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Binding of selected volatile flavour mixture to salt-extracted canola and pea proteins and effect of heat treatment on flavour binding

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ABSTRACT

Binding of homologous and heterological classes of flavours with salt-extracted canola protein isolates (CPIs) and pea protein isolates (PPIs) and the effect of heat treatment on their binding were investigated using GC/MS. Competitive binding was observed when homologous ketones were added to CPIs and PPIs and when homologous aldehydes were mixed with CPIs. Ketone mixtures performed differently than aldehydes in that 2-octanone retained more effectively than 2-heptanone and 2-hexanone by CPIs and PPIs, whereas CPIs exhibited incremental affinity to hexanal rather than heptanal and octanal. For PPIs, the presence of aldehydes increased the proteins' overall flavour-binding capacities probably due to partial unfolding of proteins revealing more binding sites as manifested by the decreased ΔH from the DSC studies. Binding of hexanal to CPIs was significantly increased with increased heating time at 95 °C, while a transition of 2-octanone retention from increasing to decreasing inferred heat-induced protein association released previously bound 2-octanone. Heat treatment at 95 °C for 30 min promoted greater competitive binding when mixed ketones and hexanal and 2-hexanone mixtures interacted with CPIs and PPIs, respectively, while dramatic increases of binding of aldehyde mixtures was observed thought out the heating processes.

1. Introduction

Among many factors influencing the mobility and release of flavour compounds within and from a food matrix, a major consideration is the chemical interaction between the food and the flavouring. A successful flavour formulation must be designed to survive a range of interactions with the food and eventually afford a sensory profile that is acceptable by consumers (Reineccius, 2006). Unfortunately, such a flavour releasing profile is not easy to be achieved especially in health-oriented low-fat foods where proteins or carbohydrates perform differently when interacting with volatile flavours compared with fat (Guichard, 2011).

Unlike carbohydrate or lipids, proteins provide complex chemical structures for interacting with flavour compounds; these include hydrophobic pockets, amino acid side chains and terminal ends (Arora & Damodaran, 2010; Reineccius, 2006; Wang & Arntfield, 2014). Both reversible weak hydrophobic interactions and irreversible strong covalent bonds may be formed between proteins and flavour compounds (Suppavorasatit & Cadwallader, 2012; Tromelin, Andriot, & Guichard, 2006). These interactions between flavour and proteins have led to dramatic reductions in desirable flavour intensity, thereby affecting the perception of flavour in various protein-based food products including soymilk (Suppavorasatit, Lee, & Cadwallader, 2012), vanillin-fababean protein slurries (Ng, Hoehn, & Bushuk, 1989a, 1989b), soy-containing crackers (Zhou, Lee, & Cadwallader, 2006), skim milk (Meynier, Garillon, Lethuaut, & Genot, 2003; Meynier, Rampon, Dalgalarrondo, & Genot, 2004), milk protein sweetened drinks (McNeill & Schmidt, 1993), and dry-cured hams (Pérez-Juan, Flores, & Toldrá, 2006).

In previous studies relating flavour binding by proteins, normally only one flavour compound was studied in an aqueous model system (Kühn, Considine, & Singh, 2006). However, when two or more flavours are mixed, compatible, cooperative or competitive binding patterns can be observed (Guichard & Langourieux, 2000). Compared with simple protein-flavour system, less emphasis has been put on competitive binding studies (Sostmann & Guichard, 1998). Only studies on β-lactoglobulin (Jouenne, Chalier, & Crouzet, 2000; Muresan & Leguijt, 1998) and 11S globulin of broad beans have been reported (Semenova et al., 2002). It can be hypothesized that when different volatile flavours are mixed,

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flavours with higher protein binding affinities should be retained more extensively than the flavours possessing lower binding affinities. Of particular interest in this work is to systematically evaluate how homologous and heterological classes of aldehyde and ketone flavours behave when combined in a single system.

Protein-flavour interactions are also highly dependent on the protein's structural state (Li, Grün, & Fernando, 2000). Any changes in protein conformation may influence flavour binding characteristics. Heat treatments are most widely used to prepare food products. The effect of heat, however, has led to conflicting results with respect to flavour binding as increases (Gkionakis, Taylor, Ahmad, & Helipoulos, 2007; Ng et al., 1989b) and decreases (Kühn, Considine, & Singh, 2008; O'Neill & Kinsella, 1988) having been reported. Kühn et al. (2006, 2008) explained that increased binding during protein unfolding and decreased binding due to protein aggregation may account for the differences. In most studies, a single heating time or temperature was used; systematically studying the development of protein flavour interactions with respect to heating time or temperature should provide additional insight into these different behaviours.

Therefore, one of the objectives of this study was to systematically evaluate the potential competitive binding phenomenon between selected volatile flavour compounds to both salt-extracted canola and pea protein isolates. In addition, clarifying the effect of heat treatment on flavour binding using the typical aldehyde and ketone flavours was another purpose of this investigation.

2. Materials and methods

2.1. Source of materials

Analytical grade flavours were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Homologous series of aldehydes (hexanal, heptanal, and octanal) and ketones (2-hexanone, 2-heptanone, and 2-octanone) were selected. Commercial yellow pea (*Pisum sativum* L.) flour was kindly supplied by Best Cooking Pulses Inc. (Portage la Praire, MB). Canola meal was obtained from Burcon NutraScience Co. (AL018, Winnipeg, Canada). All other chemicals including NaCl, K_2HPO_4, KH_2PO_4, HCl and NaOH were analytical grade and purchased from Fisher Scientific (Ottawa, Canada).

2.2. Salt-extracted canola protein isolates (CPIs)

A protein micellar mass (PMM) method from Ser, Arntfield, Hydamaka, and Slominski (2008) was adapted with minor modifications. Firstly, 50 g of finely ground (Grind Central Coffee Grinder, Guisinart) and sieved (500 μm opening, USA Standard No. 35) canola meal was mixed with 500 mL of 0.5 M NaCl by constantly stirring at medium speed on a corning PC-353 stirrer (Scientific Support, Inc., Hayward, CA) for 1 h. The mixture was then centrifuged (3000 × *g*, 4 °C, 15 min) and the supernatant (soluble protein solution) was successively filtered through four layers of Cheesecloth Wipes™ (Fisher Scientific) and two layers of each Whatman™ No. 4, 40 and 42 filter papers (90 mm) under vacuum to remove any possible debris. Clarified canola protein solution was then concentrated to 3–5 times its original volume using a Vivaflow 200 ultrafiltration unit (Vivascience AG, Hannover, Germany) equipped with a 10,000 Da molecular weight cut-off (MWCO) polyethersulfone (PES) membrane at constant pressure of 250 kPa. The retentate (concentrated protein solution) was subsequently diluted 15 times using cold distilled water and left in the refrigerator for 16 h. Instantaneous protein precipitation upon dilution indicated formation of PMM which was recovered by a second centrifugation (6000 × *g*, 4 °C, 15 min). The pellet was collected and kept frozen (−30 °C) before freeze drying (Genesis SQ Freeze Dryer, Gardiner, NY, USA).

2.3. Salt-extracted pea protein isolates (PPIs)

A method previously described by Sun and Arntfield (2010, 2011) was followed to prepare salt-extracted pea protein isolates (PPIs). Pea protein was extracted from sieved (500 μm opening, USA Standard NO. 35) yellow pea flour using 0.3 M NaCl (pea flour: sodium chloride solution = 3:10, w/v) under constant stirring for ½ hour. After the first centrifugation (4260 × *g*, 4 °C, 15 min), pea protein was recovered by diluting the supernatant in two times volume of cold distilled H_20 and refrigerating (3 °C) for 2 h. The precipitated protein sediment was collected after a second centrifugation (680 × *g*, 4 °C, 15 min) and re-suspended in small amount of distilled H_20. The resulting protein suspension was then dialyzed in 12–14,000 Da MWCO dialysis tubing (Spectra/Por Dialysis Membrane, Rancho Dominguez, CA) against 20 times cold distilled H_20 for 72 h in a refrigerator. Distilled H_20 was changed every 24 h. The desalted protein isolate was stored at −30 °C until freeze dried.

The freeze dried protein samples of salt-extracted canola and pea proteins contained 87.32 and 82.68% protein respectively using a N-to-protein conversion factor of 5.7 according to Uruakpa and Arntfield (2006) and Sun and Arntfield (2010) with a Dumas method and a FP-528 Nitrogen/Protein Determinator (LECO Corporation, St. Joseph, MI, USA).

2.4. Flavour binding to plant proteins

To bind proteins and flavours, the method of Gkionakis et al. (2007) was followed. Basically, protein and flavour stock solutions were first prepared and then mixed at specific ratio to produce an aqueous sample containing the desired concentrations of protein isolate and flavour compounds. Samples were shaken gently to reach equilibrium for binding of flavours with proteins. This was followed by the headspace gas chromatography technique for determining the binding of protein isolates with selected volatile flavour compounds.

2.4.1. Preparation of 2% protein and flavour stock solutions

2% (w/v) solutions of CPIs and PPIs were prepared in 0.01 M potassium phosphate buffer (pH 8) and subsequently placed into an ultrasonic water bath (Branson 3200, Bransonic Ultrasonic Cleaner, Shelton, CT USA) for 20 min to ensure a complete dispersion of the protein isolates (Gkionakis et al., 2007). The ionic strength was kept as low as possible to minimize the effect of salt on protein conformation.

Stock solutions of each volatile flavour compound were prepared in phosphate buffer as mentioned above at both 1000 (0.1 mL/100 mL) and 1500 ppm (0.15 mL/100 mL) and stored in amber glass bottles to prevent decomposition. These flavour stock solutions were put in an ultrasonic water bath for 1 h to ensure a thorough mixing before each use.

2.4.2. Preparation of GC/MS samples

In a typical experiment, to produce 1% (w/v) final protein solution with a flavour concentration of 250 ppm, 1 mL of 2% (w/v) protein solution was carefully loaded into a 20 mL reaction vial (22 × 75 mm, Product No.: 20-2100, Microliter Analytical Supplies, Inc., Suwanee, GA) followed by addition of 0.5 mL of buffer and 0.5 mL of flavour stock solution (1000 ppm) to reach an aliquot volume of 2 mL. The flavour solution was added last. For the competitive binding study using three different flavours, the 1500 ppm flavour stock solutions were employed and 1/3 mL of