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Temperature-pressure-time combinations for the generation of common bean microstructures with different starch susceptibilities to hydrolysis

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ABSTRACT

In common beans, starch is enclosed by natural (micro)structural barriers influencing its behaviour during processing and digestion. Such barriers and their process-induced modifications could modulate nutrient delivery if adequate processing variables could be selected. In this study, the potential of different processing variables for generating common bean microstructures with different susceptibilities to *in vitro* starch hydrolysis was assessed. A traditional thermal treatment (95 °C, 0.1 MPa) and two alternative treatments including high hydrostatic pressure at room temperature (25 °C, 600 MPa) and at high temperature (95 °C, 600 MPa) were applied to common beans following a kinetic approach. (Micro)structural properties of (mechanically disintegrated) common beans were evaluated at each processing time. Mostly free, non-swollen and birefringent starch granules were obtained after mechanical disintegration of samples subjected to high pressure at room temperature. In mechanically disintegrated samples obtained by processes involving high temperature, either in combination with high pressure or not, there was major presence of cell clusters at early processing times (7–15 min) and individual cells at intermediate and long times (≥ 45 min). Following, specific process-induced common bean microstructures were evaluated in terms of *in vitro* starch hydrolysis kinetics. Rate constants of all microstructures obtained after high temperature treatments were similar, whereas final values of digested starch and initial reaction rates exhibited differences. The variations observed in the later parameters were correlated with the starch bio-encapsulation degree. Furthermore, in samples with the same starch bio-encapsulation degree (individual cells), differences in final digested starch and initial reaction rate were hypothesised to originate from differences in cell wall porosity/fragility.

1. Introduction

Diverse processing techniques have been used ever since ancient times to increase food palatability and safety. Moreover, food nutritional quality has gained major importance for both food producers and consumers throughout time. In fact, efforts to design food products capable to enhance health and wellness of (groups of) individuals based on their specific nutritional desires and needs are nowadays increasing (Floros et al., 2010). Depending on the target consumer group needs, improving or hampering nutrient delivery can be the aim. In recent years, the need for food products providing a slow glucose release upon consumption has grown into a general consensus, driven by the worldwide epidemic levels of obesity and diabetes (Hayat, Ahmad, Masud, Ahmed, & Bashir, 2014; James, Rigby, & Leach, 2004; Tilman & Clark, 2014). Food products with controlled sugar release would represent a part of the solution to the dietary requirements of diseased individuals and to the demands of health conscious consumers that cope with the challenging current dietary patterns.

From the group of carbohydrate-rich foods, common beans exhibit a remarkably low glycaemic index (Atkinson, Foster-Powell, & Brand-Miller, 2008). Starch digestibility and glycaemic response are affected (among other factors) by food structural characteristics which, in turn, can be tailored by processing (Fellows, 2017; Floros et al., 2010). In effect, processing is being considered as a useful tool to change food structure at different lengths and scales with the aim of tailoring food digestion *in situ*. Processing alters food structure, which subsequently has an impact on food digestive functionalities such as starch digestibility (Singh, Dartois, & Kaur, 2010). In particular, microstructural properties are currently receiving increased attention since it is known that the structures formed by interaction of food constituents greatly

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affect the digestive function of foods (Parada & Santos, 2016; Singh et al., 2010; Singh, Kaur, & Singh, 2013).

In common beans, starch granules are entrapped by natural (micro) structural barriers (*e.g.* cell walls) that impede its full swelling and gelatinisation during processing. Such structural arrangement is maintained upon application of thermal processing, thus playing a fundamental part on starch digestion. Prevalence of cell walls enclosing starch after thermal processing has been observed and identified by different authors as the main responsible for the limited starch digestibility of common beans in the gastrointestinal tract (Berg, Singh, Hardacre, & Boland, 2012; Dhital, Bhattarai, Gorham, & Gidley, 2016; Tovar, De Francisco, Bjorck, & Asp, 1991; Würsch, Del Vedovo, & Koellreutter, 1986). Furthermore, it has been recently reported that starch hydrolysis in legumes is also affected by the residual molecular starch order and the binding of enzymes to cell wall components (Bhattarai, Dhital, Wu, Chen, & Gidley, 2017). However, it is not known yet if the role of cell walls on starch digestion of common beans changes as processing conditions (*e.g.* different times) and/or variables (*e.g.* high pressure) are changed, neither it is clear if this structural barrier delays or completely hinders the enzymatic action on the enclosed substrate.

The effect of processing on food structure depends on the conditions applied. In carrots for example, high temperature causes softening by inducing pectin β-elimination reactions (Sila, Melbourne, Smout, Van Loey, & Hendrickx, 2006), while a combination of high temperature and high pressure results in pronounced texture preservation due to inhibition of β-elimination by elevated pressure (De Roeck, Sila, Duvetter, Van Loey, & Hendrickx, 2008). In common beans, softening during thermal processing has been related to pectin changes in the middle lamella and cell walls, either due to pectin β-elimination or pectin solubilisation controlled by other factors (Bernal-Lugo, Parra, Portilla, Peña-Valdivia, & Moreno, 1997; Njoroge et al., 2016; Yi et al., 2016). However, to the best of our knowledge, it has not been yet reported how the sensitivity towards cell breakage/separation upon application of mechanical stresses progresses as a function of thermal processing time. Likewise, the evolution of structural properties as a result of alternative treatments involving high hydrostatic pressure combined with room or high temperature has not been studied in this food matrix. These techniques are of interest since, as observed in carrots, the effect of high hydrostatic pressure on the reactions happening during softening can be different than the effect of high temperature. Finally, although the link between process-induced structural properties and starch hydrolysis of common beans has been explored for thermal treatments of a fixed time, neither the effect of processing time nor the role of high hydrostatic pressure on such relationship is known. In this study, combination of different processing variables followed by mechanical disintegration is hypothesised to be an effective tool to generate common bean microstructures with differences in terms of starch digestibility. Hence, the present work aims at exploring, on the one hand, the potential of processes combining temperature, pressure and time for generating common bean samples with specific (micro)structural properties and, on the other hand, the susceptibility to *in vitro* starch hydrolysis of specific process-induced common bean microstructures. Since whole common beans will always be disintegrated before reaching the gastrointestinal environment (either as part of the processing chain or in the first stage of digestion), mechanically disintegrated samples are considered throughout the study.

2. Materials and methods

The experimental set-up of this study (Fig. 1) included two parts. In the first part, activities were performed to assess the potential of processing as a tool to generate different common bean (micro)structures (Section 2.2). In the second part, the susceptibility to *in vitro* starch hydrolysis of specific process-induced common bean microstructures was evaluated (Section 2.3). A kinetic approach was followed in both parts.

2.1. Plant material

Dried Canadian wonder beans (*Phaseolus vulgaris* L.) were acquired from the Kenya Agricultural and Livestock Research Organisation (KALRO), Thika Station, Kenya. The plant material was harvested and dried in Kenya during the harvesting season of April 2015. Upon arrival at the laboratory in Belgium, beans were sorted and cleaned to remove defective seeds and/or foreign elements. The cleaned material was kept at − 40 °C until use.

2.2. Processing and structural characterisation of common beans

Combinations of temperature, pressure and time (Section 2.2.1), followed by dehulling and a mechanical treatment (Section 2.2.1.4), were used to generate common bean samples with distinct structural properties. Structure was characterised at three levels: *i*) texture (macrostructural property before mechanical disintegration, Section 2.2.2), *ii*) particle size distribution and *iii*) microscopic evaluation of cellular integrity (microstructural properties after mechanical disintegration, Sections 2.2.3 and 2.2.4).

2.2.1. Processing of common beans

Common beans were initially soaked in demineralised water (ratio 1:5) for 16 h at room temperature. Soaked beans were treated by one of the following processing techniques: high temperature processing (HT, 95 °C and 0.1 MPa, Section 2.2.1.1), high hydrostatic pressure processing at room temperature (HP, 25 °C and 600 MPa, Section 2.2.1.2), or a process combining high hydrostatic pressure and high temperature (HPHT, 95 °C and 600 MPa, Section 2.2.1.3). The impact of each treatment was studied as a function of time (0–150 min). Each processing condition (temperature-pressure-time combination) was carried out once.

2.2.1.1. High temperature processing. Soaked beans were put in contact with excess demineralised water in Duran glass bottles (ratio 1:5). Afterwards, a thermal treatment at 95 °C was applied for a given period of time (water bath WNB29, Memmert, Germany). An individual bottle was used for each processing time. A non-tight closing lid was used to avoid significant evaporation and pressure build-up during heating. After treatment, the glass bottles containing the processed beans were immediately submerged in an iced-water bath until they reached room temperature.

High temperature processing was also used to generate a reference sample of reduced structural barriers. Such sample consisted of fully gelatinised free starch granules, *i.e.*, a sample with negligible physical (*e.g.* intact cell walls) and chemical (non-gelatinised starch) barriers for starch hydrolysis. For this purpose, soaked beans were first dehulled and mechanically disintegrated in order to open the cellular structure. Following, the smashed cotyledons were submerged in excess demineralised water and subjected to a thermal process for approximately 10 min in a similar way as explained in previous paragraph. The treatment achieved complete starch gelatinisation, as confirmed by the absence of birefringence upon microscopic observation using polarised light (Section 2.2.4).

2.2.1.2. High pressure processing at room temperature. Treatments involving high hydrostatic pressure were carried out in a laboratory scale high-pressure unit (Resato, The Netherlands), consisting of six individual vessels (6 × 43 ml, 20 mm inner diameter) each surrounded by a heating coil connected to a thermostat. Soaked beans (five to six per cylindrical tube) were placed in cylindrical polyoxymethylene sample holders (85 mm long, 12 mm inner diameter and 3 mm thickness) in combination with demineralised water, which was added in excess and until the tubes were completely full. Upon filling, the cylinders were closed with a movable stopper to guarantee adequate pressure transmission between the pressure medium