat protein, the later protein obtained lower scores in vanillin perception. Intuitively, we can think that larger retention produces lower flavour perception. However, the type and strength of interactions could also influence the loss of flavour perception. Since our commercial wheat protein was hydrolysed for better solubility, we can think that as a result, gluten peptides increased the number of binding sites and had better access to primary structures, including sulphur-containing residues [1], [3], [6]. Therefore, if disulphide bridges were somehow involved in wheat protein/vanillin interactions, they were probably stronger and more stable as compared to hydrophobic ones, producing larger impact on the flavour perception.

In conclusion, the impact of plant protein (wheat, soy, pea, lupin, and potato) on flavour perception was studied and tentatively correlated to the protein off-flavours and physico-chemical interactions with vanillin in aqueous systems. Understanding these protein/flavour implications is allowing the flavour industry to have better control on the flavour release and the reduction of off-flavours in plant protein based products.

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