LWT - Food Science and Technology 151 (2021) 112087

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Abrasive milling: A method to pre-fractionate testa and embryonic axis from yellow pea

Anna Cäcilie Möller [a], Albert van der Padt [a,b], Atze Jan van der Goot [a,*]

[a] Food Process Engineering, Wageningen University, PO Box 17, 6700, AA, Wageningen, the Netherlands
[b] FrieslandCampina, Stationsplein 4, 3818, LE, Amersfoort, the Netherlands

ARTICLE INFO

Keywords:
Abrasive milling
Mild fractionation
Yellow pea
Structure break-up

ABSTRACT

Making use of crops structural break-up during pearling and subsequent fractionation into starch or protein enriched fractions was investigated using stepwise pearling as a method. In first instance, pearling resulted in separation of pea testa and embryonic axis from the cotyledon. Further around 20% of the yellow pea cotyledon was pearled off and collected separately from the inner kernel. All four fractions were finely ground and their composition analysed. Due to the di-cotyledon structure of the pea, solely pearling the outer kernel couldn't be guaranteed. Therefore, the process was repeated by hand-dissection, to ensure only separation of the outer 20% cotyledon. Pearling resulted in size reduction and separation of testa, embryonic axis and the outer and inner part of the cotyledon. Although, no considerable enrichment was achieved in protein or starch content in the pearled fraction of the outer and inner cotyledon, pearling gave the opportunity to obtain the testa fraction, which according to literature is rich in dietary fibre. Moreover, the protein-rich embryonic axis was separated and collected. The testa fraction accounts for 7–8% of the whole pea and contains little protein and starch, which makes it a promising dietary fibre rich ingredient in food application.

1. Introduction

An increasing trend in food industry is shifting from animal-based proteins to plant-based proteins. This leads to the necessity to investigate methods to extract proteins from plant materials. Promising plant materials to extract proteins from are crops like legumes, as they naturally have high protein contents. To contribute to a more sustainable food production process, mild fractionation techniques were investigated (Geerts et al., 2017; Pelgrom et al., 2013, 2014) valorising the whole crop. One crop is the yellow field pea, consisting of 21–30% protein, around 50% carbohydrates, 10% crude fibres and 2–3% fat (de Almeida Costa et al., 2006). The pea is a spherical seed with an outer skin, the testa. The core of the seed is di-cotyledonous, hence contains two embryonic leaves, which function as storage organs in the pea and are connected by an embryonic axis.

The cotyledons mainly consist of protein bodies (1–3 μm) and starch granules (5–20 μm), which comprise the storage tissue of the pea cells. The concentrations of protein, fat, starch and dietary fibre change towards the outer part of the cotyledon, when compared to the inner part (Kosson et al., 1994; Otto et al., 1997). VanDonkelaar et al. (2015) showed that by step-wise pearling of the barley kernel the protein and starch content could be altered. In one fraction the protein content doubled while the starch content reduced by almost threefold. Hence, pearling provided a route for enrichment of protein and starch in different fractions. The barley is like the yellow pea a starch rich seed. Barley is mono-cotyledonous with a starchy endosperm where, similar to the pea cotyledon, the starch granules are embedded in a proteinaceous matrix including protein bodies. However, the barley kernel is distinctly different in structure compared to yellow pea. The starchy endosperm is surrounded by distinct layers including the aleurone layer, testa, pericarp and husk. These layers vary in composition and structure. Protein is next to the endosperm also present in aleurone cells in the aleurone layer. However, similarly to the pea cotyledons, the outer region of the barley endosperm is richer in protein, compared to the endosperm centre (MacGregor, 2003). Therefore, this similarity in enriched and depleted areas of protein and starch within the kernels, and the starch rich endosperm structure suggested that pearling of pea could be promising to introduce as a size reduction step while simultaneously producing enriched fractions from the pea testa and outer and inner layer of the pea cotyledon. To the best of our knowledge, so far pearling of di-cotyledon seeds beyond dehulling to produce enriched fractions has not been studied. Therefore, the aim of this research was to

* Corresponding author. PO Box 17, 6700, AA, Wageningen, the Netherlands.
E-mail address: atzejan.vandergoot@wur.nl (A.J. van der Goot).

https://doi.org/10.1016/j.lwt.2021.112087
Received 15 April 2021; Received in revised form 2 July 2021; Accepted 3 July 2021
Available online 7 July 2021

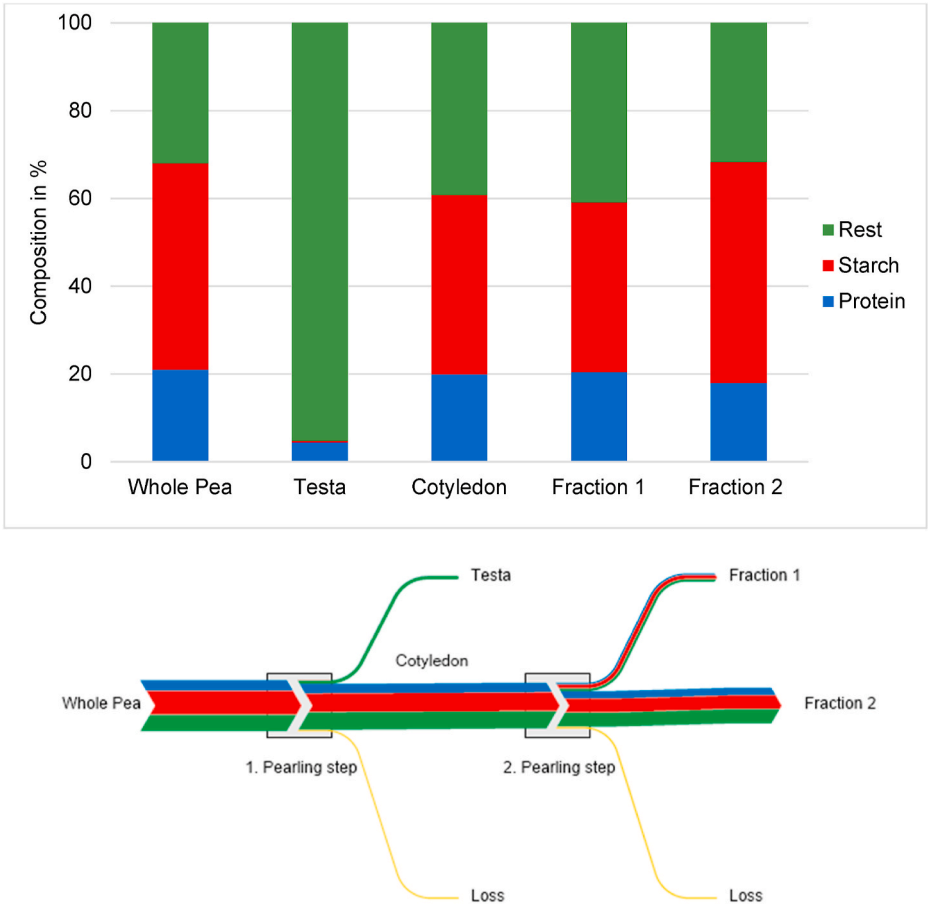

Fig. 1. The composition of the fractions obtained from the two pearling steps is depicted in a compositional bar chart and a Grassmann diagram. In the first pearling step the testa was separated from the cotyledon, subsequently the cotyeldon was pearled in a second step to obtain fraction 1 and 2. The colours indicate the protein ▮, starch ▮, rest ▮ and loss ▮ content of the streams. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

investigate the potential of pearling as a fractionation method to obtain fractions enriched in protein or starch.

2. Materials & methods

Pre-dried yellow peas (*Pisum sativum* L.), harvested in 2017 in the U. S. and stored in bulk in a warehouse silo were purchased from Alimex (Sint Kruis, The Netherlands). The dried yellow peas were pearled in a Satake TM05 testing mill. In a first step, the seeds were subjected to the pearling machine only for a short time of 10 s to separate the testa from the cotyledon which could be collected in separate baskets. Subsequently, the pea cotyledons were again subjected to the pearling and a second longer pearling step was performed of around 30 s, to pearl off around 20% w/w from the pea cotyledon. The pearled off cotyledon fraction was called fraction 1, while the remaining cotyledons were called fraction 2. Both were ground in a laboratory rotor mill prior to further analysis (Fritsch, type pulverisette 14 equipped with a 500 μm screen). The yield of both fractions was defined as the weight percentage of the pearled off fraction based on the initial weight. In a second approach testa and cotyledon were separated accordingly and the embryonic axes were hand-picked from the separated testa fraction. Furthermore, the outer layer of the pea cotyledons was dissected by hand with a sharp razor blade. And both the outer layer and the inner layer were ground for further analysis according to fraction 1 and 2. The dry matter content of the peas was determined by oven drying at 105 °C overnight, to calculate the yields. The protein content of the fractions was determined using a Dumas analysis (Nitrogen analyser, FlashEA 1112 series, Thermo Scientific, Interscience, Breda, The Netherlands) using a protein conversion factor of 5.52 (Holt & Sosulski, 1979). The total starch content was determined using the Total Starch Amyloglucosidase/α-Amylase Assay Kit (Megazyme International Ireland Ltd., Bray, Ireland).

3. Results

The whole peas (138.8 g) were dehulled, resulting in a testa fraction of 7.4% of the total pea. During dehulling around 3.0% material was lost. With the separation of the testa, the embryonic axis was concurrently separated from the cotyledons, which resulted from splitting of the peas. Some embryonic axes remained in a small pocket inside the testa, hence ended up in the testa fraction (Appendix, Figure A1). The cotyledons (124.4 g) were subsequently pearled, resulting in a fraction of the outer layer (fraction 1) of around 18.3% of the cotyledons. The remaining parts were milled and collected as the inner layer fraction (fraction 2) accounting to 78% of the cotyledons. The pearling steps resulted in a material loss of 3.7% (Appendix, Table A1). Fig. 1 depicts the composition of each fraction in grams, the colours indicating the amount of protein, starch and rest in the fractions. The Grassmann diagram is added to visualize the pearling process and yield of each fraction. The protein content in the testa was assumed to be derived from embryonic axes remaining in the testa fraction, as pea testa contain nearly no protein and starch (Bain & Mercert, 1966). Embryonic axes were hand-picked, and their composition was analysed to account to about 40% protein and 5% starch content on a dry matter basis. The remaining embryonic axes could hence account to the protein content in the testa fraction. The whole peas contained 21.0% protein and 47.0%

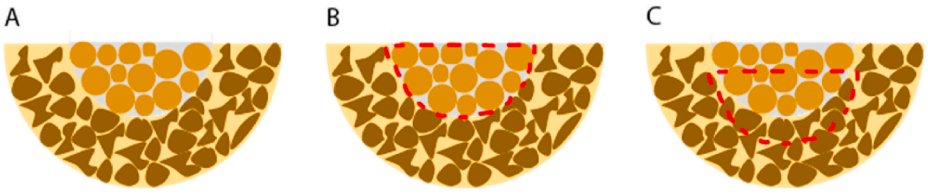

Fig. 2. Representation of a split cotyledon leaf of pea. The outer part underlined in yellow represents the assumed protein enriched outer part of the pea, while the grey underlined inner part is the assumed starch enriched part (A,B,C). The red dotted line in (B) represents the desired pearling profile, while the red line in (C) represents a less optimal pearling profile. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

starch, fraction 1 contained 20.4% protein and 38.7% starch and fraction 2 contained 18.0% protein and 50.3% starch. Hence, pearling resulted in a slight depletion (fraction1) and enrichment (fraction 2) of starch in the respective fractions, when compared to whole peas. However, a significant enrichment or depletion of protein could not be achieved in either of the fractions by pearling.

Comparing the pea seed to the barley seed, the pea is a dicotyledonous seed and splits when dehulled. It was therefore considered that upon pearling not only the outer layer was pearled off. Two possible pearling profiles are depicted in Fig. 2 (B, C). (B) represents the desired pearling profile, while (C) depicts a less optimal profile according to the hypothesis that the outer and inner layer are distinct in their starch and protein distribution.

To understand the results depicted in Fig. 1 and connect them to the hypothesis of the pearling profiles, additional experiments were performed. Both a thick and a thin layer were cut off the cotyledons, respectively and their protein content was determined. With hand dissection the cutting line in (B) was assumed to be followed, as no part of the flat inner surface of the cotyledon was dissected. Remarkably, no differences in protein content between the different outer and inner fractions could be observed (Appendix, Figure A2a), in contrast to the barley endosperm. The results of both experiments give the indication that, in contrast to barley, protein is homogeneously distributed over the cotyledon. Hence, pearling will not lead to protein and starch enrichment during structural break-up.

4. Conclusions

Pearling was shown to be a suitable first size-reduction step, prior to a milling step in the laboratory mill, the process leads to a dietary fibre enriched fraction, composed of the pea testa. Further, pearling of the cotyledon did not lead to protein or starch enrichment in the respective outer and inner fraction. It was shown that no different results are obtained for when pearling or hand-dissecting the pea cotyledon, which indicated that the structural locations of protein and starch do not allow separation by stepwise pearling. Therefore, pearling is considered effective for removing and collecting the testa, however, does not serve as a fractionation method for the pea cotyledon. Additionally, with pearling the embryo was successfully separated, it was confirmed that the embryonic axis contained a considerable amount of protein, next to only little starch, which makes it an interesting part of the pea to isolate.

CRediT authorship contribution statement

Anna Cäcilie Möller: Conceptualization, Formal analysis, Investigation, Data curation, Writing – original draft, preparation, Visualization. **Albert van der Padt:** Writing – review & editing, Supervision, All authors have read and agreed to the published version of the manuscript. **Atze Jan van der Goot:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

This project is co-funded by TKI-E&I with the supplementary grant 'TKI- Toeslag' for Topconsortia for Knowledge and Innovation (TKI's) of the Ministry of Economic Affairs and Climate Policy. The project is financially supported by the Institute for Sustainable Process Technology (ISPT), the Netherlands.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.112087.

References

de Almeida Costa, G. E., da Silva Queiroz-Monici, K., Pissini Machado Reis, S. M., & de Oliveira, A. C. (2006). Chemical composition, dietary fibre and resistant starch contents of raw and cooked pea, common bean, chickpea and lentil legumes. *Food Chemistry, 94*(3), 327–330. https://doi.org/10.1016/j.foodchem.2004.11.020

Bain, J. M., & Mercert, F. V. (1966). Subcellular organization of the developing cotyledons of Pisum Sativum L. *Australian Journal of Biological Sciences, 19*, 49–67.

Geerts, M. E. J., Mienis, E., Nikiforidis, C. V., van der Padt, A., & van der Goot, A. J. (2017). Mildly refined fractions of yellow peas show rich behaviour in thickened oil-in-water emulsions. *Innovative Food Science & Emerging Technologies, 41*, 251–258. https://doi.org/10.1016/j.ifset.2017.03.009

Holt, N. W., & Sosulski, F. W. (1979). Amino acid composition and protein quality of field peas. *Canadian Journal of Plant Science, 59*(3), 653–660. https://doi.org/10.4141/cjps79-103

Kosson, R., Czuchajowska, Z., & Pomeranz, Y. (1994). Smooth and wrinkled peas. 2. Distribution of protein, lipid, and fatty acids in seed and milling fractions. *Journal of Agricultural and Food Chemistry, 42*(1), 96–99. https://doi.org/10.1021/jf00037a015

MacGregor, A. W. (2003). Barley - origin adaptation, and production. *Food Technology*, 379–382.

Otto, T., Baik, B., & Czuchajowska, Z. (1997). Microstructure of seeds, flours, and starches of legumes. *Cereal Chemistry Journal, 74*(4), 445–451. https://doi.org/10.1094/CCHEM.1997.74.4.445

Pelgrom, P. J. M., Berghout, J. A. M., van der Goot, A. J., Boom, R. M., & Schutyser, M. A. I. (2014). Preparation of functional lupine protein fractions by dry separation. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 59*(2P1), 680–688. https://doi.org/10.1016/j.lwt.2014.06.007

Pelgrom, P. J. M., Vissers, A. M., Boom, R. M., & Schutyser, M. A. I. (2013). Dry fractionation for production of functional pea protein concentrates. *Food Research International, 53*(1), 232–239. https://doi.org/10.1016/j.foodres.2013.05.004

Van Donkelaar, L. H. G., Noordman, T. R., Boom, R. M., & Van Der Goot, A. J. (2015). Pearling barley to alter the composition of the raw material before brewing. *Journal of Food Engineering, 150*, 44–49. https://doi.org/10.1016/j.jfoodeng.2014.10.024