Impact of ageing on pea protein volatile compounds and correlation with odour

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Abstract
The positive effects of plant-based protein consumption are widely reported; nonetheless, several disadvantages such as "grassy" or "beany" off-flavours are encountered [1]. Moreover, the impact of the storage conditions, or ageing, on the evolution of the off-flavour is not well-known [2]. This study aimed to investigate the evolution of the volatile compounds and of the odour during one year using two different ageing processes. The volatile compounds were determined by HS-SPME-GC-MS and the odour by smelling the products at each time of sampling. The mechanisms involved [3-6] and the most impacting factor on the evolution of the volatile compounds were determined. Furthermore, because there is a great challenge to link instrumental data to the odour, a tentative correlation was proposed in order to be able to use instrumental data for early detection of off-flavour and to study the neo-formed compounds during storage.

Keywords: pea protein, ageing, HS-SPME-GC-MS, beany

Introduction
The consumption of plant-based protein is increasing due to their numerous positive effects. However, the consumption is limited by the "beany” off-flavour¹. Numerous works are now dealing with the off-flavour characterization, and the impact of different processes on the volatile compounds. The "beany” off-flavour is generated during the early stages of the production process of protein isolate but it can also evolve during the storage. This work is therefore threefold: - First, to investigate the evolution of the volatile compounds and odour during two ageing processes, close to usual storage conditions of vegetable proteins. - Secondly, to identify the mechanisms involved in this evolution and the most important factor. - And finally, to see if it possible to predict the evolution of the odour using data on volatile compounds.

Experimental
Two ageing processes were applied on a pea protein isolate (PPI) during 12 months. PPI was placed flat in plastic bags at 20°C exposed to light (treatment A) or at 30°C in the dark. PPI was sampled at 0, 3, 6, 9 and 12 months. At each sampling time, the odour was determined by direct sniffing of the product in a glass vial, by three experts. The volatile compounds were also analysed using headspace-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS). A 0.2 g PPI sample was weighed directly in a clear 20 mL vial. Distilled water was added to obtain a 2%/v) and a liquid/gas ratio of 2/18 (v/v). For SPME, equilibrium step and extraction step were conducted both at 40 °C under stirring at 350 rpm in the dark. The equilibrium time was 30 min and the extraction time was 60 min with a DVB/CAR/PDMS fibre. An HP 6890 Series Gas Chromatograph equipped with an HP 5973 Mass Selective Detector was used with a DB-WAX column to analyse the compounds of interest. The SPME fibre was desorbed and maintained in the injection port at 250 °C for 5 min. The programmed temperature was isothermal at 40 °C for 3 min, raised to 100 °C at a rate of 3 °C/min, and then raised to 230 °C. 100 at a rate of 5 °C/min and held for 10 min. The total run time was 59 min.

Results and discussion
Figure 1 is presenting the evolution of the profile of volatile compounds in total chromatographic area per gram of sample, sorted by chemical family, during the ageing and Table 1 is presenting the odour evolution during ageing.

Treatment A (Light – 20°C) had a strong impact on the volatile compounds: high increase of volatile compounds from 0 to 6 months, followed by a small decrease at 9 months and an equilibrium. This was linked to the odour deterioration. An increase in the aldehydes, alcohols and ketones could be observed, with the development of new compounds. The photo-oxidation due to light could be added to the other phenomena presented in Figure 2, playing a crucial role to the formation of volatile compounds.

Treatment B (Dark – 30°C) had a slighter impact on the volatile compounds, with mainly an increase at 12 months [2], as well as a very slight odour change. An increase in aldehydes and furans could be observed, that could be attributed to the Maillard reaction.

E. Guichard & J.L. Le Quéré (Eds): Proc. 16th Weurman Flavour Research Symposium, 2021.
DOI: 10.5281/zenodo.5913090
Within the range of temperatures we studied, corresponding to the usual storage of a product, the light had a higher impact on the product than the temperature.

Figure 1: Profile of volatile compounds by chemical family during the ageing.

Table 1: Odour evolution during the ageing.

| Treatment | 0 months | 3 months       | 6 months       | 9 months       | 12 months     |
|-----------|----------|----------------|----------------|----------------|---------------|
| Treatment A | Beany    | Beany, sharp, earthy | Beany+, sharp, earthy | Beany+, sharp, earthy, rancid | Beany+, sharp, earthy, rancid |
| Treatment B | Light beany, roasted | Light beany, roasted | Light beany, roasted | Beany, earthy | Beany, earthy |

Figure 2: Reactions involved during the ageing [3-6].

The odour evolution of the PPI was linked to an increase in the content of its volatile compounds. To see if it was possible to predict the odour evolution using analytical data, the results used for Figure 1 were sorted by sensory descriptors and were presented in ratios in the global profile to build Figure 3. For each compound, the sensory descriptors were looked up [7] and the compounds were classified into different attribute families like “green” or “earthy”. As the flavour investigated here was “beany”, a focus was made around the “beany” attributes, like the different types of “green”. A given descriptor was then represented as the ratio of the percentage of the cumulative amount of the volatile compounds responsible for the descriptor, to the total amount of all volatile compounds.

With this representation, it was possible to see some links between analytical data and the odour. For example, at 3 months in treatment A, an “earthy” off-flavour was smelled, as presented in Table 1, and this was linked to an increase in the proportion of volatile responsible for “earthy” notes, as presented in Figure 3.

The same way, for 3 and 6 months in treatment B, little to no changes in the odour were smelled and no big changes were seen in the ratio of compounds responsible for the off-flavour.
To conclude, an odour change was linked to a change in the ratios of compounds and the perception of a new off-flavour was linked to an increase in the ratio of compounds responsible for that attribute.

However, there is a limit to these conclusions. This representation (Figure 3) is not sufficient to describe the global sensory profile of a product and this may not work with all attributes. For example, the increase in the proportion of compounds related to “fruity” or “floral” notes in treatment A at 9 months was not perceived.

Figure 3: Profile of volatile compounds by sensor descriptors during the ageing (in ratios).

Conclusion

To conclude, the storage conditions of a product have a strong impact on the volatile compounds and odour. An evolution and deterioration of odour during time can be observed and among the conditions we checked, light had the biggest impact. It can be advised to store vegetable protein in the dark to prevent the photo-oxidation.

When presenting analytical data by sensory descriptors, it is possible to predict how compounds responsible for the different attributes are evolving. It can be used for early detection of an odour change, by detecting a change in the ratios of compounds responsible for the different attributes. It is however different from the overall odour perception. Depending on the product and the flavour or off-flavour of interest, it is important to choose and follow compounds that are correlated with that attribute.

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