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Characterisation of odour active compounds along extraction process from pea flour to pea protein extract

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ABSTRACT

Pisum sativum, rich in proteins, represents a main interest for human food. Nevertheless, pea products are underused because of their organoleptic characteristics. The extraction process of the proteins can partly explain the development of the typical flavour.

The objective of the present study was to identify odour active compounds and to follow their evolution during four steps of the process (from pea flour to pea protein).

Firstly, volatile compounds were extracted by Solvent Assisted Flavour Evaporation from each step and analysed by Gas Chromatography coupled with Mass Spectrometry and Olfactometry. Secondly, the volatile compounds, identified as odour active in pea flour and in pea protein, were followed in the intermediary steps of the process. As hypothesised, it appeared that the flavour profile is evolving during the protein extraction process. The odour active compounds are different between pea flour (first step) and pea protein powder (final step). Some compounds are disappearing earlier or later during the process, whereas some are appearing at different steps. Only few odour active compounds are common to each step.

These results lead to a better understanding of the flavour compounds of pea flour and of proteins, and of their evolution during the protein extraction process. Then, these results could permit to adapt, to improve some key parameters or to add some steps at the process in order to better control the beany flavour development.

1. Introduction

Pea (*Pisum sativum* L.) presents valuable economic and nutritional advantages. The seeds are a source of high-quality protein (Martinez-Villaluenga, Gulewicz, Frias, Gulewicz, & Vidal-Valverde, 2008) for use in animal and human food, and are easy to cultivate. However, pea seeds and pea proteins are not widely used in food applications due to their strong beany flavour (Klein & Raidl, 1986). Indeed, utilisation of legumes as nutritious and low cost source of protein for humans depends upon consumer acceptance. Typical beany flavour in pea flour and derivated product like pea protein extract have prevented these products from being exploited to their full potential for human consumption (Pattee, Salunkhe, Sathe, Reddy, & Ory, 1983). Many studies have been conducted on pea flavour (Jakobsen, Hansen, Christensen, Brockhoff, & Olsen, 1998; Murray, Shipton, Whitfield, Kennett, & Stanley, 1968; Murray, Shipton, & Whitfield, 1970; Murray, Shipton, Whitfield, & Last, 1976; Shipton, Whitfield, & Last, 1969). Some of them pointed out the effect of a specific treatment (Azarnia et al., 2011; Pariasca, Miyazaki, Hisaka, Nakagawa, & Sato, 2001; Shipton & Last, 1968) but none of them analysed the protein extraction process. The extraction process of the proteins from *P. sativum* seeds can partly explain the development of this typical flavour. The bulk of legume flavour technology comes from soybean studies. Very little or no information are available on the effect of processing on pea flour and derivated products. In order to analyse the volatile compounds responsible for the beany flavour, it is important to select a suitable method for their extraction from the food matrix. In a previous study, it appears that Solvent Assisted Flavour Evaporation (SAFE) was the most suitable method because of its good extraction capacities and its high sensory representativity of the global odour of pea flour (Murat, Gourrat, Jerosch, & Cayot, 2012). Then, to understand the impact of the protein extraction process on flavour, it is necessary to analyse the volatile compounds at several process steps.

The objective of the present study was to identify odour active compounds and to follow their evolution during four steps of the process: pea flour, total protein fraction, soluble protein fraction and pea protein powder as the final product. In this way, we could detect the moment of formation of the beany flavour compounds and propose different pathways to control the concentration levels of these compounds.

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2. Materials and methods

2.1. Pea samples

Samples were taken at different steps of the pea protein extraction process (Fig. 1): pea flour (powder), raw extract (liquid), soluble protein fraction (liquid) and the commercial pea protein Nutralys® (powder). These different samples came from a homogenous pea flour batch (batch k274, Roquette frères®) of *P. sativum*. They were stored at −18 °C in glass bottles before analyses.

Some basic chemical analyses (non volatile compounds) were done to characterise the samples. As lipoxygenase was often reported to be responsible for the main oxidative pathway in vegetables (Reynolds & Klein, 1982), the lipoxygenase activity was also measured.

Water content was determined as the loss of mass after drying in an oven until constant weight was obtained (NF EN ISO 1666–October 1994–starches–determination of moisture–methods by oven drying). Crude protein content was obtained from the Dumas method using a conversion factor of 6.25 (NF V 18–120 standard. March 1997. Animal feed–determination of nitrogen–combustion method). Crude fat content was measured with the Soxhlet method by weighting fat after hexane extraction (2nd EEC directive modified December 20, 1983. Part B).

The lipoxygenase activity was determined spectrophotometrically. A 2.5 g sample was diluted in 25 ml of borate buffer (400 mM, pH 6.0). The mixture was then centrifuged for 15 min at 25,000 *g* and at 4 °C. The supernantant was recovered and centrifuged for 30 min at 45,000 *g* and at 4 °C. The supernatant was finally diluted to 1/10 in the borate buffer. This enzymatic extract was put in contact with a substrate (linoleic acid) in a citrate (100 mM)-phosphate (200 mM) buffer (pH 6.0). The lipoxygenase activity (U/min/mg of sample) was measured at 234 nm for 30 s.

Results were reported in Table 1.

Table 1
Characterization of samples.

Sample	Pea flour	Raw extract	Soluble protein fraction	Nutralys®
Dry matter (%)	88.1	5.3	2.4	92.3
Crude protein content (%)	20.4	3.3	0.9	77.1
Crude lipid content (%)	1.27	0.25	<0.10	8.65
Starch content (%)	43.5	0.1	0.1	0.6
Lipoxygenase activity (U/min/mg)	407	<1.	<1	<40

2.2. Chemicals

Ultrapure water made with MilliQ cartridges from Millipore (Molsheim, France) was used for the pea flour and pea protein suspensions. Hexanal-d12 D62-65 (batch Z149P59) was used as the internal standard and purchased at CDN Isotopes (Cluzeau Info Lab, Sainte Foy La Grande, France). The antifoaming agent used for SAFE extractions was Rore AFE 1030 K (batch no. 0605798, Rore technologies, Vincey, France). Dichloromethane (99.8% purity) from Carlo Erba Reagents (Val de Reuil, France) was distilled before use.

2.3. Extraction of volatile compounds

Volatile compounds were extracted using the Solvent Assisted Flavour Evaporation (SAFE) method (Engel, Bahr, & Schieberle, 1999). Sample amounts used for the SAFE method were adapted for each pea extract to obtain chromatograms with peak areas suitable for analysis: 20 g for pea flour, 100 g for raw extract, 100 g for soluble protein fraction, and 20 g for commercial pea protein. All extractions and analyses were done in triplicate.

Pea sample was placed in a 500 ml glass balloon with three drops of antifoaming agent. In the case of powder samples (pea flour and commercial pea protein), 100 ml of ultrapure water was added. The balloon was then placed in a water-bath at 30 °C and the pressure was reduced to 10^{-2} mbar using a vacuum pump. The distillation continued for 2 h after the suspension in the balloon started to boil. The distillate containing the extracted volatile compounds was collected in another balloon cooled with liquid nitrogen. This water phase containing the volatile compounds was stirred three times with 10 ml of distilled dichloromethane. After liquid–liquid separation, the organic phase was collected and dried over anhydrous sodium sulphate. Then this organic fraction was concentrated in a water-bath at 70 °C using a Kuderna Danish apparatus. The extracted volume was adjusted to 290 µl with dichloromethane, and 2 µl of a 10% hexanal-d12 solution in dichloromethane was added.

2.4. GC–MS analysis

All of the volatile fraction extracts were analysed using a gas chromatograph model 6890 (Hewlett-Packard, Palo Alto, CA, USA) equipped with a non-polar column ZB1.MS (30 m × 0.25 mm × 1 µm, Zebron).

Two microliters of each extract were injected into a split-splitless injector at 270 °C, in the splitless mode. Helium was used as the carrier gas in the constant flow mode (1.5 ml min^{-1}) with a linear velocity of 44 cm s^{-1}. The chromatograph temperature was programmed from 50 °C, with an isotherm of 1 min, to 160 °C at a rate of 4 °C min^{-1},

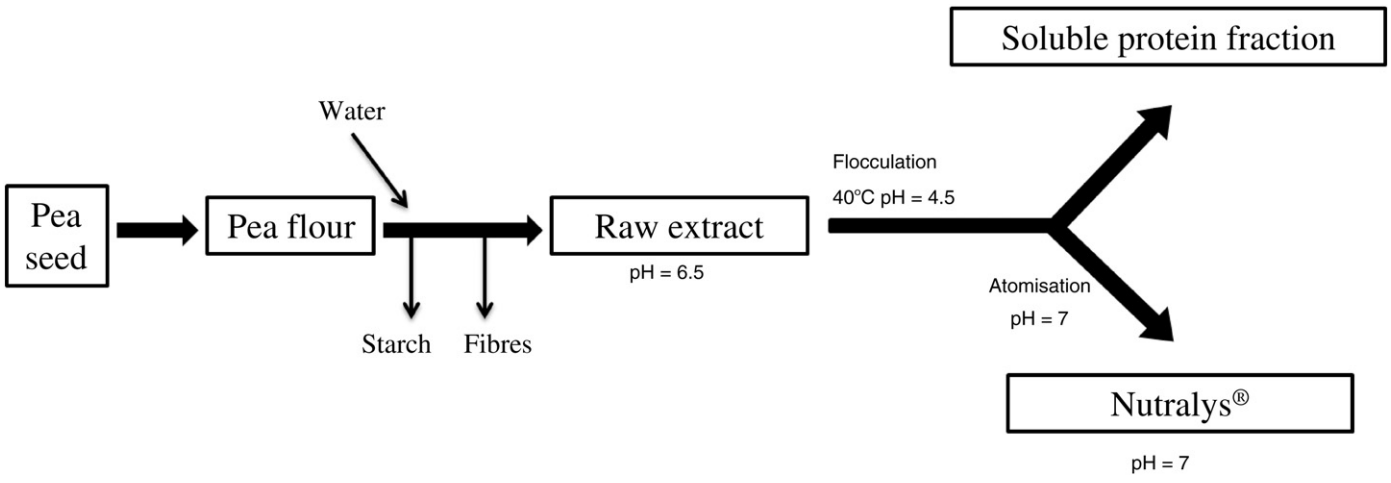

Fig. 1. Schematic description of the pea protein extraction process.