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Shear-induced structuring as a tool to make anisotropic materials using soy protein concentrate

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1. Introduction

ABSTRACT

This research presents the formation of anisotropic, and partly fibrous, semi-solid structures with Soy Protein Concentrate (SPC) using the concept of shear-induced structuring. The morphological and mechanical properties of the structures obtained are analyzed using confocal scanning laser microscopy (CSLM), and large scale mechanical deformation analysis. We present process conditions leading to the formation of anisotropic structures in SPC and found that comparable conditions did not result in anisotropy when using soy protein isolate. Results indicate the importance of the dry matter content, the process temperature and the presence of carbohydrates in structure formation. CSLM pictures show that carbohydrates form a separate phase in the system, which is oriented upon processing. The need for high temperatures also required the development of next generation shearing equipment.

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According to prognoses, providing sufficient and healthy food in a sustainable manner will soon become one of the key challenges ([Paillard et al., 2014\)](#page--1-0). An increased consumption of products from plant-based components has potential to contribute to increased food security. One route to stimulate the consumption of those products is the development of plant-based products that resemble meat. Consumer studies indicate that a key success factor for a plant-based alternative is the fact that meat-replacing products mimic the meat texture as much as possible, stressing the importance of a fibrous structure ([Hoek et al., 2011\)](#page--1-0). The challenge therefore is to develop new process concepts aimed at the formation of fibrous structures using plant materials, and apply this to a broader range of plant materials.

An important process to make meat alternatives is extrusion. In such set-up, a slit die is connected in an extruder to align proteinrich materials, and to get fibrous or layered structures. Successful formation of anisotropic structures have been reported for soy protein concentrate (SPC), soy-protein isolate (SPI) - wheat gluten (WG) blends ([Cheftel et al., 1992](#page--1-0)), and Pea Protein Isolate ([Osen](#page--1-0)

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[et al., 2014\)](#page--1-0). In case of SPI, it was stated that the addition of a polysaccharide enhanced the fiber formation in case of extrusion ([Cheftel et al., 1992\)](#page--1-0). In addition to extrusion, spinning [\(Rampon](#page--1-0) [et al., 1999](#page--1-0)) or mixing/coagulation processes [\(Kweldam, 2003\)](#page--1-0) have been reported as techniques to make fibrous materials. Almost a decade ago, we developed an additional technique to make anisotropic protein materials. Focus was initially on dairy protein ([Manski et al., 2007b](#page--1-0)); more recently we reported on the formation of fibrous structures using SPI - WG mixtures [\(Grabowska et al.,](#page--1-0) [2014\)](#page--1-0). Explorative experiments with PPI gave layered structures ([Schutyser et al., 2015\)](#page--1-0). Here, we report our results using soy protein concentrate.

The technique applied, called shear-induced structuring, was based on the use of well-defined flow conditions, and required dedicated equipment, which was developed in-house (so called shearing devices). Compared to extrusion, structuring can be done at milder conditions evidenced by a much lower specific mechanical energy input. For an up-scaled shearing device with a couette design, it was shown that the specific mechanical energy (SME) at optimum process conditions was 18.5 kJ/kg, whereas in extrusion processes the SME varies between 200 and 1200 kJ/kg ([Krintiras](#page--1-0) [et al., 2016](#page--1-0)). The low energy input facilitates scale up, because viscous dissipation hardly influenced the process, leading to much better temperature control of the product. It explains why up-Scaling of a process using well-defined flow conditions was
E-mail address: atzeian vandergoot@wurnl (A.I. van der Goot) scaling of a process using well-defined flow conditions was

possible with hardly any changes in the process conditions and similar ingredients (SPI $-$ WG blend). Scaling-up is further facilitated by the fact that flow conditions can be quantified and kept similar in both devices. These types of shearing devices allow therefore processing on a small (retail) scale and on larger (factory) scale. An additional feature of this structuring technology is that deformation (rate) and residence time are decoupled, which gives an additional process parameter to control structure formation. The use of well-defined flow is suited to perform research aimed at understanding the structure formation process. This is in contrast with extrusion, which is still considered as a black box, and the design of the process is still based on empiricism rather than scientific insights [\(Chen et al., 2010, 2011\)](#page--1-0). Besides, the use of welldefined flow fields facilitates linking to studies in which model materials are investigated. Research on model materials present multiple examples of highly anisotropic structures by applying simple shear flow, though often in a diluted dispersion. From studies on model system, it became evident that the creation of an anisotropic structure requires the presence of structural domains that are susceptible to shear flow ([Sprakel et al., 2008; Van Loon](#page--1-0) [et al., 2014\)](#page--1-0). Alignment of domains (e.g. particles or micelles) into an anisotropic structure depends critically on achieving the right combination of interactions between domains, but also applying the right amount of shear flow to compensate the movements in the system (e.g. Brownian's motions and shear induced motions). Since our ambition is to structure plant materials without additives or chemical modifications, these domains should be naturally present in the plant material ideally. SPC is a raw material that potentially obeys those requirements naturally, because it consists of protein and carbohydrates, which are unlikely to be mixed on molecular scale. An additional advantage of using SPC instead of SPI is that production of SPC requires less intensive fractionation, which further increases the gain in sustainability and reduced raw materials costs ([van der Goot et al., 2016\)](#page--1-0). SPC is currently used as main raw material in existing meat alternatives that are often produced using extrusion. To make an anisotropic structure, a high extrusion temperature of about 140° C seems necessary ([Fang et al.,](#page--1-0) [2014; Liu and Hsieh, 2008\)](#page--1-0). In this study, we present our findings with SPC being structured in a next-generation shearing device. Besides, we hypothesize on the differences in structuring behavior between SPI and SPC.

2. Materials and methods

2.1. Materials

Soy Protein Concentrate (SPC) (Barentz) contained at least 67 wt % of soy protein on a dry basis, less than 1.5 wt% free fats, 8 wt% moisture and 8 wt% ash, according to the manufacturer's specifications (Solae Europe S.A).

Soy Protein Isolate (SPI) (Barentz) contains at least 90 wt% soy protein and has a fat content less than 1 wt%, based on information provided by the manufacturer (Solae Europe S.A., Missouri 63110 USA). It was used in combination with Soy Fiber to understand the role of protein and carbohydrates and their functional properties in the structure formation process. Differential scanning Calorimetry (DSC measurements) revealed differences in behavior of protein in SPC and SPI. In case of SPC, a clear thermal transition in SPC around 69 \degree C was observed, which could be related to protein denaturation. It was found that SPI did not show any thermal transitions anymore, suggesting complete denaturation of protein as a result of the separation process. Soy Fiber, abbreviated as SF, (Solae Europe S.A) is composed of insoluble fiber, soluble fiber and protein. SF contains at least 75 wt.dietary fiber on dry basis and no more than 12 wt% moisture. It was used to mimic the composition of SPC by combining it with SPI to understand its function in formation of fibrous structure.

Rhodamine B (Sigma R 6626, Sigma Aldrich) and Calcofluorwhite (Fluka, 18909, Sigma Aldrich) were used as staining agent when preparing samples for Confocal Laser Microscopy.

2.2. Methods

2.2.1. Structure formation

2.2.1.1. Equipment. Given the expected high processing temperature necessary for structure formation, we developed a next generation shearing device that is able to resist a water vapor pressure of 5 bars corresponding to a water boiling temperature of about 150 °C. The new device allows processing far above 100 °C. The design of a shearing device was based on equipment used in previously reported research ([Grabowska et al., 2014; Habeych et al.,](#page--1-0) [2008; Peighambardoust et al., 2004\)](#page--1-0). Fig. 1 shows the image of the upgraded version of the shear cell. High temperatures can be reached because of the improved closing system preventing water evaporation [\(Fig. 2\)](#page--1-0). An extra seal is added to create a pressure chamber in the system (indicated with a red arrow). Compressed air is used to keep the shear cell under pressure, preventing moisture evaporation at high temperatures. This new device can be used to process high-moisture materials up to a temperature of almost 150 \degree C successfully.

2.2.1.2. Preparation of the protein blends. Protein blends with different dry matter concentration $(35-50 \text{ wt})$ of soy protein concentrate (SPC) in water were prepared by thorough mixing by hand for $15-20$ min prior to processing. Approximately 90 g of the premix was placed inside the device, which was pre-heated to the set temperature. SPI dispersions were processed in a similar manner. Additionally, a blend consisting of SPI and SF was studied under shear flow. SPI (33.4 wt%) was hydrated for $15-20$ min with 55 wt% demineralized water and subsequently 11.6 wt% SF was added prior to structuring in the shear cell.

2.2.1.3. Structuring process. Process conditions used are shown in

Fig. 1. Upgraded version shear cell.

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