Pasta highly enriched with vegetables: From microstructure to sensory and nutritional aspects

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LIST OF ABBREVIATIONS

BP	Broccoli powder
HMBP	Broccoli powder in-house produced
CBP	Commercial broccoli powder
BPulp	Broccoli pulp
SPS	Sweet potato starch
PGS	Pre – gelatinized starch
DWS	Durum wheat semolina
QB	Quartz beads
FG	Fish gelatin
G*	Complex modulus
CLSM	Confocal laser scanning microscopy
FITC	Fluorescein 5-isothiocyanate
HC	Hydrocolloid
LBG	Locust bean gum
GG	Guar gum
KG	Konjac glucomannan
HPMC	Hydroxypropyl methylcellulose
XG	Xanthan gum
WBC	Water binding capacity
CL	Cooking loss
SI	Swelling index
HPLC	High-performance liquid chromatography
GLs	Glucosinolates

ABSTRACT

A lifestyle that combines poor food choices with very low or no physical activity can result in the development of diseases such as obesity, and this is affecting a growing number of children. One of the most effective strategies to fight obesity combines physical activity and the consumption of low energy-dense foods, such as vegetables. Vegetables are known to have health benefits but are often non-appealing to children/adolescents due to their bitterness, undesired texture, and their low satiating capacity. One of the possible solutions to increase vegetable intake by children is to incorporate vegetables in a food matrix they like. Several studies have shown that pasta is very much appreciated by children, making it an ideal candidate for the development of vegetable-enriched foods. In this work, dried broccoli powder (BP) was used to enrich pasta-like products. We have investigated aspects that are important to sensorial properties and aspects related to possible health benefits. One aspect relevant to sensorial properties is rheology. The rheology of sweet potato starch (SPS) dough was drastically affected by high volume fractions of BP. This was caused by the swelling of the broccoli powder, up to a maximum of 7.6 times their original volume. In order to control this high swelling capacity of the particles two approaches were followed and both resulted in the prevention of particle swelling. The first was the use of hydrocolloids with high water binding capacity (e.g. xanthan gum) and the second was the use of a different matrix (durum wheat semolina (DWS)). DWS pasta did not show to be greatly affected by the incorporation of high amounts of broccoli powder. The acceptability of pasta products was assessed using a test panel. The results showed that all samples tested (0 – 30%BP) were acceptable, where 30% BP turned out to be on the limit of acceptability. Glucosinolates (GLs) are phytochemicals that are associated with the health benefits of broccoli. An increasing volume fraction of broccoli powder resulted in an increasing content of glucosinolates in dried cooked pasta. At volume fractions higher than 20% BP this effect levels off. From this work, we can conclude that as much as 20% BP can be added to DWS pasta to improve nutritional properties (in terms of GLs) while maintaining acceptable sensorial properties.

CHAPTER 1

GENERAL INTRODUCTION

1. Introduction

Worldwide, people's lifestyle is continuously changing [1, 2], and with respect to eating habits, it is changing in an unhealthy direction [3, 4]. Both developed and developing countries are experiencing a nutrition transition [5, 6]. This phenomenon is characterized by a decrease in physical activity and a too low consumption of vegetables and grains [1, 5, 7]. Eating habits are now characterized by an increase in the consumption of high energy-dense foods, i.e. foods with a high amount of calories per gram of food [8-10]. This lifestyle is one of the factors for the development of diseases such as obesity [1, 2, 10, 11], which is now acknowledged as a global epidemic [6, 12-15]. In turn, obesity has been linked to the development of other chronic diseases such as type II diabetes, hypertension, coronary heart disease and several types of cancer [4, 10, 16-18]. When it comes to childhood obesity, concerns increase, since there are strong indications that it will persist into adulthood [19-23]. One of the most effective strategies to fight this problem involves the combination of physical activity and the consumption of low energy-dense foods, such as vegetables, at an early age [8, 24, 25]. A hurdle for implementing this strategy is the fact that children often dislike vegetables [26, 27]. Vegetables are known to have health benefits but are often non-appealing to children/adolescents due to their bitterness, undesired texture, and their low satiating capacity [28-30]. One of the possible solutions to increase vegetable intake by children is to incorporate vegetables in a food matrix that they do like. Several studies have shown that pasta is very much appreciated by children [27, 31, 32], making it an ideal candidate for the development of vegetable-enriched foods. Besides that, pasta is also regarded by the WHO and FDA as a good matrix to be enriched [33-36]. The enrichment of pasta products is an old practice, e.g. protein enrichment is dating back more than five decades [37]. Enriching pasta with ingredients, like vegetables powder, represents challenges since the incorporation of particles will dilute the continuous matrix, resulting in more solids leaching into the cooking water and consequent undesirable textural properties (such as high stickiness and low firmness) [38-40]. This hampers the development of vegetable-enriched pasta with high fractions of vegetables. Commercially available vegetable–enriched pasta contains only 2 - 3% (w/w) of vegetable powder.

2. Pasta-like products

Pasta and noodles are staple food products in many countries. They differ from each other in many aspects, mostly in the "raw-material" used for their production. Pasta is usually produced from durum wheat semolina, whereas noodles are produced either from common wheat flour (and salt), or starches from different sources. In the latter case they are referred to as starch noodles [41, 42].

2.1. Pasta

Traditionally being an Italian product, pasta has become a worldwide consumed product due to its ease of transportation, handling, cooking and long shelf-life [43]. The most common method for the production of pasta is through extrusion. In this process, the flour is mixed with water (usually about 30 – 35% moisture [44, 45]), resulting in the formation of a dough that is forced through a die and then dried [46]. Due to its unique proteins that will form a very strong and visco–elastic network, wheat is the preferred cereal for the production of flour for pasta-making. Among wheat, durum wheat semolina is regarded as the best raw-material [43, 44, 47, 48]. The composition of durum wheat semolina can be divided into 3 main constituents, the main fraction being starch, varying between 70 and 80% of the total weight, followed by proteins, reaching up to 15% of the total weight and the remaining part is composed of small amounts of fiber, lipids, vitamins and minerals [43]. The proteins in durum wheat semolina are a mixture of albumins, globulins, glutenins and gliadins. The last two are capable of interacting with each other and with other components, forming intra and intermolecular disulphide bonds that will result in the development of a three dimensional visco–elastic gluten network [43, 48, 49]. Besides controlling the visco–elastic properties, protein content and composition also determine the quality of the flour and consequently the quality of pasta (such as firmness and extensibility) [44, 50, 51]. The level of adhesion and interaction between the starch granules and the protein matrix also has a contribution to the final product quality [52]. Durum wheat semolina is regarded to be the best raw-material for the production of pasta because of its high protein content, resulting in the formation of a dense gluten network [46, 53]. Gluten is responsible for the development of dough during mixing and extrusion, entrapping the starch granules in its network [54]. This visco–elastic network restricts starch swelling, maintaining the structure of pasta during cooking, and thus preventing cooking losses [47].

Besides this, gluten is also responsible for the elasticity and the *"al dente"* bite of pasta [46, 50]. This stresses the importance of gluten since the quality of pasta is mostly determined by its textural properties and cooking quality [55-57].

2.2. Starch noodles

Starch noodles are produced from pure starch, mostly derived from cereals (e.g. rice, maize), tuber/roots (e.g. sweet potato, cassava) and legumes (e.g. mung bean, pea) [58]. Starch is composed of two polysaccharides, amylose and amylopectin, and its characteristics depend on the properties of these two molecules (e.g. ratio amylose-amylopectin, composition, structure). In starch systems that do not contain gluten, a matrix is formed by pre-gelatinizing part of the starch [46, 58]. This process is responsible for the development of the visco–elastic properties of the dough. Gelatinization happens when starch granules are heated in excess of water, causing absorption of water and swelling to many times their original size [59]. As a consequence of this heating and swelling, the granules burst open allowing amylose to leach out, resulting in an increase of the viscosity of the suspension and in the formation of a continuous amylose phase [60-62]. In this continuous phase, the swollen starch granules are embedded. Inside the swollen starch granules there is still some amylose present which is surrounded by the amylopectin [62]. The quality of the starch noodles depends mostly on the physico-chemical, thermal, and rheological properties of the starch itself [58]. But even between the same type of starch, changes can occur due to differences in growing and local conditions [59]. When it comes to starch noodles, mung bean starch is regarded as the best raw material for the production of this type of noodles [42, 58, 63]. The quality of mung bean starch noodles has been attributed primarily to the high amylose content of the starch, its chemical structure and composition, and restricted swelling of the starch granules upon gelatinization [41, 58, 63-65]. Although it has unique properties, several attempts have been made to produce noodles from other starches with similar characteristics to mung bean starch, as this raw-material is rather expensive [66, 67]. Some of the different sources of starch that have been studied are tapioca [67], beans [68, 69], potatoes [42, 64, 65, 69, 70], peas [63, 71], maize [67, 72] and rice [66, 70]. Considering the many different varieties, wide availability and low price, sweet potato starch is a good substitute for mung bean starch in the production of starch noodles [42, 58]. Moreover, sweet potato starch noodles are already a very popular group of noodles in a large part of Asia and some sweet potato starch varieties can result in noodles with similar properties to that of mung bean starch [42, 58, 64, 73].

3. Enriched pasta-like products

The enrichment of pasta-like products already began more than five decades ago with the addition of soy protein [37]. Since then, enrichment, mostly with vegetable and legume flours, is common only in pasta made from durum wheat semolina, and only a few studies were conducted on the enrichment of starch noodles. Several reasons for the enrichment of pasta have been pointed out in literature, such as nutritional improvement, use of local raw materials, use of cereal by-products, production of gluten-free pasta or development of products with additional health benefits [74]. Nutritional improvement has been discussed most often since pasta lacks the essential amino acids lysine and threonine [33]. The enrichment often has a negative effect on the structural properties of this type of products, as the replacement of gluten proteins will dilute the network and thereby weakening it [36]. As far as structural changes are concerned, rheological measurements can give very valuable information [75] and several models have been proposed to describe rheological properties of these systems.

4. Rheology

As many other food products, pasta enriched with vegetables can be regarded as particle-filled systems, in which the vegetable powder and the starch granules are regarded as the particles. The concentration of particles will determine whether these systems can be regarded as dilute or concentrated suspensions. In the latter systems, the geometry, size distribution and arrangement of particles will determine the maximum packing fraction, which is the maximum volume fraction of particles that can be added to the suspension [76]. When the concentration of particles in a system is below the maximum packing fraction, it is called a filled system. When the system is at the maximum packing fraction, it is called a closely-packed system. Considering the type of arrangement between particles, the maximum packing fraction of identical spherical particles can be 0.52 for cubic arrangement, 0.64 for random close packing and 0.74 for a hexagonal close packed arrangement [77]. If the particles are deformable, the maximum packing fraction can go up to 0.96 [78].The rheological properties of closely packed systems are mostly influenced by the volume fractions and mechanical properties of the particles [79]. Theoretical models are available to describe the behavior of systems with different degrees of packing. When systems have a very low concentration of particles (dilute regime < 5%), their rheological behavior can be described by a variation of the Einstein equation (1906) [80]:

$$G^* = G_c^* . (1 + 2.5\phi) \tag{1.1}$$

In this generalized equation, $G*$ corresponds to the complex modulus of the system, G_c^* corresponds to the complex modulus of the continuous phase and ϕ is the volume fraction of the dispersed particles [80].

When the system has a concentration of particles slightly above the dilute regime, its rheological properties can be described by empirical models such as the one based on Batchelor (1977) [81]:

$$G^* = G_c^* . (1 + 2.5\phi + 6.2\phi^2) \tag{1.2}$$

When the concentration of particles in the system is high, the system's rheological properties can be described by mean field theories such as the Frankel and Acrivos model (1967) [82]:

$$G^* = \frac{9}{8} G_c^* . \left[\frac{\tilde{\phi}^{\frac{1}{3}}}{1 - \tilde{\phi}^{\frac{1}{3}}} \right] \tag{1.3}$$

In this generalized equation, $\tilde{\phi} = \phi / \phi_m$ where ϕ_m is the volume fraction of particles, at close packing [82].

When the concentration of particles in a system is below the maximum packing fraction, it is called a filled system, but if the concentration of particles approaches the high packing fraction, the system can become a cellular system. Alongside with particle-filled systems, cellular systems are also common among food products [79]. Cellular systems are characterized by the presence of cells filled with gas or liquid, surrounded by a soft-solid matrix or by the presence of large percentage of voids of air, also known as porous systems [79, 83]. The rheological properties of such systems are mostly determined by the mechanical properties of the cellular components [79].

5. Quality parameters of pasta-like products

The quality of pasta and starch noodles is defined by their cooking quality (cooking losses and swelling index), and their textural and other sensorial properties [34-36, 58]. Cooking loss is the amount of material that detaches from the product and goes into the cooking water and the swelling index is the amount of water that the pasta and noodles take up. For both pasta and starch noodles, high quality products should have low cooking losses. With regard to processability and textural and sensorial properties, starch noodles should have a low degree of stickiness, to facilitate the separation of the strands during the drying process, and should also be translucent and elastic [34, 41, 42, 64]. High quality pasta products are usually defined as having low stickiness and high firmness [34, 43, 46]. The enrichment of pasta-like products above a certain concentration often results in a negative effect on the products' properties, decreasing their quality [84]. In literature, the most reported problems of the enrichment of pasta-like products include a detriment in cooking quality, textural and sensorial properties [85, 86]. More specifically, the addition of flours other than wheat increases cooking losses, decreases firmness and increases stickiness of pasta and usually enriched products have a lower acceptability than pasta that has not been enriched [43, 44, 87]. These negative effects have been linked to the dilution of the network, since wheat flour or starch are replaced by vegetable/legume flours that do not contain proteins capable of forming a network [43, 85, 88]. To summarize, the ingredients and their mutual interactions determine the microstructure of the pasta-like products and, to a large extent, their quality characteristics.

6. Aim and Outline of thesis

Much has been done on the enrichment of pasta-like products, mostly with the aim of increasing the nutritional aspects, which focus mainly on the chemical characterization of the products. Properties such as rheology, microstructure and texture of enriched dough and pasta-like products has received less attention. The aim of this thesis has been to produce broccoli enriched pasta-like products with high volume fractions of broccoli powder and determine relations between product microstructure, rheological, textural, sensorial and nutritional properties.

In this thesis we first describe the effects of incorporating different amounts of broccoli particles into starch matrices (chapter 2). In the following chapters, we explored other matrices (chapters 3 and 4) with high volume fractions of particles in relation to their textural properties and we end with a discussion on the effect of the enrichment on the sensorial and nutritional properties (chapter 5). Finally we put our findings into perspective in the general discussion (chapter 6).

In **chapter 2**, we discuss the effects of adding broccoli particles produced in-house to sweet potato starch dough and also the effect of the particles addition on the rheological properties and microstructure of this matrix. The swelling capacity of the broccoli particles was studied to investigate its effect on the microstructure. We have found that these broccoli particles can swell to almost 8 times their original volume, when dispersed in an aqueous solution. For the addition of 20% (V/V) broccoli particles, this large degree of swelling, results in a large increase in the modulus of these systems. At these high concentrations, these systems can be described as closely packed systems that can behave like cellular materials, whereas systems with lower volume fractions of particles are still considered dispersions of particles in a gelled matrix. In **chapter 3**, several hydrocolloids with different water binding capacities were added to the sweet potato starch matrix containing different concentrations of broccoli particles. The influence of water distribution on the properties of the noodles was studied. We found that the hydrocolloids with the highest water binding capacity (hydroxypropyl methylcellulose (HPMC) and xanthan gum (XG)) were able to prevent both the broccoli particles and the starch granules from swelling. Besides rheological and microstructural properties, also texture and cooking properties of the cooked noodles were investigated and HPMC and XG also improved the cooking quality and some textural properties of the enriched pasta-like products. In **chapter 4**, two different matrices, sweet potato starch and durum wheat semolina, as well as broccoli particles with different properties (e.g. swelling index) were investigated. Sweet potato starch and durum wheat semolina were used to produce noodles and pasta, respectively. With the same amount of broccoli powder incorporated, pasta made from durum wheat semolina showed to be much less affected by the incorporation of the broccoli particles than noodles made from sweet potato starch. Unlike sweet potato starch noodles, durum wheat semolina pasta contains gluten that forms a very strong elastic network that prevents the broccoli particles from swelling. **Chapter 5** focuses on the nutritional, textural and sensorial properties of the enriched pasta-like products. For the nutritional characterization, the retention of glucosinolates (water soluble phytochemicals present in broccoli) in the pasta-like products after cooking was investigated. Between sweet potato starch noodles and durum wheat semolina pasta there was not a large difference in the amount of retained glucosinolates. The most significant observation was that dried cooked samples with 30% broccoli powder contained the same amount of glucosinolates as the samples with 20% broccoli powder, most likely related with the higher cooking losses of the samples with the higher concentration of broccoli powder. In the final chapter (**chapter 6**), the results of the previous chapters are compared with the findings from other authors and some suggestions for future work are presented.

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CHAPTER 2

INFLUENCE OF SWELLING OF VEGETABLE PARTICLES ON STRUCTURE AND RHEOLOGY OF STARCH MATRICES

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ABSTRACT

High volume fractions of dried broccoli particles (up to 20% V/V) were incorporated in a starch dough. The concentration of pre-gelatinized starch was varied between 10 and 30%. The addition of 20% (V/V) dried broccoli powder causes a significant increase in the shear modulus. For pure starch systems, the modulus increased with increasing pre-gelatinized starch concentration, whereas the shear modulus of starch systems containing broccoli particles decreased with increasing pre-gelatinized starch concentration. From viscosity measurements in the dilute regime, the swelling capacity of the broccoli particles was determined. When dispersed in water the dried broccoli particles can swell to up to 7.6 times, and this swelling capacity has a significant effect in the rheological behavior of starch dough systems. When volume fractions up to 20% (V/V) are incorporated, the system acts as a cellular material, instead of a gelled matrix with dispersed particles. This observation was confirmed with confocal scanning laser microscopy.

Keywords: Swelling index, Rheology, High volume fractions, Vegetable noodles

1. Introduction

Worldwide, the number of obesity cases has tripled in the last two decades and has now reached epidemic proportions [1, 2]. Several authors have shown that children tend to prefer food with high energy-density, due to the high palatability of sugar and fat, over nutrient-rich foods, such as vegetables [3-7]. The consumption of nutrient-rich food, in combination with physical activity, plays a protective role in the onset of chronic diseases, such as obesity, and could therefore be one possible solution for the child obesity problem [8-10]. From literature it is known that pasta and noodles, a low/medium energy dense food, is one of the food types that children like most, but vegetables are very much disliked [11-13]. Considering the previous, one strategy to increase the intake of vegetables by children is to incorporate vegetables in pasta or noodle-like products. The incorporation of vegetables or fruits in pasta products is not uncommon, but commercial products tend to contain either low concentrations of dried vegetables, or vegetable pulp (which often contains around 90% of water). Studies that consider pasta or noodle products with a high content of *dried* vegetable particles are far less common. Petitot et al [14] reported that the incorporation of 35% (w/w) split pea and fava bean powder had a very significant effect on these type of matrices, resulting in higher cooking loss and very firm and rubbery pasta. Several other authors have studied the incorporation of vegetable/fruit powders in these type of matrices, but their main focus was on parameters such as increase of protein content/quality, substitutes for gluten, increase of indigestible carbohydrates of pasta, increase of total phenolic content, and general nutritional enrichment [14-23]. The effect of the addition of high concentrations of dried vegetable particles on physical properties such as the complex shear modulus has received little attention. For complex food systems, rheological measurements can provide valuable information that can be used for the design and development of new food products, and for the processing of these products [24]. The rheological response of the dough is directly related to the quality of the end product and when major changes are seen in the dough rheology, quality parameters such as firmness, elasticity and stickiness will not meet the standard criteria [23, 24]. The aim of this work was to study the effect of the incorporation of low and high volume fractions of dried broccoli powder, on the structure and rheological properties of starch noodle dough.

2. Materials and Methods

2.1. Materials

Sweet potato starch (SPS, commercial grade) was kindly provided by Henk Schols, Food Chemistry Group, Wageningen University and high molecular weight fish gelatin was purchased from Multi Products B.V. (Amersfoort, The Netherlands). Quartz beads (QB) (diameter = 25 – 53 µm) were obtained from Tatsumori LTD. (Koriyama-city Fukushima, Japan), and broccoli was bought in the local supermarket, Albert Heijn (Wageningen, The Netherlands). Deionized water was used to prepare all samples.

2.2. Broccoli powder (BP) preparation

Broccoli was washed under running tap water, cut in small pieces and frozen at – 22 °C in a commercial freezer (Bosch). After approximately 16 h, the broccoli pieces were taken to the freeze-drier (Christ Epsilon 2-6D, Salm and Kipp, The Netherlands), for which particular freeze-drying settings can be found in Table 2.1.

Table 2.1 Freeze-drying settings for broccoli powder.

Time (H)	Temperature (°C)	Vacuum Pressure (mBar)
1	- 20	1000
2,5	-15	1000
3,2	-15	1
5,7	-10	1
9,2	4	1
18,8	4	0,001

After the broccoli was dried, it was immediately ground (Waring, commercial blender) until a fine powder was obtained. In order to compare the effect of the broccoli particles with model quartz beads, the broccoli powder was sieved in a sieving machine (Retsch® ZM 200, Germany) using 4 sieves with different mesh sizes (25, 53, 71 and 112 µm). Broccoli powder with a particle size distribution between 25 – 53 µm was used for all samples.

2.3. Sweet potato starch/broccoli dough preparation

Sweet potato starch (SPS) dough with a moisture content of 55% (V/V) was prepared according to Chen et al [25]. All the concentrations were expressed in % (V/V) and for that the densities of the broccoli powder and the starch were measured (Anton Paar DMA 5000, Graz, Austria). The density values were 1.35 and 1.3 g.cm^{-3}, for broccoli powder and sweet potato starch, respectively. The concentration of pre-gelatinized starch (PGS) was varied between 10 and 30%. Usually, for the pre-gelatinization of starch, a ratio starch:water of 1:9 is used. In this case, because of the high concentration of pre-gelatinized starch, this ratio could not be kept for all the concentrations. The ratios used can be found in Table 2.2.

Table 2.2 Ratios starch:water for the pre-gelatinization of starch.

PGS (% V/V)	Ratio starch:water	
	Blank sample	Broccoli sample
10	1:9	1:9
15	1:8	1:9
20	1:6	1:9
25	1:4	1:9
30	1:4	1:9

A recipient containing the amount of starch to be pre-gelatinized was placed in a water-bath at 100 °C. The water was mixed with the starch and the solution was stirred until it became translucent. After this step, the recipient containing the pre-gelatinized starch was moved to a water-bath at 40 °C. To this mixture, the rest of the starch and the water were added gradually to facilitate mixing. Stirring was continued until a uniform dough was obtained (figure 2.1a). The preparation of the dough containing broccoli powder is very similar to the preparation of the blank dough. In this case, broccoli powder replaced part of the starch, therefore, the ratio starch:water for the pre-gelatinization of starch could be kept at 1:9. The broccoli powder was added after the pre-gelatinization. When the recipient with the pre-gelatinized starch goes into the water-bath at 40 °C, the remaining ingredients, including the broccoli powder, were added to the solution and were stirred until the broccoli was evenly dispersed (figure 2.1b).

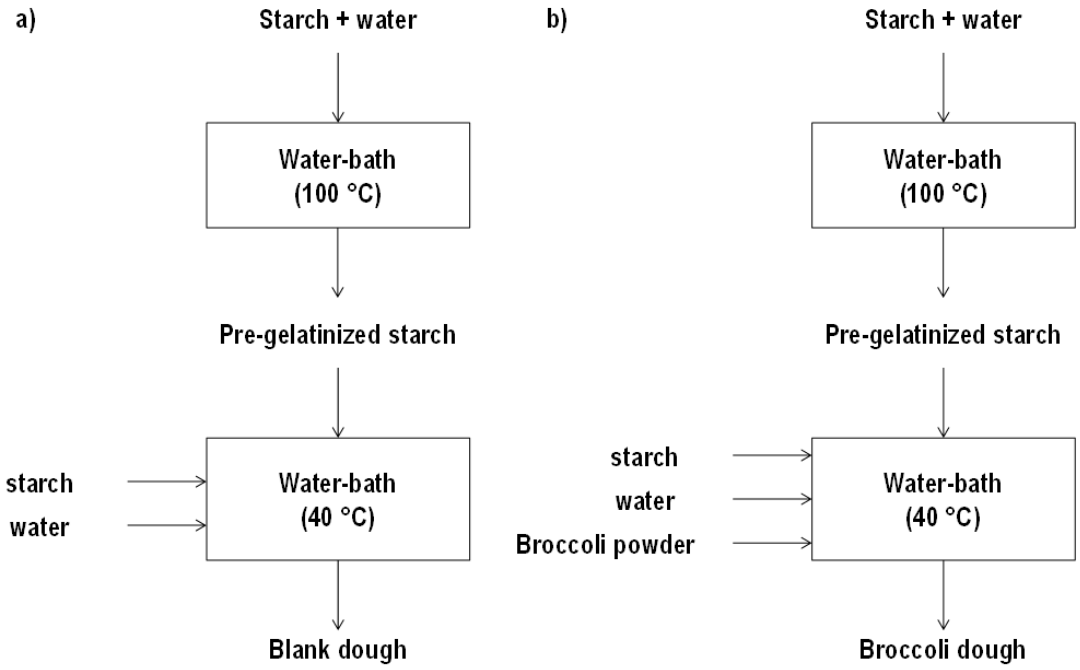

Figure 2.1 Schematic overview of the preparation of the blank noodle dough (a) and broccoli noodle dough (b).

2.4. Fish gelatin gel preparation

Stock solutions of 8% (w/v) fish gelatin (FG) were prepared according to the method described by Gilsenan and Ross-Murphy [26]. Gelatin granules were soaked in deionized water for 2.5 h at room temperature, followed by mechanical stirring in a water-bath at 70 °C during 15 min. When broccoli particles or quartz beads were added to the solution, the samples were mechanically stirred until a homogeneous distribution of the particles was obtained. The gelling temperature of this gelatin is 8 °C.

2.5. Shear rheology

Rheological experiments were performed with a Paar Physica MCR 301 (Anton Paar, Austria) stress-controlled rheometer. For the FG system, a concentric cylinder with a diameter of 17 mm was used. Before gelation, the samples were pre-sheared for 10 min at 20 °C, at a shear rate of 250 s^{-1}. This was followed by a time sweep of 1 h at 5 °C, at a strain of 0.01%, and a frequency of 1 Hz. For the system containing starch, serrated parallel plates with a diameter of 25 mm (PP25) were used. A resting period of 15 min was used after the sample was loaded, followed by a time sweep of 20 min at 25 °C, at a strain of 0.01% and a frequency of 1 Hz. For both systems, the time sweep was followed by a strain sweep, from 0.001 to 10%, with a frequency of 1 Hz, at 25 °C and 5 °C for starch dough and FG, respectively. All the time sweep tests were in the linear visco–elastic (LVE) region (data not shown).

2.6. Swelling behavior in dilute aqueous suspensions

The swelling behavior of the broccoli particles was determined using a capillary viscometer (Ubbelohde). A stock suspension of dried broccoli particles was used to prepare a series of dilutions with known volume fractions. The relative viscosity of these dispersions was determined at 25 °C. For low volume fractions, the relative viscosity of a suspension can be described by the Einstein equation (2.1):

$$n_r = 1 + 2.5\phi \tag{2.1}$$

Here η_r corresponds to the relative viscosity, i.e. the ratio of the viscosity of the suspension and the viscosity of the continuous phase, and ϕ is the volume fraction of the dispersed particles. The swelling factor can be extracted from the slope of the relative viscosities versus the volume fraction of the dry particles, divided by 2.5.

2.7. Confocal Laser Scanning Microscopy (CLSM)

Both blank and broccoli samples were analyzed by CLSM. The samples were prepared as described before (in section 2.3), and post-stained with a solution of 0.25% (w/w) Fluorescein 5-isothiocyanate (FITC) and 0.025% Rhodamin B. FITC will preferentially stain starch and Rhodamin B will preferentially stain protein. CLSM images, acquired in 1024x1024 pixel resolution, were recorded at 20 °C on a LEICA TCS SP5 Confocal Laser Scanning Microscope, equipped with an inverted microscope (model Leica DMI6000) and with a set of four visible light lasers (Leica Microsystems (CMS) GmbH., Mannheim, Germany). The excitation/emission wavelengths for FITC and Rhodamin B were 488/518 and 568/625 nm, respectively.

3. Results and Discussion

3.1. Shear rheology

In the production of pasta from wheat flour, mainly gluten proteins are responsible for the cooking and textural properties, by providing water absorption capacity, cohesiveness, viscosity, and elasticity to the dough [27, 28]. In pure starch products, that do not contain gluten, a matrix is formed by pre-gelatinization of part of the starch [16, 29]. When starch granules are heated in excess water, they absorb water and swell many times their original size. As a consequence of this swelling, the granules burst open and amylose leaches out. This results in an increase of the viscosity of the suspension and a continuous gel phase is formed by amylose. In this continuous phase, amylopectin and swollen starch granules are embedded [30-33]. The influence of different concentrations of PGS in samples with and without broccoli powder was studied and the results are shown in figure 2.2a and 2.2b, respectively. In the samples without broccoli (figure 2.2a), an increase in the complex modulus (G*) was seen as the concentration of PGS increased. The opposite was seen for samples containing 20% dried broccoli powder, where G* decreased up to a factor of 4 as the concentration of PGS increased (figure 2.2b).

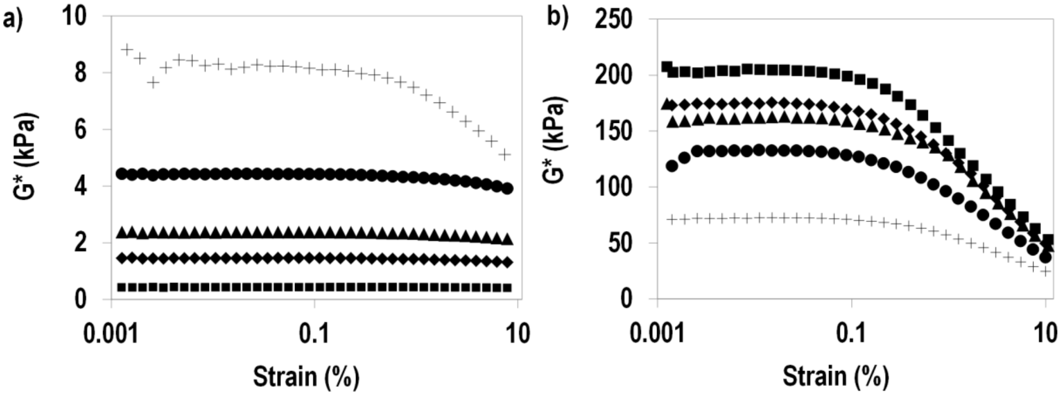

Figure 2.2 Rheological response of blank SPS dough (a) and SPS dough containing 20% BP (b) as a function of PGS concentration: 10% PGS (■), 15% PGS (◆), 20%PGS (▲), 25% PGS (●) and 30% PGS (+).

Moreover, the incorporation of broccoli powder into this type of matrix, shows a significant increase in the G* compared to samples without broccoli powder (figure 2.3). This increase is roughly between 1 and 2 orders of magnitude. Mean field theories, such as Krieger and

Dougherty [34] for dispersions of hard sphere particles predict a far smaller increase of the G* at comparable particle volume fractions. The significant increase of the modulus upon addition of broccoli, and the unusual dependence on PGS concentration could be explained by the fact that at 20% (V/V) of dry broccoli particles, the structure of the sample is not a dispersion of broccoli particles and starch granules in an amylose network, but a closely packed system of particles "glued" together by the amylose. During sample preparation the freeze-dried broccoli powder absorbs water and swells, leading to a significant increase in the effective volume fraction of particles, and resulting in a strong increase of the complex shear modulus.

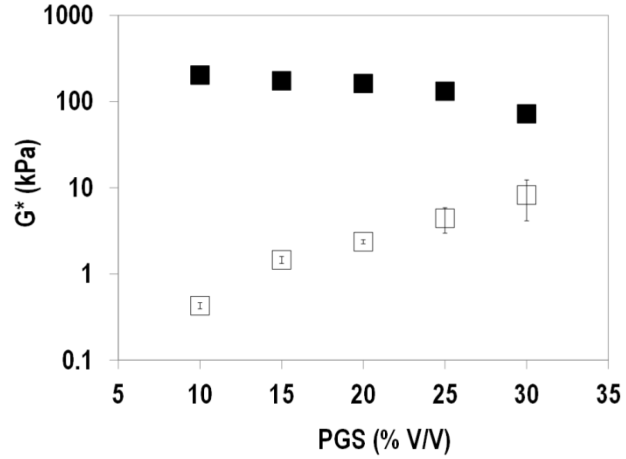

Figure 2.3 Comparison of the rheological response between blank (□) and 20% BP samples (■) as a function of the PGS concentration.

The decrease in G* as the fraction of PGS increases could be due to the fact that when the pre-gelatinized fraction is increased, the total volume fraction of particles decreases (because the total volume fraction is the sum of the volume fraction of the starch granules and the volume fraction of the broccoli particles) and the modulus of the closely packed system decreases.

3.2. Swelling behavior in dilute suspensions

To quantify the swelling behavior of the broccoli powder particles, viscosity measurements were performed on suspensions of broccoli powder in the dilute regime, with volume fractions between 0.0125 and 0.45% (dry basis), using an Ubbelohde viscometer (figure 2.4). From figure 2.4, the swelling factor was calculated by dividing the slope of the curve by 2.5, to get to a value of 7.6 for the swelling factor. This indicates that if the swelling of the broccoli particles is unhindered, the particles can swell by a factor of 7.6 (note that when the highest concentrations in figure 2.4 are multiplied by the swelling factor, the new concentration value is still within the dilute regime, < 5.0% V/V).

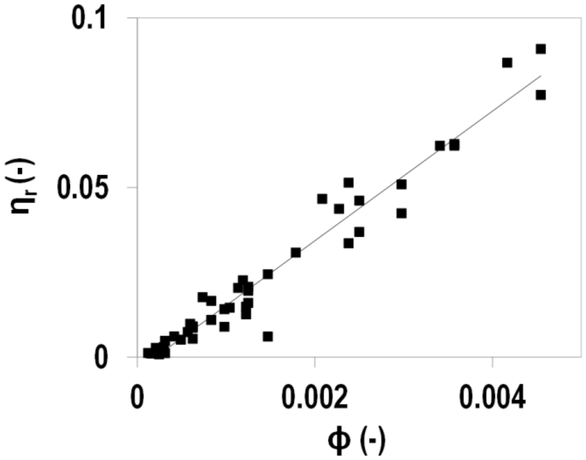

Figure 2.4 Swelling behavior of broccoli powder produced in-house. The data points were fitted with the Einstein equation (η_r = 1+ 2.5ϕ) with R^2 = 0.951.

To exclude the effect of the starch matrix and to confirm the importance of the swelling of the broccoli particles for the rheology of the samples, broccoli powder was dispersed into a more simple matrix (fish gelatin), and the rheology of this system was compared with that of a model system, consisting of quartz beads and fish gelatin (figure 2.5). Figure 2.5 also shows data for the modulus as a function of the corrected volume fraction of the broccoli particles, calculated using a swelling factor of 7.6. The lines in figure 2.5 are determined by fitting a visco–elastic generalization of the Frankel and Acrivos model [35] (Eq. 2.2) to the experimental data. In this model, the G* of a dispersion of hard spherical particles is given by

$$G^* = 9/8.\ G_c^*.\,[\tilde{\phi}^{1/3} / (1-\tilde{\phi}^{1/3})] \qquad (2.2)$$

where G_c^* is the G* of the continuous phase, $\tilde{\phi} = \phi / \phi_m$, and ϕ_m is the volume fraction of particles, at close packing. In spite the fact that our broccoli particles are not spherical nor hard, the model still describes the data fairly well.

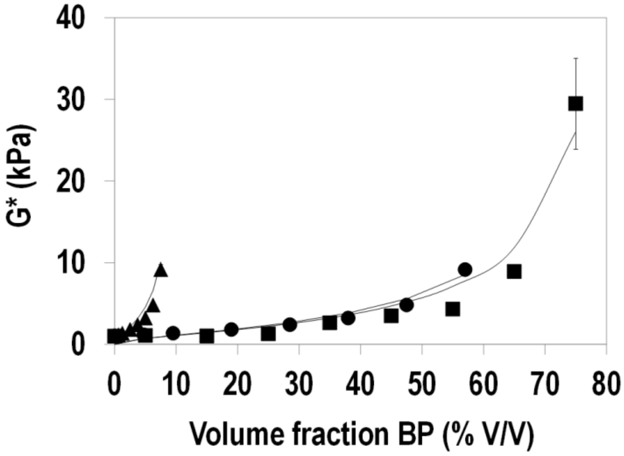

Figure 2.5 Comparison between the rheological response of FG gel containing QB (■), and FG gel containing BP, based on the volume fraction of dry particles (▲) and the swollen particles (●). Lines represent a fit with the generalization of the model of Frankel and Acrivos (1967).

From the results in figure 2.5, it is clear that, if the swelling of the particles is not taken into account, the system containing "dry" broccoli particles has a much steeper increase of the G* with particle volume fraction, then the system containing quartz beads. When the swelling is taken into account, the rheological response of the broccoli dough becomes very similar to that of the model system. This indicates that the broccoli particles also swell in more concentrated regimes and are very polydisperse, much like the quartz beads. By fitting the generalized Frankel and Acrivos model with the experimental data, the values of 11 and 83% were obtained as the theoretical maximum volume fraction for the systems containing broccoli powder in the dry and swollen state, respectively. The theoretical maximum volume fraction for the model system containing quartz beads was 84%. This confirms our hypothesis that at 20% dried broccoli powder, the system is not a dispersion of particles in an elastic matrix, but, due to the swelling capacity of the particles, forms a close-packed system, and can be considered a cellular material (figure 2.6).

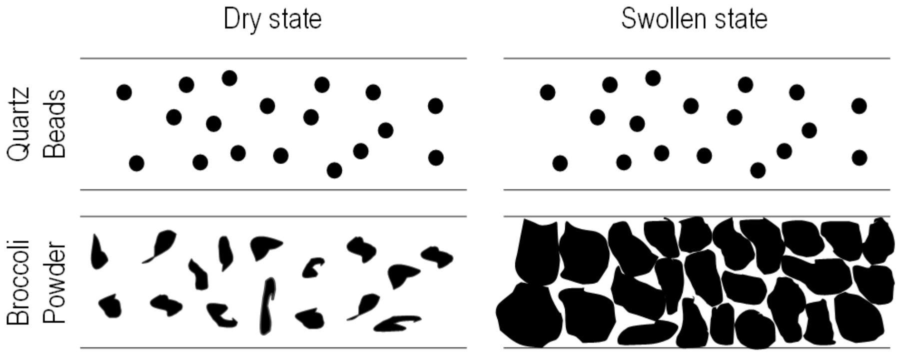

Figure 2.6 Schematic overview of the structure of the samples.

3.3. Confocal Laser Scanning Microscopy (CLSM)

Samples with 0, 4 and 20% (V/V) broccoli powder and with 10 and 30% PGS were analyzed with CLSM (figures 2.7 a – f). The samples with 4 and 20% broccoli powder were prepared as a representative of a low and a high fraction of particles. All the samples were labeled with a solution of 0.25% FITC and 0.025% Rhodamin B. FITC will preferentially label starch and Rhodamin B will preferentially label protein, but (to a lesser degree) Rhodamin B can also label starch, and FITC can also label protein [36-38]. Figure 2.7a shows a sample that does not contain any broccoli powder and contains a small amount of PGS. In this sample, a very small amount of matrix is present, labeled in green, and it is completely filled with starch granules, labeled both in red and in green. This happens because both labeling agents are able to label starch. Comparing figure 2.7a (10% PGS) with figure 2.7d (30% PGS), the difference in the volume fraction of matrix present becomes clear. A higher percentage of starch pre-gelatinization results in a higher volume fraction of matrix and a lower amount of incorporated starch granules in the matrix. When broccoli powder is incorporated in the matrix, the starch is replaced by an equal volume of broccoli powder. In figures 2.7b, 2.7c, 2.7e, 2.7f, the starch granules and matrix are labeled in green and the broccoli powder labeled in red/orange. In these samples, Rhodamin B is labeling the protein present in the broccoli, which is around 20% of the dry matter. In figure 2.7b the broccoli powder and the starch granules are densely packed and glued together by the matrix.

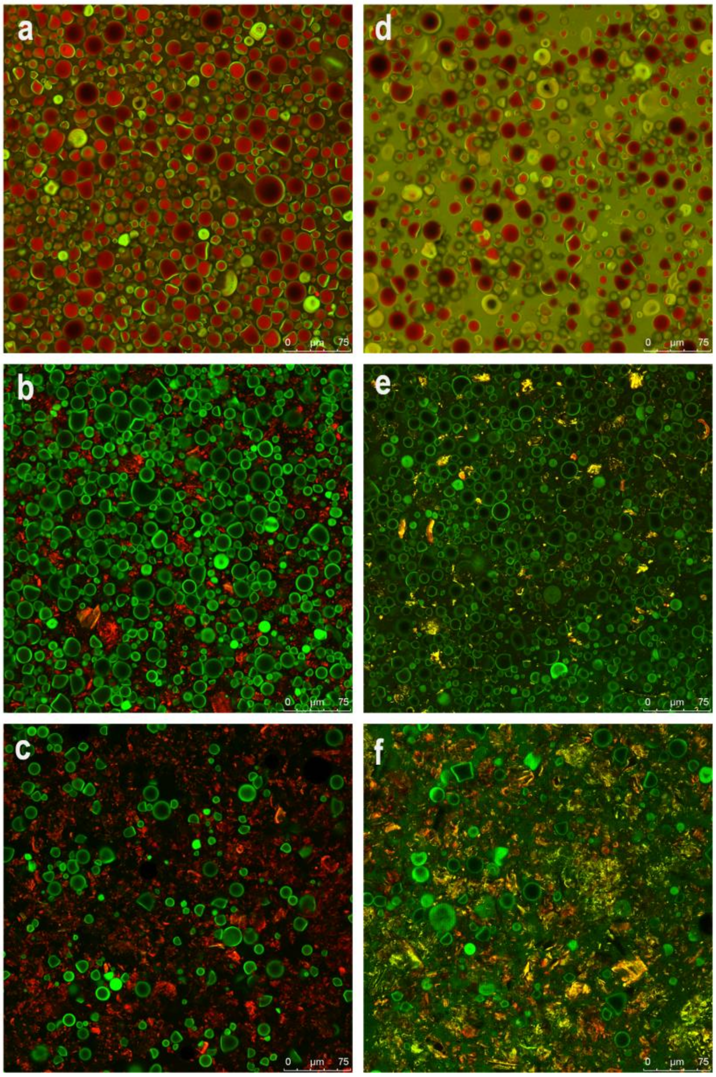

Figure 2.7 CLSM images of the starch matrices containing 0, 4 and 20% BP in 10% PGS (a – c, respectively) and in 30% PGS (d – f, respectively). Scale bar is 75 µm.

Comparing this sample with the sample containing the same amount of broccoli particles and 30% PGS (figure 2.7e), we see that the latter has a higher volume fraction of matrix available with fewer starch granules, evenly dispersed, compared to the matrix with 10% PGS. When the samples containing 20% broccoli powder, with different amounts of PGS, are compared, it is possible to see two different systems. The sample with 20% broccoli powder and 10% PGS is a closely packed system of broccoli particles and starch granules glued together by the matrix, and the sample with 20% broccoli powder and 30% PGS is a starch gel filled with broccoli particles and fewer starch granules. When the amount of PGS increases, the total volume fraction of particles, which is the sum of the starch granules and the broccoli particles, decreases, leading to a weaker system. In samples containing 20% broccoli powder and 10% PGS (figure 2.7c) black regions are present in the matrix, meaning that the samples were inhomogeneous. The sample with the same amount of broccoli particles and 30% PGS (figure 2.7f) was more homogeneous. This shows that at these high particle loadings, it is difficult to mix all ingredients and get a homogeneous structure.

The results obtained from CLSM are in accordance with the rheological measurements. In the CLSM pictures it was possible to observe the effect of the difference in PGS concentration and the addition of broccoli particles. The blank samples can be considered as a gelled matrix with starch granules incorporated. When broccoli powder is added to the system with 10% PGS, the volume fraction of particles increases and the system became close packed, behaving like a cellular material. G* is proportional to the volume fraction. When the concentration of PGS increased to 30%, the G* of the system decreased as a result of a decrease of the *total* volume fraction (broccoli particles and starch granules). This was also confirmed by the CLSM pictures, where it was observed that more matrix was formed as a result of the gelatinization of the starch particles, thereby decreasing the volume fraction of particles present in the matrix.

4. Conclusions

The addition of high volume fractions of dried broccoli powder to starch noodles has a significant effect on the rheology of these systems. We have shown that the swelling of the vegetable particles is a major factor in the structure and rheology of starch noodles containing broccoli particles. In dilute suspensions the broccoli particles can swell to approximately 7.6 times their original size. This high swelling capacity limits the amount of particles that can be incorporated into a starch matrix. As a result of the swelling, the noodle dough at volume fractions above about 10% dried particles is not a dispersion of particles in an elastic matrix, but in fact a cellular material, consisting of swollen vegetable particles, glued together by amylose.

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CHAPTER 3

CONTROLLING RHEOLOGY AND STRUCTURE OF SWEET POTATO STARCH NOODLES WITH HIGH BROCCOLI POWDER CONTENT BY HYDROCOLLOIDS

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ABSTRACT

Incorporating high volume fractions of broccoli powder in starch noodle dough has a major effect on its shear modulus, as a result of significant swelling of the broccoli particles. Several hydrocolloids with distinct water binding capacity (locust bean gum (LBG), guar gum (GG), konjac glucomannan (KG), hydroxypropyl methylcellulose (HPMC) and xanthan gum (XG), were added to systems with 4 and 20% (V/V dry based) broccoli particles, and the effect of this addition on dough rheology, mechanical properties and structure of cooked noodles was investigated. Hydrocolloids with low (LBG and GG) and intermediate (KG) water binding capacity had no significant effect on shear rheology of the dough. Adding hydrocolloids with high water binding capacity (HPMC and XG) decreased the shear modulus of dough with 20% broccoli powder significantly. CLSM analysis of cooked noodles showed that in samples containing xanthan gum there was also an inhibition of swelling of starch granules. Strength and stiffness of cooked noodles with 20% broccoli powder were higher for samples containing XG, than samples without XG. The cooking loss and swelling index of samples with added hydrocolloids were slightly lower than samples without hydrocolloids. Our results showed that hydrocolloids with high water binding capacity can be used to control the degree of swelling of broccoli powder and starch granules in starch noodle products, and thereby control both dough rheology and textural properties of the cooked noodles.

Keywords: Noodles, Vegetable particles, Particle swelling, Hydrocolloids, Rheology

1. Introduction

Incorporating high volume fractions of vegetable particles in pasta-like products can increase the nutritional value of these products [1-3]. But this incorporation has a significant effect on the rheological properties of the dough as described in chapter 2, and also on the properties of cooked noodles [4]. For example, the addition of legume flours to durum wheat semolina has been reported to cause deterioration in the cooking quality and textural properties of these products [4-7]. In chapter 2, we incorporated 20% (V/V) of dried broccoli powder in a starch matrix, and observed that this incorporation caused an increase in the shear modulus of the dough by two orders of magnitude, in comparison with samples without broccoli. This rather large increase in shear modulus was found to be caused by the swelling of the broccoli powder. The swelling behavior of the powder was studied and it was found that in dilute dispersions they can swell to up to 7.6 times their original volume. As a result of this swelling, at high volume fractions of particles (> 11% V/V dry basis) the system can no longer be considered a dispersion of particles in an elastic matrix, but is in fact a cellular material in which starch granules and vegetable particles are closely packed. In the latter type of system, the modulus of the system is determined by the volume fraction and mechanical properties of the particles, whereas in a system that consists of a gelled matrix with dispersed particles, the modulus is mostly influenced by the particle volume fraction and mechanical properties of the gel matrix [8]. The significant swelling of the powder limits the amount of broccoli that can be incorporated in the starch noodles. In the work described in this chapter, different hydrocolloids were added to the starch-broccoli system in an effort to limit the degree of swelling of the particles. Five hydrocolloids (locust bean gum, guar gum, konjac glucomannan, hydroxypropyl methylcellulose and xanthan gum) were selected based on their distinct water binding capacity. We investigated the effect of the addition of these hydrocolloids on the swelling of the broccoli particles, by studying the rheology and microstructure of noodles with 4 and 20% (V/V) broccoli powder. Textural properties of the cooked noodles were also studied. Several authors have studied the effect of hydrocolloids on starch and found that some hydrocolloids are capable of limiting the gelatinization of the starch granules [9-14]. Based on this, our expectation was that a hydrocolloid with a high water binding capacity could diminish the swelling of the broccoli and starch particles, by competing with the particles for the available water. Limiting the extent of swelling can be used to incorporate more broccoli particles in the noodles, or to control their textural properties.

2. Materials and Methods

2.1. Materials

Sweet potato starch (SPS, Mong Lee Shang), was kindly supplied by Deximport (Barendrecht, The Netherlands). The hydrocolloids (HC), xanthan gum (XG), locust bean gum (LBG) and guar gum (GG) were kindly provided by Cargill Texturizing Solutions (Sas van Gent, The Netherlands). Konjac glucomannan (KG) and hydroxypropyl methylcellulose (HPMC) were purchased from Konjac Foods (Sunnyvale, USA) and Sigma-Aldrich (St. Louis, USA), respectively. Broccoli powder (BP) was prepared according to the method described in section 2.2 of chapter 2. Commercial pasta, tagliatelle verdi (1.5% spinach powder, Mamma Lucia) was bought in supermarket Real (Guetersloh, Germany) and it was used for comparison since comparable commercial noodle products are not available on the market. Deionized water was used to prepare all samples.

2.2. Sweet potato starch and hydrocolloid dough preparation

The sweet potato starch and hydrocolloid dough was prepared by dissolving the hydrocolloid powder in the water used for the pre-gelatinization of the starch. When the hydrocolloid was completely dissolved, 10% of the total starch was added to the solution for pre-gelatinization. For pre-gelatinization, the ratio starch:water used was 1:9 and it took place in a water-bath with boiling water. The solution (hydrocolloid + water) was mixed with the starch and stirred until a homogeneous solution was obtained (≈ 1 – 2 min). After this, the dough consisting of pre-gelatinized starch and hydrocolloid was moved to a water-bath at 40 °C and the rest of the starch and water were added gradually to facilitate mixing. Stirring was continued until a uniform dough was obtained. The temperature of 40 ^{o}C is far below the gelatinization temperature, so most of the starch is not swollen and present as granules. Three different sets of samples were prepared: a so-called blank dough, with broccoli powder but no hydrocolloid added, a dough with hydrocolloid and no broccoli powder added, and a dough with hydrocolloid and broccoli powder added. Broccoli powder, 4 and 20% (V/V) was incorporated after the starch dough was prepared and the solution was stirred for 5 – 6 min. When broccoli powder was included, part of the starch was replaced by an equal volume of broccoli powder. The noodle recipe was based on a total water content of 55% (V/V).

2.3. Noodle production

The blank dough was placed in 10 ml syringes (Plastipak, Italy) and the noodles were produced by depressing the plunger of the syringes with a texture analyzer (TA.XT Plus, Stable Micro Systems, Surrey, UK), at 2mm/s and with a load cell of 5 kg. After the noodles were produced, they were dried in an oven at 42.5 ± 2.5 °C for 4h. The vegetable noodles were produced by passing the dough through a commercial sheeting/cutting machine and dried for 5h at 42.5 ± 2.5 °C. Different times were used for the drying step so that the blank and vegetable samples had the same moisture content. Until further analysis, all the samples were kept in a desiccator. Two different methods for the production of blank and vegetable noodle were used because, when the moisture content was kept constant, the blank dough was too liquid for the sheeting/cutting-machine and the vegetable dough was too tough to be extruded through a syringe.

2.4. Water Binding Capacity

The water binding capacity (WBC) of the hydrocolloids and broccoli powder was measured by two different methods, the Baumann capillary method, done according to Wallingford and Labuza [15], and the centrifugation method, according to Elhardallou and Walker [16]. In the Baumann capillary method, the determination of the WBC was made by placing approximately 10 mg of hydrocolloid on top of the glass filter and measuring the water uptake over time, until equilibrium was reached. Water evaporation was also taken into account. In the centrifugation method, 1 g of sample was weighed into 50 ml plastic tubes and the samples were centrifuged (Avanti J-26 XP Beckmann, Beckmann Coulter, USA) at 16,040 g for 1 h. For both methods an average of three measurements was taken.

2.5. Shear rheology of dough

Rheological experiments were performed on the dough samples with a Paar Physica MCR 301 (Anton Paar, Austria) stress-controlled rheometer with serrated parallel plates with a diameter of 25 mm (PP25) and a gap of 1 mm. After loading the sample in the rheometer, all the samples had a resting period of 15 min. A time sweep of 30 min at 25 °C, at a strain of 0.01% and a frequency of 1 Hz was performed after the resting period. Subsequently, a strain sweep was done, with strains from 0.001 to 10%, with a frequency of 1Hz, at 25 °C, during

30 min. All the values that were used from the rheological measurements were taken from the linear visco–elastic region. In order to study the effect of each hydrocolloid, the results were expressed in terms of a relative complex modulus, $G_r^* \equiv G_{SPS+BP\,(+HC)}^*/G_{SPS\,(+HC)}^*$, where $G_{SPS+BP\,(+HC)}^*$ corresponds to the G^* of the SPS matrix containing broccoli powder (and hydrocolloid added) and $G_{SPS\,(+HC)}^*$ is the G^* of the SPS matrix (and hydrocolloid added).

2.6. Confocal Laser Scanning Microscopy of dough and cooked noodles

Both dough and cooked noodles were analyzed by confocal laser scanning microscopy (CLSM). The samples were prepared as described before in this chapter (Section 2.2) and were analyzed the same day. The uncooked dough (before being processed into strands) was cut with dissecting blades in a cubic shape with dimensions of 3×3×3 mm (approximately). The cooked noodles, that had a rectangular shape and a cross section area of approximately 5 mm^2, were cut in pieces of 3 mm length. Both dough and noodles were post-stained with a solution of 0.25% (w/w) Fluorescein 5-isothiocyanate (FITC) and 0.025% Rhodamin B in water. FITC will preferentially stain starch and Rhodamin B will preferentially stain protein. CLSM images, acquired in 1024x1024 pixel resolution, were recorded at 20 °C on a LEICA TCS SP5 Confocal Laser Scanning Microscope, equipped with an inverted microscope (model Leica DMI6000) and with a set of four visible light lasers (Leica Microsystems (CMS) GmbH., Mannheim, Germany). The objective used for all experiments provided a 10 (HC PL APO 10x/0.40 CS) or 20 (HC PLAPO 20x/0.70 IMM/CORR CS) magnification with a zoom of 1 or 2. The samples were sliced with a dissecting blade to create a smooth surface for the CLSM. The excitation/emission wavelengths for FITC and Rhodamin B were 488/518 and 568/625 nm, respectively.

2.7. Cooking quality

Cooking loss (CL) and swelling index (SI) were determined according to Tudorică et al [17]. All tests were performed in duplicate. For the cooking loss, the cooking and rinsing water of each sample were evaporated at 105 °C. The residue was weighed and reported as a percentage of the weight of the dry starch noodles before cooking. The swelling index of the cooked noodles was determined after drying the cooked sample at 105 °C and calculated as $SI \equiv (w_{\text{cooked noodle}} - w_{\text{after drying}})/(w_{\text{after drying}}) * 100$,

where $w_{\text{cooked noodle}}$ is the weight of the noodle after cooking and $w_{\text{after drying}}$ is the weight of the dried noodles.

2.8. Texture Analysis of cooked noodles

Prior to texture analysis, the noodles with 4 and 20% BP were cooked for 6.5 and 5 min, respectively. The optimal cooking time was determined according to Collado et al [18]. After that, they were rinsed, drained and analyzed within 20 min, being at room temperature at the moment of analysis. The texture analyses experiments were performed using a TA.XT Plus Texture Analyser (Stable Micro Systems, Surrey, UK). Texture parameters were measured under tension using the tensile grip A/GT and a 5 kg load cell (pre-test speed, test-speed and post-test speed of 1, 3 and 10 mm/s, respectively, and a trigger force of 5 g for noodles with 20% BP, and 0.5 g for noodles with 4% BP). Foamy material was placed between the upper and lower grip of each side to prevent breaking of the noodle strands at the edges. The grips were always tightened with the same distance between them, which was measured with a digital caliper (Mitutoyo, USA). Each single strand tested had a cross sectional area of approximately 5 mm² and a length of 40 mm. The apparent fracture stress ($\sigma_f \equiv F/A$), Hencky strain ($\varepsilon_H \equiv \ln L/L_o$) and the Young's modulus (E_u) were determined. F is the extension force, A is the cross sectional area of the starch noodle, L_o is the original length, and L the current length. The fracture stress (Pa), Hencky strain (-) and Young's modulus (Pa) are related to the strength, extensibility and stiffness of the noodles, respectively [8]. Reported values are an average of two different samples of the same concentration, each measured in triplicate.

3. Results and Discussion

3.1. Water Binding Capacity

The water binding capacity (WBC) values of the hydrocolloids and the broccoli particles are presented in Table 3.1. The Baumann and centrifugation method gave slightly different values for the same hydrocolloid. The hydrocolloid with the highest water binding capacity was XG (24.1 ml H_20/g XG), followed by HPMC (18.2 ml H_20/g HPMC), KG (15.5 ml H_20/g KG), GG (11.2 ml H_20/g GG) and LBG (8.0 ml H_20/g LBG). Wallingford & Labuza [15] and Sánchez, Bartholomai & Pilosof [19] have tested three of the hydrocolloids that we tested, namely XG, GG and LBG with the Baumann capillary and they found the same order of WBC (XG with the highest WBC, GG lower than XG and LBG with the lowest WBC). The water binding capacity mentioned in the remainder of this discussion refers to the results obtained with the Baumann capillary method.

Table 3.1 Water binding capacity of the hydrocolloids and broccoli powder determined by two different methods, Baumann and centrifugation.

	Water Binding Capacity	
	Baumann (ml H_20/g solids)	**Centrifugation** (g H_20/g solids)
XG	24,1 ± 0,757	32,4 ± 4,038
HPMC	18,2 ± 1,458	-
KG	15,5 ± 1,102	15,0 ± 2,343
GG	11,2 ± 0,135	12,9 ± 1,873
LBG	8,0 ± 0,583	11,7 ± 0,666
SPS	6,3 ± 0,471	5,4 ± 2,192
Broccoli freeze-dried	5,2 ± 0,477	4,6 ± 0,778
Commercial broccoli powder	4,6 ± 0,089	-
Commercial broccoli powder sieved	4,8 ± 0,103	4.3 ± 0.529

3.2. Shear Rheology of dough

In chapter 2 we have seen that the swelling of the broccoli particles had a very large effect on the rheological properties of starch dough with broccoli particles incorporated. It was observed that when broccoli particles were added to a system with 10% pre-gelatinized starch, the complex modulus increased by more than 2 orders of magnitude. The swelling behavior of these particles was studied and it was found that, in a dilute regime, they can swell to up to 7.6 times their original volume. As a result of the swelling of the particles, at a volume fraction of 20% the dough is not a dispersion of solid particles in an elastic matrix, but in fact a cellular material, in which broccoli particles and starch granules are closely packed.

From figures 3.1 to 3.5 it is possible to see that the addition of different hydrocolloids to sweet potato starch dough gave different results for the systems containing 4 and 20% BP.

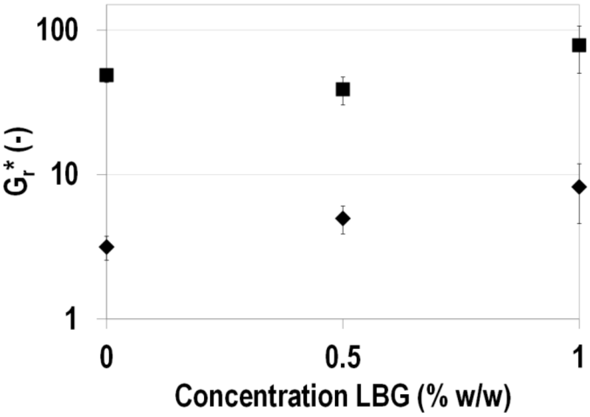

Figure 3.1 Effect of LBG on the rheological properties of starch dough with 4 and 20% BP (♦ and ■, respectively).

In figure 3.1 the systems containing LBG are shown. In the samples with 4 and 20% BP no significant difference was observed between the blank sample and the ones with 0.5 and 1% LBG. The samples containing GG are shown in figure 3.2.

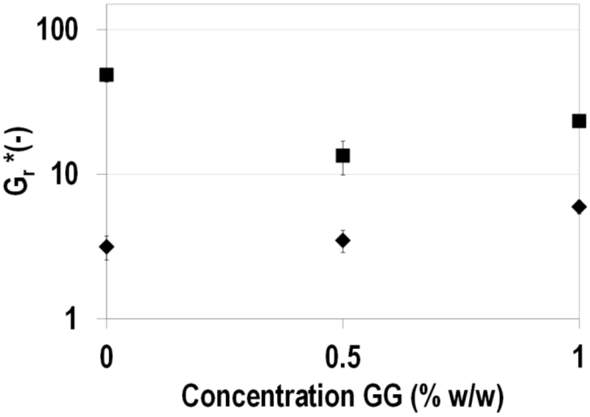

Figure 3.2 Effect of GG on the rheological properties of starch dough with 4 and 20% BP (♦ and ■, respectively).

When GG was added to the sample containing 4% BP, no differences were seen in comparison with the blank sample. In the sample containing 20% BP and GG, it was possible to observe a small decrease in the modulus for both 0.5 and 1% hydrocolloid, with respect to the blank. Figure 3.3 shows the effect of KG. When KG was used, a similar trend was seen for systems containing 4 and 20% BP. At 0.5% KG, there was an increase in the modulus and with 1% KG, the modulus decreases again, and was in the same range as the sample without KG.

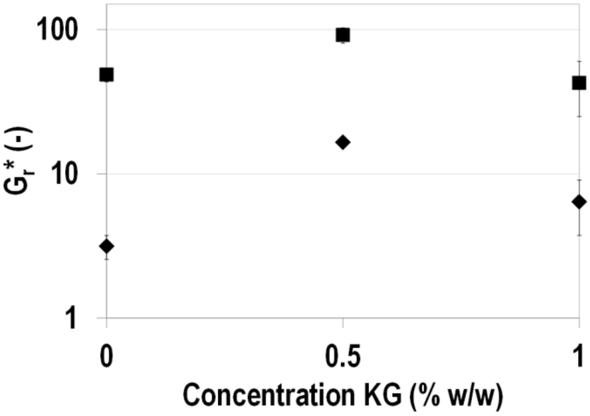

Figure 3.3 Effect of KG on the rheological properties of starch dough with 4 and 20% BP (♦ and ■, respectively).

In figures 3.4 and 3.5 it is possible to see the effect of HPMC and XG, respectively. For both hydrocolloids, compared with the blank, no differences were observed in the modulus of the sample with 4% BP, independently of the concentration of the hydrocolloid used.

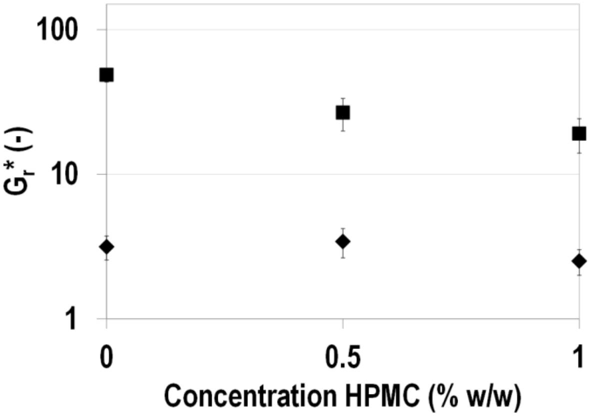

Figure 3.4 Effect of HPMC on the rheological properties of starch dough with 4 and 20% BP (♦ and ■, respectively).

At 20% BP, both hydrocolloids lower the modulus of the system. For HPMC the decrease was smaller than for XG and the difference between 0.5 and 1% HPMC was not significant. For XG, the difference between the blank sample and 0.5% XG was significant, and between the blank sample and 1% XG the difference in relative complex modulus was almost 1 order of magnitude. From these results we can say that only the hydrocolloids with the highest water binding capacity, namely HPMC and XG, were capable of lowering the modulus of the systems with 20% BP incorporated. The fact that this decrease was seen in the dough, which was not cooked yet (and where most of the starch granules are not swollen), suggests that HPMC and XG prevented the swelling of the broccoli particles to some extent, decreasing the total volume fraction of particles, and consequently the modulus of the system. Unlike what we observed for dough containing broccoli powder, when XG was added to solutions of only sweet potato starch, an increase in the elastic properties was observed (data not shown). The same results were found by Choi & Yoo [20].

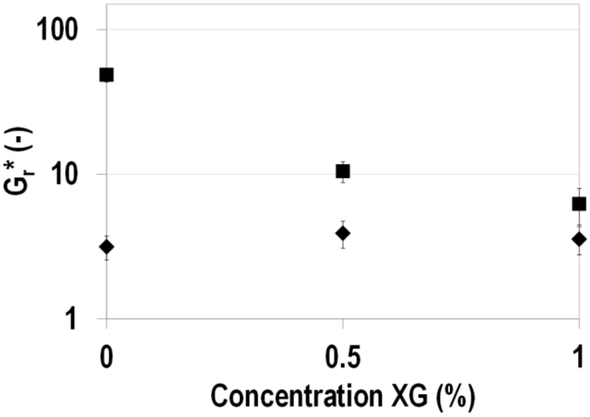

Figure 3.5 Effect of XG on the rheological properties of starch dough with 4 and 20% BP (♦ and ■, respectively).

This behavior was also observed when XG is added to other types of starch [21]. This result suggest that the decrease in swelling of the broccoli particles is very significant and actually overcomes the increase in the modulus observed when XG is added to pure starch dough.

3.3. Confocal Laser Scanning Microscopy of dough and cooked noodles

Samples of dough and noodles with 4 and 20% BP were analyzed with CLSM. Only the blank samples (without hydrocolloids added) and the samples containing LBG and XG were analyzed, since very small differences were observed in the rheological measurements for the rest of the hydrocolloids tested. LBG was chosen because it was the hydrocolloid that showed the smallest effect on dough rheology, and XG because of the very large effect that was visible in the rheological measurements. All samples were post-labeled with a solution of 0.25% FITC and 0.025% Rhodamin B. FITC will preferentially label starch and Rhodamin B will preferentially label protein, but (to a lesser degree) Rhodamin B can also label starch, and FITC can also label protein [22-24]. Using this labeling method it was not possible to distinguish the hydrocolloids, as they can be labeled by both labeling agents.

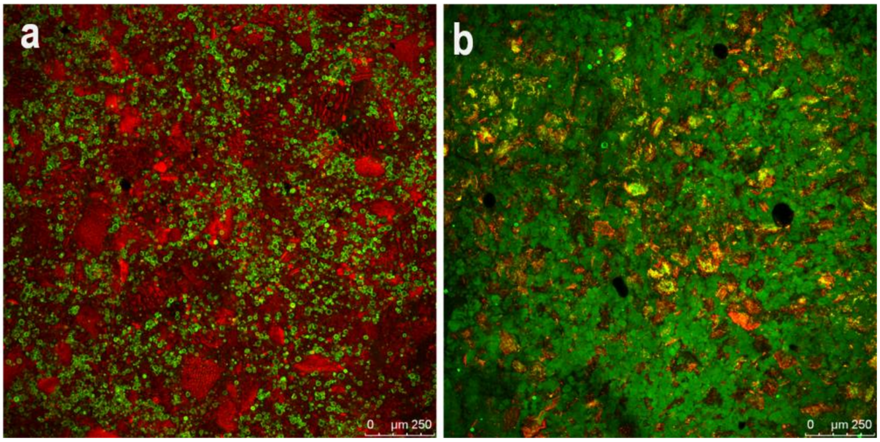

Figure 3.6 CLSM pictures of noodle dough with 20% BP (a) and cooked noodle with 20% BP (b).

Figures 3.6a and 3.6b show the 20% BP dough and cooked noodle, respectively, without hydrocolloid added. From these pictures it is possible to see that the starch granules that were intact in the dough were completely swollen in the cooked noodles. Figures 3.7a and 3.7b show the 20% BP dough and cooked noodle with 1% LBG added. These pictures confirm what was observed in the rheological measurements, where no difference was seen in the dough when LBG was added. In fact, these pictures are very much comparable with the previous ones (figures 3.6a and 3.6b), where no hydrocolloid was added.

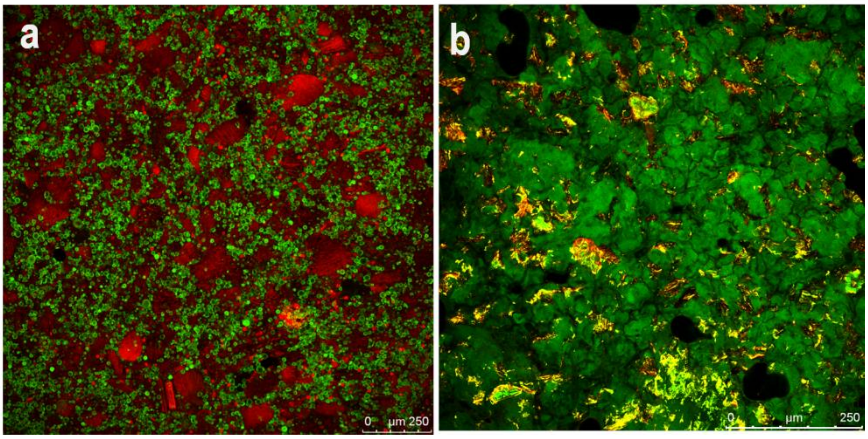

Figure 3.7 CLSM pictures of noodle dough with 20% BP and 1% LBG (a) and cooked noodle with 20% BP and 1% LBG (b).

In figures 3.8a and 3.8b, the dough and noodle containing 20% BP with 1% XG added can be seen. In the dough picture (figure 3.8a), no significant differences were seen compared to figures 3.6a and 3.7a.

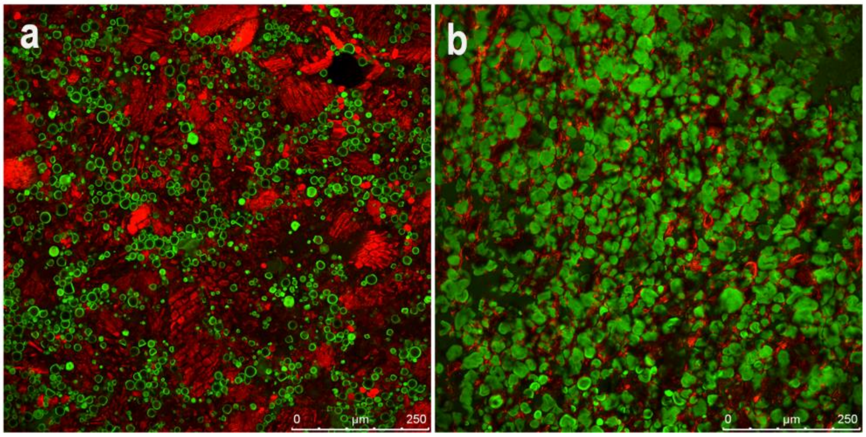

Figure 3.8 CLSM pictures of noodle dough with 20% BP and 1% XG (a) and cooked noodle with 20% BP and 1% XG (b).

In the cooked noodles containing 20% BP, XG has a very large effect on the structure of these systems, in contrast with the samples without hydrocolloid and with LBG added. We see that the starch granules were still intact, suggesting that the XG prevented granule swelling and starch gelatinization. The intact starch granules result in a higher total volume fraction of solid particles, in comparison with samples without XG. Since these high volume fraction systems behave like cellular materials (like we have seen in chapter 2), in which the mechanical properties have a strong dependence on the volume fraction of particles, an increase in the total volume fraction should result in an increase in stiffness and strength of the noodles [8]. As we will see in section 3.5, this is confirmed by the results of the textural measurements (see figure 3.12). In the samples with 4% BP (data not shown), both hydrocolloids had the same effect as in the 20% BP samples. LBG showed no effect in the dough and in the cooked noodles, whereas XG prevented the starch granules from further gelatinization upon cooking. These results were also observed by other authors, who saw that the addition of XG protected the starch granules from gelatinization [9, 25-27].

3.4. Cooking quality

Cooking quality is a very important parameter in the determination of the acceptability of noodles by consumers [17, 28]. Noodles are considered good when they are firm and elastic, have low cooking losses and good surface conditions, related with low stickiness [29-31]. Cooking loss (CL) and swelling index (SI) both contribute to the cooking quality and the results of these parameters are shown in figures 3.9 and 3.10, respectively. As reference, commercial pasta containing 1.5% spinach powder (Mamma Lucia) was also analyzed. The results for the CL of the system with 4% BP are presented in figure 3.9a. In this figure it is possible to see that LBG was the only hydrocolloid that did not decrease the CL as it had the same value as the blank sample. All the other hydrocolloids decreased the CL significantly.

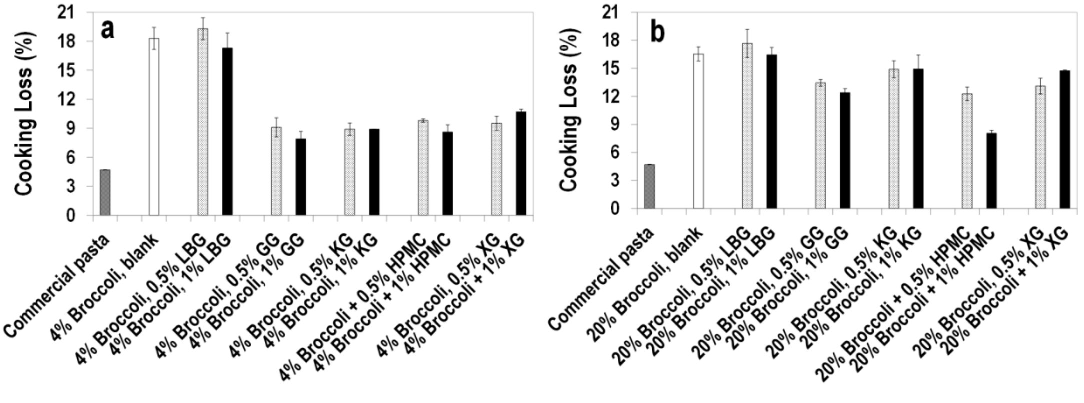

Figure 3.9 Cooking losses of the 4% BP noodles (a) and 20% BP noodles (b).

The commercial sample had a CL approximately four times lower than the blank sample. The samples containing GG, KG, HPMC and XG were all in the same range and despite the decrease that these hydrocolloids produced in the CL, our samples still had a higher CL than the commercial sample. No differences were observed between the samples with 0.5 and 1% hydrocolloid.In figure 3.9b, the results for the CL of systems with 20% BP are presented. In these samples, the hydrocolloids had a less pronounced effect than in the samples with 4% BP. Again in these samples, LBG did not have an effect in the CL, and neither did KG. They gave a similar CL to the blank sample. The samples with GG, XG and 0.5% HPMC had a slightly lower CL than the blank sample and the sample with 1% HPMC was the one with the lowest CL. However, this sample was still slightly higher than the commercial sample. The results of the SI, for the noodles containing 4% BP, are presented in figure 3.10a. In this figure it is possible to see that the different hydrocolloids had different effects on the SI of these systems.

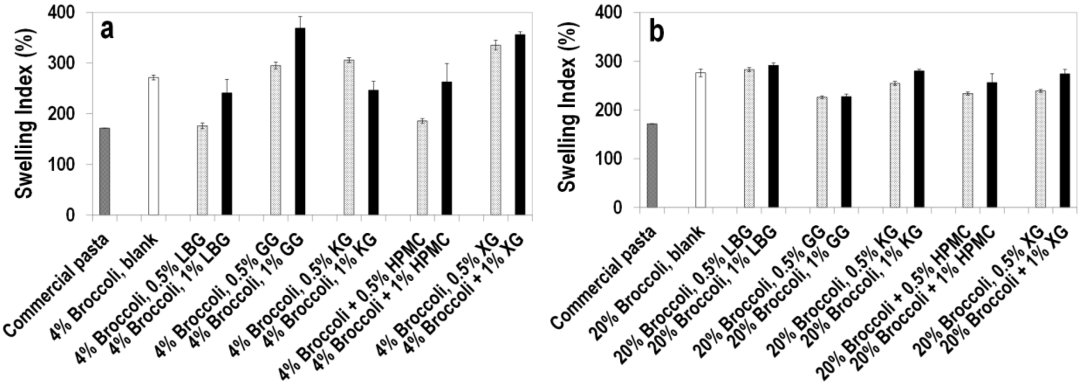

Figure 3.10 Swelling index of the 4% BP noodles (a) and 20% BP noodles (b).

The noodles containing 1% LBG, 0.5% GG, 0.5% KG, 1% KG and 1% HPMC had the same SI as the blank sample, so they did not affect this parameter. The noodles with 0.5% LBG and 0.5% HPMC were lower than the blank and in the same range as the commercial sample. The noodles with 1% GG and, 0.5 and 1% XG even increased the SI, in comparison with the blank sample. The SI of the noodles with 20% BP is presented in figure 3.10b. The SI of these systems was always higher than the commercial sample, and no large differences were seen between the hydrocolloids and the blank. For the cooking quality it was expected that the noodles containing hydrocolloids with the highest water binding capacity, would also have better cooking quality. This trend cannot be clearly observed in our samples. The fact that the same hydrocolloid shows different results for the noodles with 4 and 20% BP, suggests that more important than the water binding capacity of the hydrocolloids, is the amount of particles incorporated in the matrix. Other factors such as structure or charge of the hydrocolloids might also be responsible for the differences as suggested by Kim & Yoo [32] and Umadevi Sajjan & Raghavendra Rao [33].

3.5. Texture Analysis

The noodles were characterized in terms of texture parameters, such as stiffness, extensibility and strength. Figures 3.11 and 3.12 show these parameters for the samples with 4 and 20% BP, respectively. Blank samples (with no hydrocolloid) with 4 and 20% BP were produced as a control. Regarding the strength of the noodles, the samples containing 4% BP (figure 3.11a) and 1% KG and 1% XG were in the same range as the commercial sample. The blank sample had the lowest strength together with 0.5% LBG and 0.5% GG. The other hydrocolloids had a higher strength but still lower than the commercial sample. The results for strength of the systems with 20% BP can be seen in figure 3.12a. In these noodles the blank sample had a lower strength than the commercial sample, and all hydrocolloids were in the same range as the blank sample, except for XG. The samples with 20% BP and 1% XG had a higher strength and were in the same range as the commercial sample. The sample with 0.5% XG had an even higher strength than the commercial sample. Regarding the parameter extensibility, in figure 3.11b the results for the systems with 4% BP are shown. The extensibility of the samples with 4% BP seemed to follow a trend, in which the addition of 1% hydrocolloid decreased the extensibility of the noodles further compared to the addition of 0.5%. For some hydrocolloids this trend was more significant than for others. The majority of the hydrocolloids decreased the extensibility of the noodles, in comparison with the blank sample, to the range of the commercial sample. The blank sample had the highest extensibility and the noodles with 0.5% GG, 0.5% KG and 0.5% HPMC were also in this range of extensibility. The samples containing 20% BP (figure 3.12b) are less extensible then the samples with 4% BP and also less extensible than the blank sample. Besides that, the hydrocolloids did not affect this system, as the samples with hydrocolloids are all in the same range as the blank sample. These results suggest that the volume fraction of particles incorporated is a more important factor for the extensibility then the type and concentration of hydrocolloid used. The results of the stiffness of the noodles with 4% BP can be seen in figure 3.11c. In this figure we see that most of the hydrocolloids did not have an effect on the stiffness of these noodles, as they present the same value of stiffness as the blank sample. However, the stiffness of the commercial sample is much higher than the blank sample and only 0.5% KG and 1% XG are in the same range as the commercial sample. The sample containing 1% KG increased the stiffness beyond the commercial sample. Figure 3.12c shows the stiffness of the noodles with 20% BP. In the noodles with 20% BP, all the hydrocolloids were in the same range together with the blank sample.

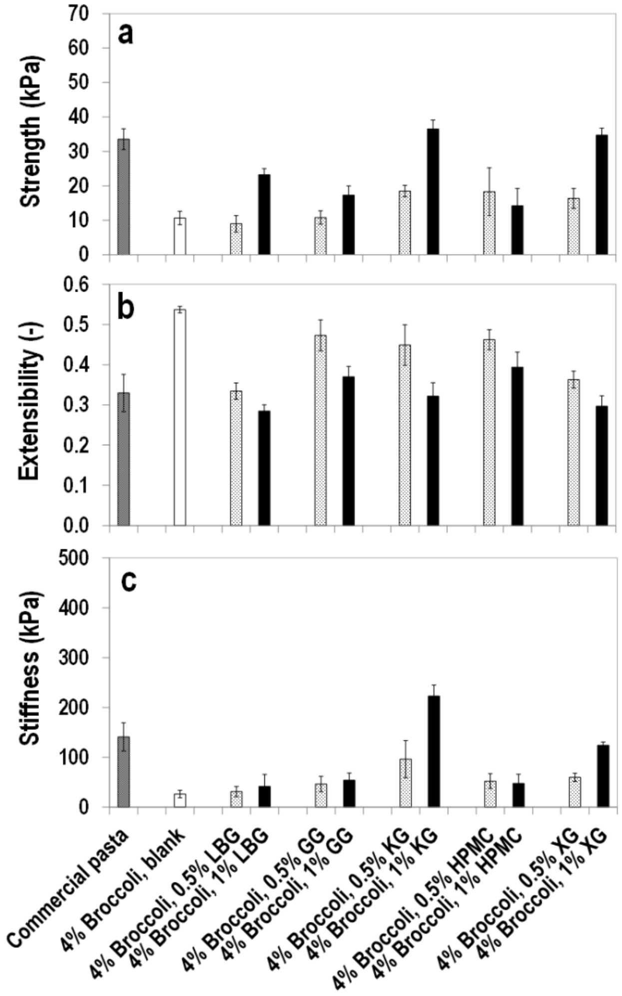

Figure 3.11 Texture parameters: Strength (a), Extensibility (b) and Stiffness (c) of 4% BP noodles.

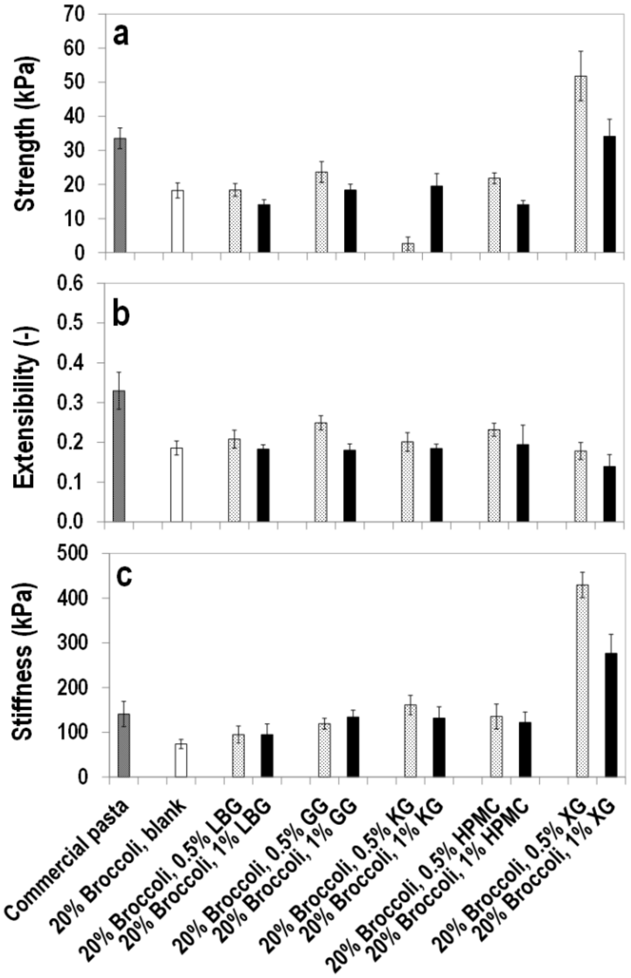

Figure 3.12 Texture parameters: Strength (a), Extensibility (b) and Stiffness (c) of 20% BP noodles.

The only exception is XG that increased the stiffness of the noodles with 20% BP. For the sample with 0.5% XG the increase was even more pronounced than for 1% XG. An increase in noodle firmness, upon the addition of XG was also observed by Brennan & Tudorica [34]. As we observed on the CLSM pictures, XG inhibits the starch granules from swelling and dissolving. A possible explanation for the decrease seen in strength and stiffness of noodles with 20% BP in going from 0.5 to 1% XG added is that at 0.5% XG there is enough hydrocolloid present to prevent the starch granules from swelling and dissolving. This results in a higher the total volume fraction of solid particles (starch granules and broccoli particles) compared to cooked noodles without XG. When more XG is added, this extra amount of XG will result in further limitation of the swelling of the broccoli particles, decreasing the total volume fraction of particles and weakening the system (figure 3.13).

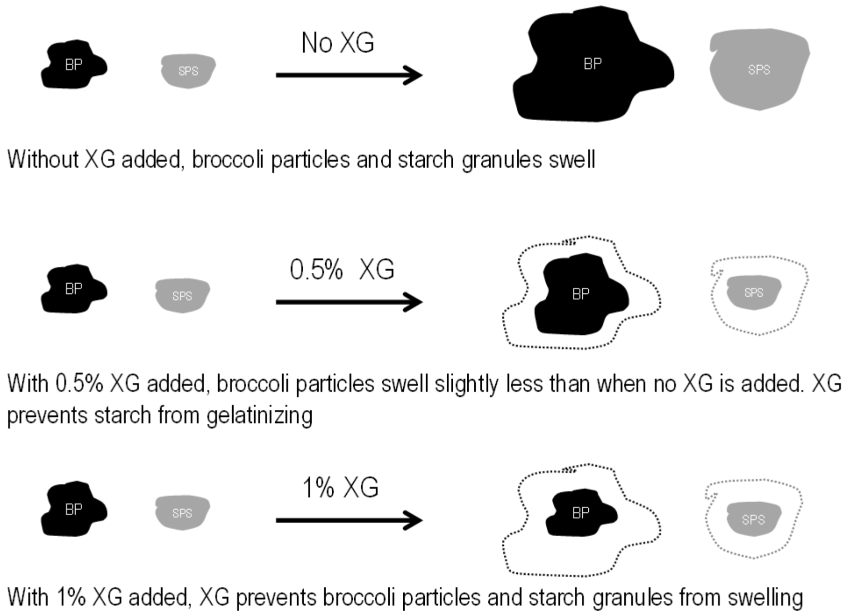

Figure 3.13 Schematic representation of the influence of different concentrations of xanthan on broccoli powder (BP) and starch granules (SPS).

4. Conclusions

The addition of HPMC and XG had a clear effect on the shear rheology of starch dough with 20% BP. As a result of the significant swelling of the broccoli powder the system with 20% BP is a cellular material, in which the modulus had a strong dependence on the total volume fraction of particles (broccoli powder and starch granules). The hydrocolloids bind part of the available water, which results in less swelling of the broccoli powder. The systems with added hydrocolloids with high WBC have a lower total volume fraction of particles than dough produced without hydrocolloids, and therefore had a lower modulus. The hydrocolloid that showed the largest decrease in the complex shear modulus in systems with 20% BP was XG, which was also the hydrocolloid with the highest water binding capacity. In the systems containing 4% BP no significant differences were seen in the shear rheology of the dough upon addition of hydrocolloids. These systems are gelled matrices with broccoli particles and starch granules dispersed in them. Their modulus is predominantly determined by the matrix, and is less affected by the swelling of the broccoli particles.

For cooked noodles CLSM images showed a clear effect of XG on noodles with 20% BP. In cooked noodles with XG most of the starch granules were intact, whereas in noodles without XG granules showed significant swelling. Regarding the texture of the systems with 4 and 20% BP, we saw that all the hydrocolloids either increased or maintained the strength and stiffness of the noodles, in comparison with the blank sample. But for most of the hydrocolloids these values were still lower than the values of a commercial pasta. XG increased the stiffness and the strength of the noodles with 20% BP far beyond the commercial sample, which we believe was caused by the fact that the XG limited starch granules swelling and dissolution, resulting in a much harder cellular material. Based on our observations we can conclude that hydrocolloids with high water binding capacity can control the degree of swelling of vegetable particles and starch granules in starch noodle products, affecting both dough rheology and textural properties of the cooked noodles.

Acknowledgements

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CHAPTER 4

EFFECT OF MATRIX AND PARTICLE TYPE ON RHEOLOGICAL, TEXTURAL AND STRUCTURAL PROPERTIES OF BROCCOLI PASTA AND NOODLES

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ABSTRACT

Durum wheat semolina (DWS) pasta and sweet potato starch (SPS) noodles were incorporated with different volume fractions and types of broccoli powder (up to 20% V/V). The incorporation of high volume fractions of broccoli powder produced in-house in SPS noodles increased the modulus of the dough and the stiffness and strength of the noodles as these broccoli particles can swell to up to 7.6 times their original volume. This effect was smaller when commercially available broccoli powder was used, as this powder can only swell 2.6 times. These results were confirmed by CLSM images. The incorporation of the two types of broccoli powder in DWS showed little effect on the rheology of this system. Since there is no difference between the water binding capacity of SPS and DWS, the small effects on rheology observed in the DWS system are due to the strong gluten network that can prevent the broccoli particles from swelling.

Keywords: Sweet potato starch noodles, Durum wheat semolina pasta, Broccoli powder, Swelling index, Rheology

1. Introduction

In the current lifestyle, with increasing numbers of obesity, the demand for new food products that have some additional health benefits is increasing rapidly [1]. Both durum wheat semolina (DWS) pasta and starch noodles are very popular foods around the world and are considered healthy and an ideal food to be enriched with nutrients [2-4]. Pasta has a low fat content, contains no cholesterol and has a low glycemic index [5]. Similar to pasta, starch noodles also have a low glycemic index, and are referred to as a source of resistant starch. Resistant starch is unavailable for digestion and therefore can be a possible solution to fight obesity [6]. The enrichment of pasta and noodle products by nutrients will therefore be valuable for dietary benefits. Several studies have already reported successful (protein) enrichment of DWS pasta, especially with a variety of flours from peas and beans, such as pigeon pea [7], soy [8], common bean [9], lupin [10], corn germ [11], chickpea [12], faba bean and split pea [13]. Besides beans, also dried green leaves such as spinach and amaranth have been incorporated [14]. Although a lot of examples can be found for DWS, enrichment of sweet potato starch (SPS) noodles is not a common practice. There are only a few studies available that describe the enrichment of SPS noodles [15-17]. A possible explanation for the preference of fortification of DWS systems instead of SPS might be related to the most significant difference between these two materials, which is the presence of gluten in DWS [18]. High quality pasta is obtained because of gluten [19]. This protein provides a certain visco–elasticity and cohesiveness to the dough and contributes to the water holding capacity of the cooked pasta [20]. In starch systems, a possible solution to compensate for the lack of gluten is to pre-gelatinize part of the starch, which will act as a binder between the starch particles [21]. In chapter 2, we have investigated the effect of loading high volume fractions of broccoli particles into a SPS matrix. The results show that adding high volume fractions (>10% dry volume) has negative effects on the quality of these products. We have showed that this effect was caused by the swelling of the broccoli particles, thereby increasing the effective volume fraction to such extend that the amylose matrix was interrupted [22]. In chapter 3, we have shown that one way to overcome that problem is to use hydrocolloids with a high water binding capacity, such as xanthan gum, to limit the swelling of the particles. Water diffusion and water holding capacity are therefore essential parameters that influence the quality of pasta and starch products. Both DWS and SPS are known to be able to bind water up to twice their own weight [23, 24]. However, the gluten present in DWS forms a very strong network, whereas starch cannot form a network and acts only as a binder between the starch granules [25, 26]. As a result, we expect that using SPS or DWS with high loadings of broccoli will have different effects on the structure and quality of these products. Therefore, in this chapter we have studied the effect of the incorporation of different types and concentrations of broccoli particles on the structure of these different matrices (DWS and SPS). The broccoli particles differ in swelling behavior and are added either in a dry or wet state. This induces different water migration and network formation of the filled pasta and noodle products, leading to different properties of the dough and the cooked pasta/noodles.

2. Materials and Methods

2.1. Materials

Sweet Potato starch (SPS, Mong Lee Shang) was kindly supplied by Deximport (Barendrecht, The Netherlands) and Durum wheat semolina (DWS) was purchased at a local windmill (De Vlijt, Wageningen). Broccoli was bought in a local supermarket (Spar, Wageningen, The Netherlands) and the commercial broccoli powder (CBP) was purchased from Z Natural Foods, LLC (West Palm Beach, USA). Deionized water was used to prepare all samples.

2.2. Water binding capacity (WBC)

The water binding capacity of the SPS and the DWS was measured according to the method used by Medcalf & Gilles [27] with minor changes. Briefly, 2.57 (± 0.12) g of sample (SPS, pre-gelatinized and not pre-gelatinized, and DWS) were added to 35 ml of water and centrifuged at 16,040 *g* for 1h. The supernatant was removed and the pellet was drained for 10 min and weighed.

2.3. Broccoli powder and pulp preparation

The broccoli powder was produced in-house (HMBP) for which the method is described in section 2.2 of chapter 2. Both HMBP and CBP had a particle size distribution between 25 – 53 µm. Broccoli pulp (BPulp) was prepared from fresh cut broccoli florets frozen in liquid nitrogen and immediately blended in a kitchen machine (“Thermomix” Vorwerk, The Netherlands). The BPulp was not sieved and was used as is.

2.4. Swelling behavior of broccoli particles

The swelling behavior of the dried broccoli powder (BP) was determined using capillary viscometry (Ubbelohde), following the method described in section 2.6 of chapter 2. A suspension of dried broccoli particles was used to prepare a series of dilutions with volume fractions between 0.02 and 0.4%.

2.5. Sweet potato starch/broccoli-sweet potato starch dough/noodles preparation

The preparation of the SPS dough and the SPS dough with broccoli incorporated was based on the method described in section 2.3 of chapter 2 with minor changes. In the present study, the pre-gelatinized starch was always set at 10% and the ratio starch:water for the pre-gelatinization of starch was 1:9. Moisture content was always set at 55% (V/V). The starch to be pre-gelatinized was mixed with the appropriate amount of water and placed in a water-bath at 100 °C. The solution was stirred until it became translucent (approximately 1 min after stirring and observed visually). After this step, the solution containing the pre-gelatinized starch was moved to a water-bath set at 40 °C. To this mixture, the remainder of the starch and water was added. Stirring was continued until a uniform dough was obtained (approximately 6 min). Samples with 4, 10 and 20% BP were produced and the BP concentrations always refer to V/V. For broccoli containing doughs, part of the starch was replaced by broccoli powder and was added after a blank uniform dough was obtained. The dough was stirred for 1 more minute to evenly disperse the broccoli powder. The doughs were then transferred to 5 ml syringes (Plastipak, Italy) of which the tip of the syringe was removed. The noodles were produced by pressing the plunger of the syringes at a controlled rate and the created noodles were collected in cooking water. The noodles with 4 and 10% BP were cooked for an optimal cooking time of 30 sec and the noodles with 20% BP for 1 min. After that, the noodles were rinsed with cold water, drained and analyzed within 20 min. The cooked noodles had an average cross-sectional area of 10.0 ± 1.4 mm^2.

2.6. Durum wheat semolina/broccoli-durum wheat semolina dough/pasta preparation

The DWS dough with and without broccoli (blank) was prepared by mixing the dry ingredients with water (35% V/V) and kneading by hand for 4 min for the doughs with 4 and 10% BP, and 5 min for the dough with 20% BP. After that, the dough was passed 5 - 7 times through a commercial sheeting/cutting machine to ensure homogeneous mixing, and the pasta strands were cooked in boiling water. The blank strands were cooked for 8 min and the strands with 4, 10 and 20% BP were cooked for 8, 7 and 5 min, respectively. After that, the strands were rinsed with cold water and drained, and analyzed within 20 min. The cooked strands had an average cross-sectional area of 10.2 ± 0.8 mm^2.

2.7. Optimal cooking time

The optimal cooking time for the SPS noodles and the DWS pasta was determined according to Collado et al [28].

2.8. Shear Rheology of dough

Shear rheology experiments were performed as described in section 2.5 of chapter 3. The rheological measurements were expressed in terms of complex modulus since we were interested in the comparison between the different samples. Besides that, the low values for the loss tangent (<0.26) indicated that the loss modulus is not relevant in these samples and the behavior is dominated by the storage modulus.

2.9. Confocal laser scanning microscopy of dough and cooked noodles/pasta

Both dough and cooked noodles/pasta were analyzed by confocal laser scanning microscopy (CLSM). The samples were prepared as described before (section 2.5 and 2.6, for SPS dough/noodles, and DWS dough/pasta, respectively), and post-stained with a solution of 0.25% (w/w) Fluorescein 5-isothiocyanate (FITC) and 0.025% Rhodamin B in water. The image acquisition was done according to the method described in section 2.6 of chapter 3.

2.10. Texture Analysis of cooked noodles/pasta

The texture analyses experiments were performed according to the method described in section 2.8 of chapter 3. Each single strand that was tested had a length of 15 mm. Reported values are an average of two different samples of the same concentration, each measured at least 4 times.

3. Results and Discussion

In this work, durum wheat semolina (DWS) and sweet potato starch (SPS) were used for the production of DWS pasta and SPS noodles, respectively. Starch dough is structured by pre-gelatinized starch whereas DWS dough is structured by the gluten network [28]. DWS is considered to be the best raw material for the production of pasta because of the gluten network that provides cohesiveness and visco–elasticity to the dough [20, 29]. As water diffusion and water absorption are important parameters in the fortification of pasta/noodles with broccoli particles, the water binding capacity of the SPS (both powder and gelatinized) and the DWS was measured (Table 4.1).

Table 4.1 Water binding capacity of sweet potato starch (SPS) and durum wheat semolina (DWS).

	Water Binding Capacity (mg H_2O/g powder)
SPS	1.91 ± 0.04
PGSPS	1.88 ± 0.04
DWS	2.03 ± 0.03

The results show that the difference between the SPS samples (either in powder or gelatinized) is not significant (difference between them is smaller than the error margin), and the difference between SPS and DWS is only about 5%. Therefore, this parameter cannot be responsible for any differences between matrices.

3.1. Swelling behavior of broccoli particles

In chapter 2 we have reported the influence of the swelling of the broccoli particles in SPS matrices. In the study described in that chapter we found that the dried broccoli powder produced in-house could swell to up to 7.6 times their original volume. In the present study we have also investigated broccoli powder that is commercially available (Z Natural Foods, LLC). To understand the effect of different types of particles, we have determined their swelling behavior. In figure 4.1, the results of the viscosity measurements are presented. Dividing the slope of the fitted line by 2.5, a swelling factor of 2.6 was obtained, almost 3 times lower than the broccoli powder we have produced in house.

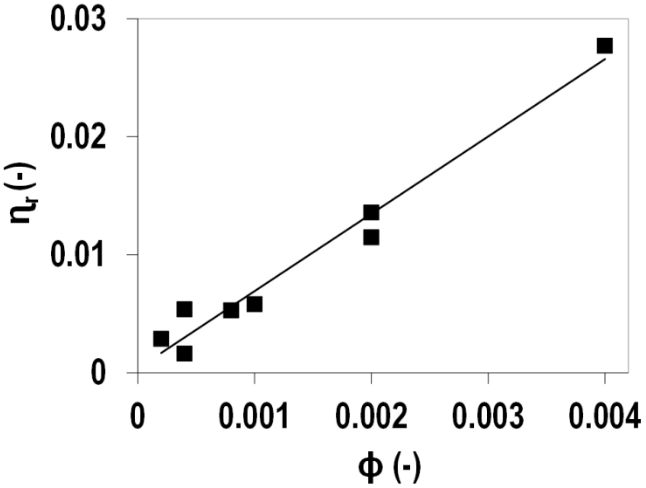

Figure 4.1 Swelling behavior of commercial broccoli powder. The data points were fitted with the Einstein equation ($\eta_r = 1 + 2.5\phi$) with $R^2 = 0.969$.

3.2. Shear rheology

Several authors have reported that the total or partial replacement of DWS dilutes the gluten-network, resulting in a weaker network and consequently in a final product with reduced quality [29, 30]. However, there is very limited information about the dough rheology of systems filled with vegetable powder.

3.2.1. Effect of the matrix

The rheological responses of the blank SPS dough and blank DWS dough are shown in figure 4.2, when the volume fraction of BP is zero. The dough made of DWS has a modulus almost two orders of magnitude higher than the SPS dough. This is caused by the fact that DWS contains gluten which is known to produce a very strong visco–elastic network, formed by chemical cross-linking of protein polymers [25]. For starch-based matrices, no covalent cross-links exist, explaining the much lower modulus of the starch systems.

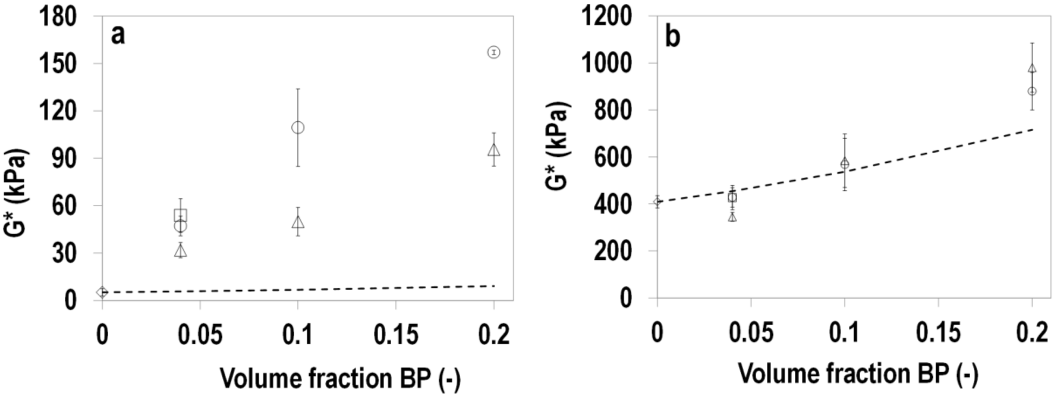

Figure 4.2 Rheological behavior of SPS dough (a) and DWS dough (b) with different concentrations of broccoli powder (0, 4, 10 and 20%) and different types of broccoli powders, BPulp (□) CBP (△) and HMBP (○).Blank sample is shown by ◊. Dashed lines represent the complex modulus calculated with Batchelor model [31] taking the dry volume fraction as the fitting parameter.

3.2.2. Effect of the type and concentration of broccoli powder

In figure 4.2 the rheological behavior is shown of SPS dough (figure 4.2a) and DWS dough (figure 4.2b), with different concentrations of broccoli powder (0, 4, 10 and 20%) and different types of broccoli added (BPulp, CBP and HMBP). The differences between the types of broccoli powders are related to the swelling behavior of dried powders (CBP and HMBP) and the water present in the pulp (BPulp). Independent of the type of particles, the total water content of the final dough is the same. So for dried particles, most of the water is incorporated in the matrix and diffuses into the particles upon production. For the pulp, most of the water is already incorporated in the particles and diffuses into the matrix. Due to the high amount of water present in the BPulp (86.4%), it was only possible to incorporate 4% of BPulp (on a dry basis, equal to 29.2% of wet particles). The 4% BPulp, CBP and HMBP have the same total amount of broccoli present in the end product. In figure 4.2a, in the system containing SPS, the concentration and type of powder influence the rheological response of this system. The addition of 4% (V/V) broccoli powder already increases the modulus significantly. The modulus of samples with 4% BPulp (represented by squares) and 4% HMBP (represented by circles) are slightly higher than the modulus with 4% CBP (represented by triangles). This can be explained by the swelling capacity of the broccoli powders; the CBP has a much lower swelling capacity and therefore results in a lower effective volume fraction and lower modulus. For SPS dough, the same trend is visible in the samples with 10% and 20% BP, where the modulus of the samples with HMBP is again higher than the modulus of the samples with CBP. The sample containing 20% CBP has a modulus even lower than the sample containing 10% HMBP. These results are an indication that the particles are completely swollen. Table 4.2 shows the values of the maximum broccoli volume fraction reached when the particles are completely swollen (based on their swelling capacity of 2.6 for the CBP and 7.6 for the HMBP).

Table 4.2 Volume fraction of particles after considering the swelling factor.

	4%	10%	20%
CBP (× 2.6)	10.4	26	52
HMBP (× 7.6)	30.4	76	-

The sample with dry volume fractions of 10% CBP and 4% HMBP would have volume fractions equal to 26% CBP and 30% HMBP if the particles swell completely. These volume fractions are very close and indeed so are the shear moduli of these samples (figure 4.2a). Again assuming complete swelling, the dry volume fractions of 10% HMBP and 20% CBP becomes 76 and 52%, respectively, which would explain why the 10% HMBP has a higher shear modulus than the 20% CBP sample. The addition of particles with an inhomogeneous size distribution does not seem to influence the rheological properties different than a homogeneous size distribution as there is no difference between 4%BPulp and 4%HMBP. So, for SPS doughs, the modulus is determined by the effective volume fraction of the incorporated particles, and therefore depends strongly on the swelling capacity of the particles.

Regarding the system with DWS, in figure 4.2b, the effect of the swelling of the particles seems to be less dominant. The samples containing 4% broccoli powder with either BPulp, CBP or HMBP show the same modulus as the blank sample, indicating that these low concentrations as well as the particle type do not have a significant effect on the rheological properties of this system. The samples with 10% broccoli powder are slightly higher than the blank and the samples with 20% broccoli are the ones with the highest modulus. No significant differences are seen between CBP and HMBP in the samples with 10 and 20% broccoli powder.

In both SPS and DWS systems, the increase in the modulus is caused by the increase in the volume fraction of particles incorporated. However, in the SPS system this effect is much larger because of the swelling of the broccoli particles. The fact that there are no differences in the modulus of the DWS samples with the different types of broccoli powder added is an indication that the gluten network can prevent the particles from swelling and that this system is only affected significantly by the incorporation of broccoli particles at volume fractions much higher than 4%.

To evaluate the swelling of the broccoli particles in the different matrices, we have compared the experimental values of the moduli to expected values calculated according to the Batchelor model [31], based on dry volume fractions. In this model, the complex modulus of a dilute suspension is given by

$$G^* = G_c^* .(1 + 2.5\phi + 6.2\phi^2) \tag{4.1}$$

where G_c^* is the complex modulus of the continuous phase and ϕ is the particles' volume fraction. In figure 4.2, the dashed line corresponds to calculated values for volume fractions up to 20%. In figure 4.2a, we see that the experimental data points are not in agreement with the calculated values, which reveals that the actual volume fractions are much higher than the ones based on dry volume, indicating that swelling of the broccoli particles takes place when they are incorporated in SPS matrices. For the DWS matrix (figure 4.2b), the experimental data points are close to the calculated values. This shows that in the DWS case the volume fractions are close to their dry volume fraction, indicating no swelling. Since the water binding capacity of SPS and DWS is very similar, it is the mechanical properties of the gluten matrix that prevent the broccoli particles from swelling.

3.3. Confocal Scanning Laser Microscopy

All the samples made from SPS (0, 4, 10 and 20% BP), both dough (figures 4.3 a – h) and cooked noodles (figures 4.4 a – f), and some samples made from DWS, also dough (figures 4.5 a – d) and cooked pasta (figures 4.6 a – d) were post-stained with a solution of 0.25% FITC and 0.025% Rhodamin B and analyzed by confocal laser scanning microscopy. In figure 4.3a the microstructure of the blank SPS is shown. In this picture it is possible to see that the sample is composed of a very little amount of matrix and a large amount of starch granules. The starch is labeled in both red and green since FITC will preferentially label starch and Rhodamin B will preferentially label protein, but (to a lesser degree) Rhodamin B can also label starch, and FITC can also label protein [32-34]. Figure 4.3b shows the sample containing 4% BPulp. The microstructure of this sample is characterized by the presence of a few very large broccoli powder particles and more very small particles. This was expected since this sample did not undergo particle size separation. The samples containing 4% CBP and 4% HMBP are shown in figures 4.3c and 4.3d, respectively. These samples present a very similar microstructure, with a few small broccoli powder particles evenly dispersed in the matrix. Figures 4.3e and 4.3f show the samples with 10% CBP and 10% HMBP, respectively. Despite having the same concentration of broccoli powder, the sample with 10% HMBP seems to have more and larger broccoli powder particles than the sample with 10% CBP. These results are in agreement with the swelling capacity of the broccoli powders, as HMBP can swell almost 3 times more than CBP. The samples with 20% CBP and 20% HMBP are shown in figures 4.3g and 4.3h and they show the same trend as the samples with 10% BP. The sample with HMBP seems to have a larger volume fraction of broccoli than the sample with CBP. Moreover, when comparing the samples containing 20% CBP and 10% HMBP the latter seems to have more broccoli than the former. This can also be explained by the swelling of the particles, since 10% would become 76% of HMBP upon complete swelling and 20% CBP would become 52% of swollen CBP. The 10% HMBP dough had a higher shear modulus than the 20% CBP, and the figures 4.3f and 4.3g confirm our hypothesis that this is due to a higher effective volume fraction of the 10 % HMBP systems.

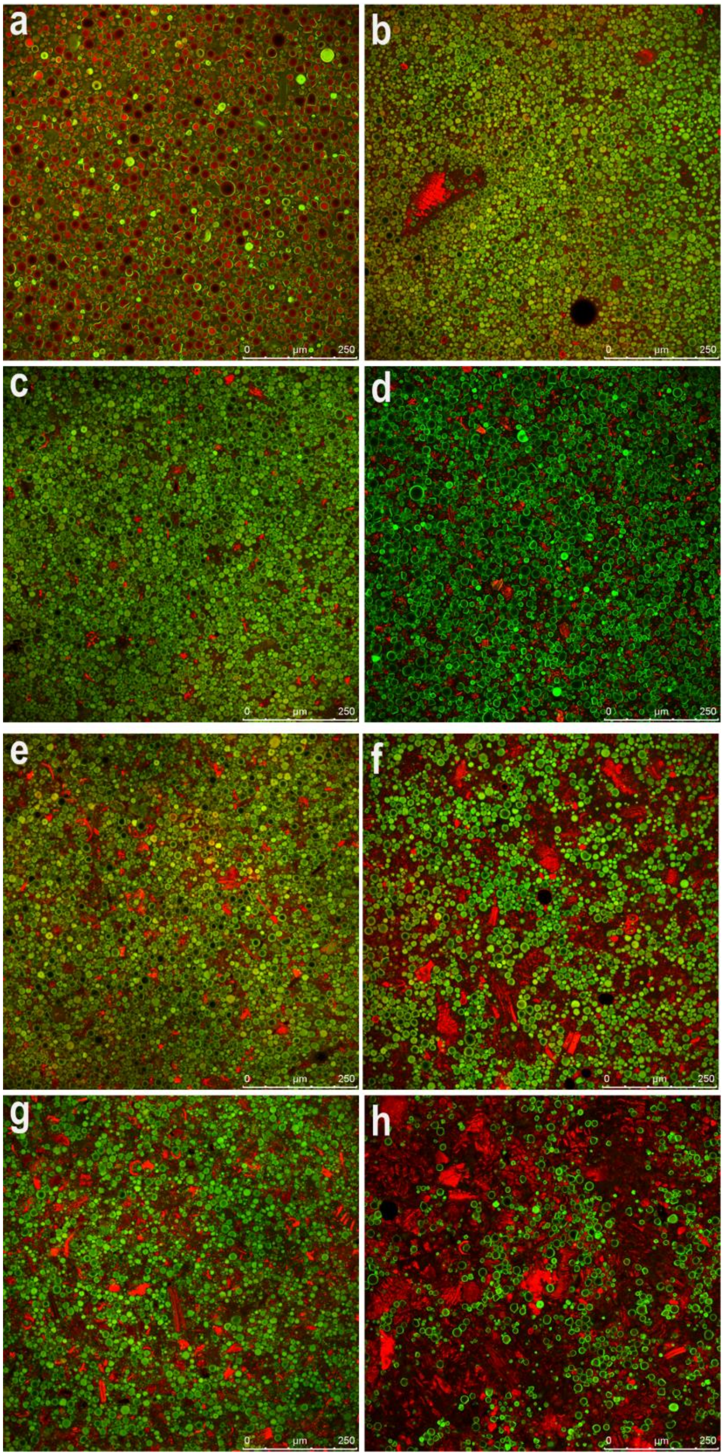

Figure 4.3 CLSM images of the blank sweet potato starch dough (a), sweet potato starch dough with 4% BPulp (b), 4% CBP (c), 4% HMBP (d), 10% CBP (e), 10% HMBP (f), 20% CBP (g) and 20% HMBP (h).

The microstructure of the cooked SPS noodles can be seen in figures 4.4 a – f. In these samples the starch granules are completely gelatinized but the trend is the same as the one found in the SPS dough. The samples containing HMBP seem to have a higher amount of broccoli powder particles than the samples containing the same concentration of CBP.

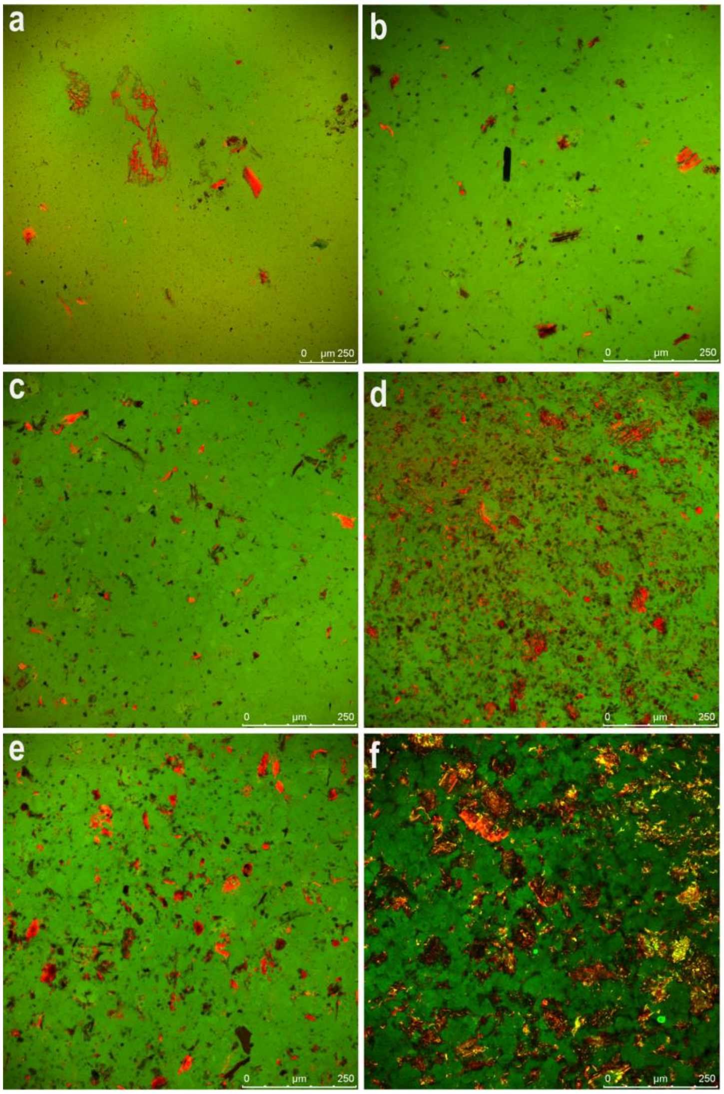

Figure 4.4 CLSM images of the cooked sweet potato starch noodle with 4% BPulp (a), 4% CBP (b), 10% CBP (c), 10% HMBP (d), 20% CBP (e) and 20% HMBP (f).

The pictures from DWS dough can be seen in figures 4.5 a – d. Figure 4.5a shows the microstructure of the blank DWS dough. In this sample, the gluten network is labeled in red and the starch granules are labeled in green. When broccoli powder is added to this system, it was not possible to distinguish between the protein network (gluten) and the protein present in broccoli, both labeled in red. In figure 4.5b, the sample with 4% BPulp is shown and in this sample it is possible to distinguish some of the broccoli powder particles because of their shape/size (red particle inside the black circle). BPulp is the only sample that was not sieved and thus it contains an inhomogeneous particle size distribution, all the other broccoli powders were sieved and have a smaller average particle size.

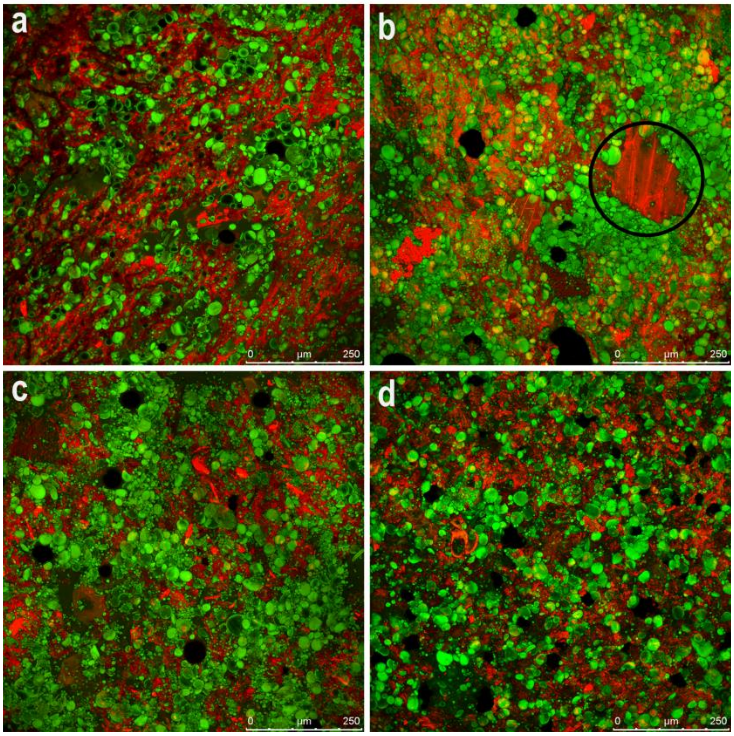

Figure 4.5 CLSM images of the blank durum wheat semolina dough (a), durum wheat semolina dough with 4% BPulp (b), 20% CBP (c) and 20% HMBP (d). The blank circle in figure b depicts a very big broccoli particle.

Figures 4.5c and 4.5d show the samples containing 20% CBP and 20% HMBP. In both samples it is not possible to see any differences. The dilution of the gluten network that has been reported in literature when (part of the) DWS is replaced, could not be observed with this labeling technique/agents. Figures 4.6 a – d show the microstructure of the cooked DWS pasta. In these pictures it is also not possible to distinguish differences in the systems. The fact that we see only minor differences between the samples confirms that the swelling of the broccoli particles is inhibited by the gluten network.

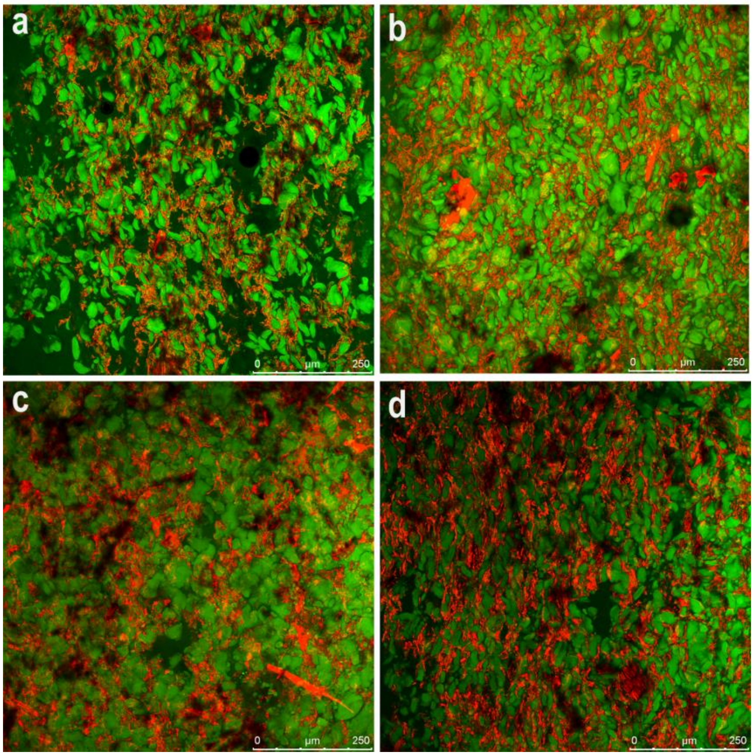

Figure 4.6 CLSM images of the cooked blank durum wheat semolina pasta (a), durum wheat semolina pasta with 4% BPulp (b), 20% CBP (c) and 20% HMBP (d).

3.4. Texture Analysis

The texture of the cooked SPS noodles and the DWS pasta strands was analyzed, and the results can be seen in figures 4.7 and 4.8, respectively. Figure 4.7a shows the strength of the SPS noodles. Despite the large error bars, the strength of the SPS noodles seems to follow the same trend found in the dough rheology and the CSLM pictures, in which more swollen HMBP was observed than for CBP. The strength increases with particle concentration, and the strength of the samples with HMBP is always higher than the strength of the samples with CBP, especially the sample with 20% HMBP that shows a significant higher strength than the sample with 20% CBP. The extensibility of the SPS noodles can be seen in figure 4.7b.

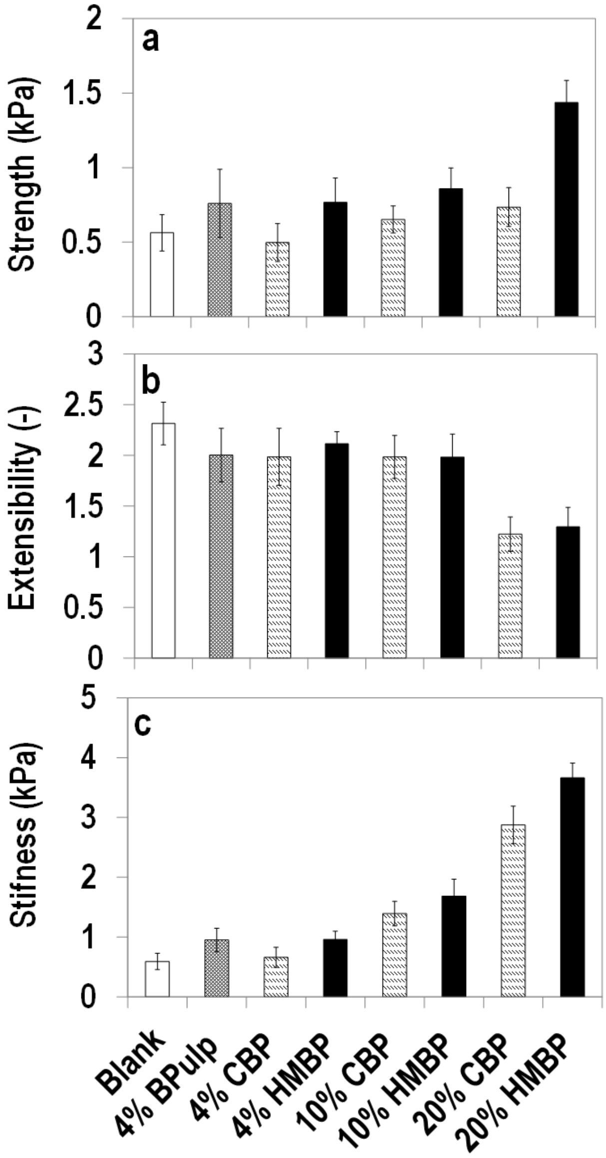

Figure 4.7 Texture parameters, strength (a), extensibility (b) and stiffness (c) of the fresh sweet potato starch noodles.

No significant differences are visible between the different broccoli powder types and also not between the blank and the samples with 4 and 10% BP. The samples containing 20% CBP and 20% HMBP show a lower extensibility than the samples with 4 and 10% BP. As shown by the CLSM pictures, the SPS samples with 4 and 10% BP have a sufficiently large amount of matrix present which is enough to hold the particles together. At higher concentrations, the particles are disrupting the matrix, and that is reflected in the lower extensibility of the samples with 20% BP. In figure 4.7c it is possible to see the effect of the different concentrations and types of broccoli powders on the stiffness of these samples. The stiffness of the samples containing 4% BP is similar to the blank sample, but increases as the concentration of BP increases. However, the different types of broccoli particles induce very small differences in the stiffness of the noodles containing 4 and 10% BP.

The largest difference seen between different particle types is in the sample with 20% CBP and 20% HMBP. The sample containing 20% HMBP has a higher stiffness than the sample with 20% CBP. This is in agreement with the dough rheology results and the microscopy pictures, where the sample with 20% HMBP shows a higher modulus and a higher effective volume fraction of particles than the sample with 20% CBP.

In figure 4.8 the texture analysis of the DWS pasta strands is shown. Figure 4.8a shows the results of the strength of the DWS pasta. No differences are visible between the different samples. The extensibility of the DWS pasta is shown in figure 4.8b. As the concentration of broccoli powder increases, the extensibility of theses strands decreases, but the decrease is very subtle. Nevertheless, the incorporation of broccoli powder implies that less DWS is present to form a network. The decrease in the extensibility as the concentration of particles increases is probably the result of a weaker gluten network caused by the replacement of DWS. The parameter stiffness can be seen in figure 4.8c and all the samples have a slightly higher stiffness than the blank sample, but given the large error bars, these differences are not significant. This means that neither the different concentrations nor types of broccoli powder have an effect on the stiffness of the DWS matrix, which is determined by the properties of the gluten network. In summary, the gluten provides the DWS pasta strands with a strong network that is very little affected by the type and concentration of particles incorporated. Unlike DWS pasta, the SPS noodles have shown to be very sensitive to both type and concentrations of broccoli particles added. The fact that there are no differences between 4% BPulp and 4% HMBP indicates that the systems (both SPS and DWS) are not affected by the different water content present in the powders. On the other hand, the particles with different swelling capacities (2.6 for CBP and 7.6 for HMBP) give larger effects on the changes in structure with HMBP being the one that shows the larger effects.

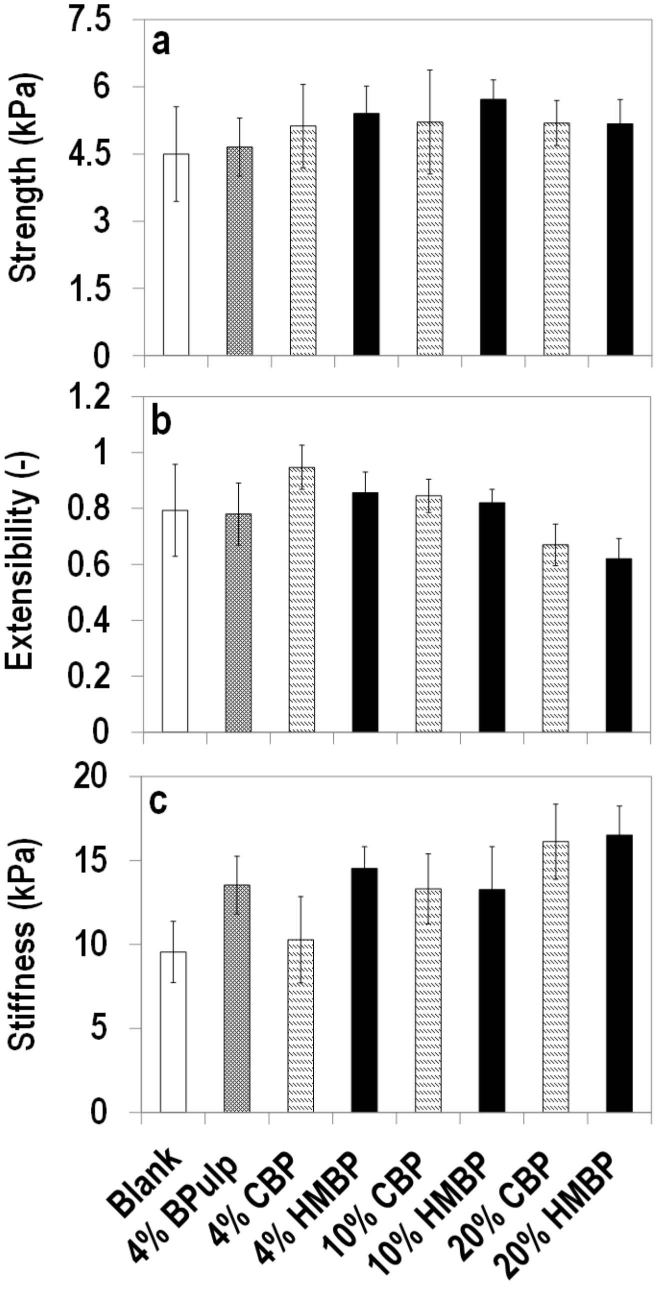

Figure 4.8 Texture parameters strength (a), extensibility (b) and stiffness (c) of the fresh durum wheat semolina pasta.

4. Conclusion

In this work we have studied the effect of the addition of different types and volume fractions of broccoli powder particles to different matrices through dough rheology, microscopy and texture analysis. Incorporating broccoli powder into a sweet potato starch (SPS) matrix has a negative effect on structure of these systems. The higher the concentration and swelling capacity of broccoli powder added, the larger is the disruption caused in the microstructure of these systems. Adding higher amounts of broccoli powder implies that more starch is replaced by broccoli powder and therefore there is less starch available to form a matrix. Since it is the starch matrix that will glue the particles together, less matrix formed will lead to an easily disrupted matrix. The incorporation of wet particles, which are completely swollen, causes the same effect as the dry particles with the higher swelling capacity. Unlike SPS, durum wheat semolina (DWS) it is not significantly affected by either the different volume fractions or type of broccoli powder incorporated. The DWS system remains unaltered as a consequence of its gluten proteins that form a very strong and elastic network that is capable of preventing the broccoli particles from swelling. In comparison with SPS, DWS is a more suitable raw material for the production of the pasta filled with vegetable particles, since it does not show to be greatly affected by either the type or concentration (up to 20% V/V) of broccoli particles added.

Acknowledgments

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CHAPTER 5

HIGH AMOUNTS OF BROCCOLI IN PASTA-LIKE PRODUCTS: NUTRITIONAL AND SENSORIAL EVALUATION

This chapter is based on a manuscript that has been accepted for publication:

Silva, E., L. Gerritsen, M. Dekker, E. Van der Linden, and E. Scholten, *High amounts of broccoli in pasta-like products: nutritional evaluation and sensory acceptability*

ABSTRACT

Pasta and noodles were enriched with concentrations of broccoli powder (BP) up to 30% (V/V). In chapter 4 we have shown that the enrichment of pasta and noodles with high amounts of broccoli powder had a large effect on the rheological and textural properties of these products, as a result of structural changes. One of the consequences was the increased cooking losses in the samples enriched with high concentrations of broccoli powder. Developing pasta-like products with nutritional benefits requires that the nutrients are retained within the matrix during the preparation, and leakage during the cooking step should be prevented. This chapter focused on the nutritional and sensorial evaluation of pasta and noodles highly enriched with broccoli powder. Broccoli is widely known for its anti-carcinogenic effects associated with glucosinolates (GLs). Therefore, we have investigated the concentration of these phytochemicals in dried and cooked pasta and noodles. We have found that glucosinolates present in the pasta and noodles increased linearly with the volume fraction of BP up to 20%. Sensory evaluation showed that all the samples were considered acceptable. From both a nutritional and sensorial point of view, we were able to add a maximum of 20% BP with acceptable sensorial and textural properties. For higher percentages, the change in microstructure led to higher losses of nutrients and a lower acceptance. For a concentration of 30% BP, the glucosinolates concentration found in the pasta and noodles did not increase further than the ones with 20% BP, possibly caused by the high cooking losses of these samples. Therefore, incorporation of 30% BP does not lead to additional health benefits over incorporation of 20% BP. Nevertheless, concentrations of 20% BP are much higher than the concentrations found in commercial products.

Keywords: sweet potato starch noodles, durum wheat semolina pasta, broccoli powder, Glucosinolates, sensory evaluation

1. Introduction

Nowadays the demand for new food products with additional health benefits is increasing [1, 2]. Due to an unhealthy lifestyle, more health-related issues arise. Since people are more aware of these health issues, there is an increasing demand for more healthy food [3], especially for the new generation. Several studies indicate that a diet rich in fruits and vegetables plays a protective role against the onset of some chronic diseases. Consequently, it would be beneficial if children eat more vegetables [4-7]. However, children tend to dislike and avoid eating vegetables. On the other hand, they do like pasta-like products [8]. Therefore, we have proposed that incorporating vegetables into pasta and noodles might increase children's vegetable intake (chapter 2). Vegetables such as broccoli have been widely investigated because of the relation between its secondary metabolites, denominated Glucosinolates (GLs), and health benefits [5, 9, 10]. Glucosinolates are by themselves primarily inactive. Upon consumption or processing, these phytochemicals are hydrolyzed by an endogenous enzyme, myrosinase, and form breakdown products. These breakdown products include isothiocyanates that are associated with anti-carcinogenic effects [9, 11-13]. When studying the content of GLs in food stuffs it is important to inactivate myrosinase to exclude the effect of enzymatic breakdown of GLs [10, 14]. Although many more components are present in broccoli, we will mainly focus on GLs in this study as a representative of healthy components. Besides the health benefits of vegetables, some starchy foods, due to its low-glycemic index, are part of a recommended diet that reduces the risk of chronic diseases and are also considered a suitable food to be enriched [15-17]. In chapter 4, we have reported the production and physical characterization of sweet potato starch noodles and durum wheat semolina pasta, enriched with different amounts and types of broccoli powder (BP) [18]. In that chapter, we have seen that the incorporation of BP into pasta and noodles has detrimental effects on the dough rheology and textural properties of these products, caused by the significant swelling of the BP. These changes become also apparent during the cooking process, as samples with higher volume fractions of BP have a weaker structure, resulting in higher cooking losses. Therefore, in order to produce pasta-like products enriched with broccoli powder that will still have some additional health benefits, it is necessary that the structure of these products can cope with the high volume fractions of particles to retain the healthy components. The aim of the work described in this chapter was to investigate the effect of different concentrations of broccoli particles on the changes in textural and sensorial properties of the pasta and noodles, and the ability of these products to retain the added broccoli particles and the subsequent glucosinolates. We hypothesize that only a limited amount of broccoli particles can be added to increase the amount of glucosinolates, due to changes in the microstructure of the systems. These parameters were tested at different stages in the preparation of the noodles and the pasta.

2. Materials and Methods

2.1. Materials

Sweet Potato starch (SPS, Mong Lee Shang) was kindly supplied by Deximport (Barendrecht, The Netherlands) and Durum wheat semolina (DWS) was purchased at a local windmill (De Vlijt, Wageningen). Broccoli was bought in a local supermarket (Albert Heijn, Ede, The Netherlands) and the commercial broccoli powder was purchased from Z Natural Foods, LLC (West Palm Beach, USA).

2.2. Chemicals

The HPLC grade solvents methanol and acetonitrile, used for extraction and chromatography, respectively, were purchased from Biosolve (Valkenswaard, The Netherlands). The internal standard glucotropaeolin was bought from the Laboratory of Biochemistry, Plant Breeding and Acclimatization Institute at Radzikow, Blonie, Poland. Sulphatase type H – 1, from *Helix pomatia* (cat. No. S9626) with an activity of 16,020 U/g of solids and DEAE Sephadex A-25 were bought from Sigma-Aldrich. Milli-pore water was used in the samples undergoing HPLC analysis. Demineralized water was used to prepare the samples and tap water was used to cook the pasta for the sensory test.

2.3. Broccoli powder preparation

Broccoli florets were separated from the stem and only the florets were used, hereafter referred to as broccoli. Broccoli was washed under running tap water and cut into small pieces of approximately 3×3 cm. To inactivate the endogenous myrosinase, portions of 300 g of broccoli were placed in a 1 L container and blanched in a microwave for 4 min 50 sec at

900W (DAEWOO, Model KOC-87-T Korea) [19]. After blanching, the samples were first cooled on ice, subsequently frozen in liquid nitrogen and immediately blended in a kitchen machine ("Thermomix" Vorwerk, The Netherlands). The frozen broccoli powder was then placed in aluminum trays and freeze dried (GRInstruments, Model GRI 20-85 MP 1996, The Netherlands) until a constant weight was obtained (approximately 6 days). After being dried, broccoli powder was sieved in a sieving machine (Retsch® ZM 200, Germany) using three sieves with different mesh sizes (25, 53 and 125 µm). Commercial broccoli powder was also sieved following the same method. The broccoli powder produced in-house will hereafter be referred to as HMBP, whereas commercial broccoli powder will be referred to as CBP.

2.4. Preparation of pasta for sensory evaluation

For the sensory evaluation, durum wheat semolina (DWS) pasta with 10, 20 and 30% broccoli powder was prepared in a lab extruder (La Monferrina, Model Molly, Italy). The concentrations of broccoli powder always refer to V/V %. A blank sample, with no broccoli powder added was used as a control and Tagliatelle Verdi (Grand'Italia, Italy) with 2% spinach was used for comparison with a commercially available enriched product. The preparation of the pastas with different amounts of broccoli powder required different moisture contents to obtain a good dough consistency for the extrusion process. The blank and the pasta with 10% BP had a moisture content of 30%, the 20% BP pasta a moisture content of 35% and the pasta with 30% BP had a moisture content of 40%. The dry ingredients were mixed first, then the water was added and the dough was kneaded inside the extruder for approximately 10 min until a dry and grainy dough was obtained. After kneading, the dough was extruded into pasta strands (cross-sectional area of 7.98 ± 0.19 mm^2) and cut into strands of approximately 10 cm. Cooking of the pasta and sensory evaluation took place one day after the pasta was produced, and the pasta was kept in the fridge overnight before use.

2.5. Sensory evaluation

A sensory evaluation of the different pastas was carried out in order to evaluate the acceptability of the enriched products. The sensory evaluation was performed in one day over five sessions and the pasta products were cooked before being served in each session. The optimal cooking time varied between 2.25 – 4.25 min for our fresh pasta samples (Table 5.1) and was 8 min for the (dry) commercial sample. After cooking, the samples were rinsed with cold water, drained and their sensory evaluation started within 20 min, the samples being at room temperature at that moment.

Table 5.1 Cooking times (minutes) of fresh pasta for the sensory evaluation and texture analysis.

	Cooking time (min)
Commercial pasta	8 ± 0.5
Blank	3.25 ± 0.25
10% BP	4.25 ± 0.25
20% BP	3 ± 0.25
30% BP	2.25 ± 0.25

The sensory evaluation was based on quantitative methods and two tests were performed: an acceptance test and an attribute diagnostics test. The sensory evaluation was carried out by 47 consumer panelists (20 men and 27 women, 28.7 ± 8.8 years old). The acceptance test (liking) was performed separately from and prior to the attribute diagnostic test. In the acceptance test the panelists evaluated pasta (identified with 3 digit random codes) for acceptability based on how much the products were liked, considering overall liking and liking of color, texture and taste (structured scale from 1 = dislike intensely to 9 = like extremely) [20]. The products were considered acceptable when their mean values were above 4.5 (between the scores for 'like slightly' and 'like moderately'). After the first test, the attribute diagnostics test was done and the panelists were asked to evaluate the enriched pastas based on the intensity of three sensory attributes, described to them as follows:

Firmness: the amount of force required to bite with your teeth through the pasta strands;

Stickiness: the amount of force required to remove pasta strands that adhere to your teeth;

Vegetable flavor: the intensity of the vegetable flavor in the mouth (for example, broccoli flavor or spinach flavor).

These parameters were evaluated in a non-structured line scale (12 cm) where the left anchor represented the lowest intensity of a particular attribute and the right anchor represented the highest intensity. Drinking water was provided for palate cleansing between each sample [21].

2.6. Statistical analysis

Statistical analyses were performed using SPSS software (IBM). Descriptive statistics and non-parametric test (Wilcoxon) were used to determine the mean and the standard deviation of the sensory attributes and to determine statistical differences between samples.

2.7. Preparation of pasta and noodles for glucosinolates analysis

SPS noodles and DWS pasta were prepared according to the methods described in sections 2.5 and 2.6, respectively, in chapter 4. Samples with 10, 20 and 30% BP were produced and the BP concentrations always refer to V/V. Pasta and noodles were cooked to their optimal cooking time (Table 5.2). After that, the noodles were rinsed with cold water, drained and analyzed within 20 min.

Table 5.2 Cooking times (minutes) of fresh and dried pasta and noodles produced for the GLs extraction.

	Pasta		**Noodles**	
	Fresh	Dried	Fresh	Dried
Blank	8 ± 0.5	9 ± 0.5	0.7 ± 0.17	-
10% BP	7 ± 0.5	8 ± 0.5	0.7 ± 0.17	4.5 ± 0.25
20% BP	5 ± 0.5	6 ± 0.5	1 ± 0.25	4.5 ± 0.25
30% BP	2 ± 0.5	5 ± 0.5	1 ± 0.25	5.5 ± 0.25

Dried samples were also prepared for the GLs extraction and for that, both pasta and noodles were dried in an oven at 43 °C until constant weight (approximately 24 h). Broccoli powders with different particle size distributions (25 – 53 μm, 53 – 125 μm, > 125 μm and 'not sieved (NS)') and different swelling capacities were used.

2.8. Glucosinolates extraction

In order to determine the amount of glucosinolates (GLs) that were added to the pasta and noodles, the amount of GLs in the pure broccoli powders was investigated. For that, 0.2 g of (dried) BP were used. For the determination of GLs in pasta and noodles, 2.5 g of fresh and cooked samples and 1.5 g of dried samples were used. GLs extraction was done based on the method described by Verkerk et al [22]. Samples were weighed into 15 ml disposable tubes and 4.8 ml of pre-heated 100% methanol (incubated at 80 °C for at least 60 min) and 400 µl of 3mM glucotropaeoline (internal standard) were added to the sample. The samples were then incubated at 80 °C for 20 min and mixed every 5 min using a vortex. Following incubation, the samples were centrifuged at 2500 RPM for 10 min. The supernatant was collected in a new 15 ml disposable tube and the pellet was re-extracted two more times with 4 ml of a pre-heated 70% methanol solution. The supernatant of the re-extractions was combined with the supernatant of the first extraction and stored in the freezer until desulphation.

2.9. Glucosinolate purification and desulphation

Purification of GLs took place in a DEAE Sephadex A-25 anion exchange column and followed the method described by Oerlemans et al [14] with minor modifications. The DEAE Sephadex column, with a height of 1.5 cm, was prepared inside a 2 ml syringe. The syringe was placed in a glass tube, the column was washed twice with 1 ml of milliQ water and 2 ml of supernatant (containing extracted GLs) were added. Subsequently, the syringe was then placed in a new glass tube and 75 µl of sulphatase solution (25 mg/25 ml) were added to the column and this was incubated overnight at room temperature. After approximately 16.5h, the desulphated glucosinolates were eluted with (3×0.5ml) milliQ water and the eluate was filtered using a 0.45 µm filter (13 mm, Alltech, Deerfield, IL, USA).

2.10. HPLC analysis

Desulfoglucosinolates were separated and analyzed by high-performance liquid chromatography (HPLC) using a Lichosphere RP–18 column (Merck, Darmstadt, Germany). Elution of desulfoglucosinolates was carried out at a flow rate of 1 ml/min in a total running time of 25 min and with an injection volume of 20 µl. The gradient elution was as follows:

100% Milli-pore water and 0% acetonitrile for 2 min, from 0 – 8% acetonitrile between 2 and 7.5 min, up to 25% acetonitrile between 7.5 and 14 min, 25% acetonitrile until 18 min, back to 0% acetonitrile between 18 and 20 min and 0% acetonitrile until 25 min. Glucosinolates were detected at a wavelength of 229 nm and were identified based on the retention time and spectra of the internal standard (glucotropaeolin) and reference materials. The relative response factor of each glucosinolate was used for its quantification.

2.11. Texture Analysis of cooked pasta

The pasta products produced in the lab extruder were cooked according to their optimal cooking time (Table 5.1) and their texture was analyzed using the same method as described in section 2.8 of chapter 3. All the strands tested had a length of 15 mm and an average cross-sectional area of 7.98 ± 0.19 mm^2. Reported values are an average of at least five measurements.

2.12. Confocal laser scanning microscopy of dough noodles/pasta

Pasta and noodle dough were analyzed by confocal laser scanning microscopy (CLSM). The samples were prepared as described before (section 2.7), and post-stained with a solution of 0.25% (w/w) Fluorescein 5-isothiocyanate (FITC) and 0.025% Rhodamin B in water. The image acquisition was done according to the method described in section 2.6 of chapter 3.

3. Results and Discussion

The texture of noodles and pasta is very dependent on the microstructure, which is affected by the incorporation of vegetable particles. The microstructure of the vegetable-filled matrices is dependent on the continuous matrix (gluten or starch) and the swelling of the dispersed vegetable particles. Figure 5.1 shows an example of the effect of the incorporation of broccoli particles. Figure 5.1a shows the uncooked noodle dough from sweet potato starch with 20% of vegetable particles, whereas figure 5.1b shows the pasta dough prepared from durum wheat semolina. As can be observed, the doughs have different microstructures. In the case of the noodle prepared from starch, we observe a very closed packed system, in which the pre-gelatinized starch is used to glue the starch and vegetable particles together.

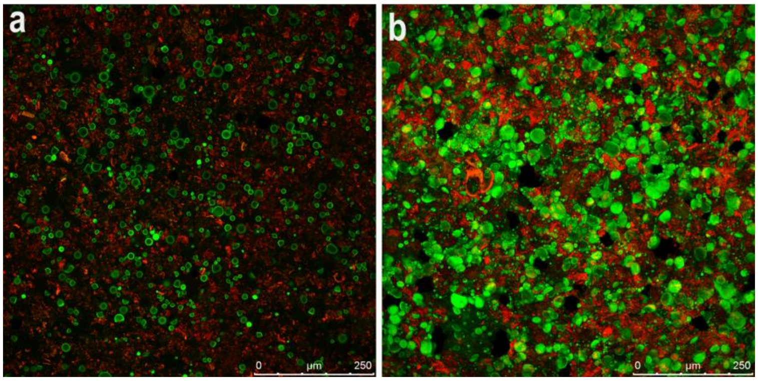

Figure 5.1 Confocal laser scanning microscopy of the sweet potato starch dough with 20% broccoli powder (a) and durum wheat semolina dough with 20% broccoli powder (b).

The close-packing is a consequence of the swelling ability of the vegetable particles (up to a factor of 7.6) and the high amount of starch granules. The effect on the microstructure has already been extensively discussed in chapter 2. In the case of the durum wheat semolina dough, the particles are assumed to still be dispersed in a continuous network of gluten. The dough microstructure is related to several parameters during cooking, such as the cooking loss and the swelling index (not presented). We have observed that the cooking losses for durum wheat pasta only changes with 10% as a consequence of the incorporation of 20% particles, whereas for the starch noodles, we observed an increase in the cooking losses of 180%. High cooking losses will not be preferred for vegetable-enriched products, as all beneficial nutrients will not be retained in the cooked pasta. As starch and gluten matrices show differences in microstructure, we expect that the capacity to maintain the incorporated vegetables will be different.

3.1. Effect of different particle sizes of HMBP and CBP

The amount of glucosinolates for the different particle sizes, namely 25 – 53 µm, 53 – 125 µm, > 125 µm and 'NS' (not sieved) as well in home-made (HMBP) as in commercial (CBP) broccoli powder is shown in figure 5.2. We can see that there is no significant difference between the different particle sizes of HMBP. All particle sizes had an approximate average GLs content of 10.98 ± 0.38 µmol/g dw, which is within the limits of 0.6 – 59.3 µmol/g dw reported by Verkerk et al [10] as being the range of published GLs content of broccoli. CBP presented a much lower GLs content than the HMBP (all the samples < 0.7 µmol/g dw). In the

CBP there is a small difference between the different particle sizes, but this difference was not found to be significant.

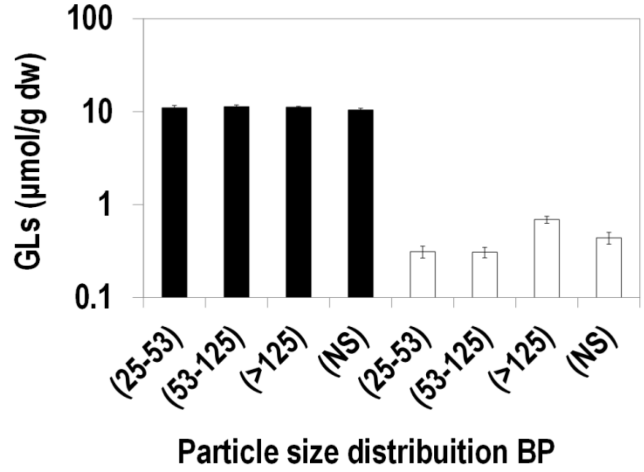

Figure 5.2 Glucosinolates content in different types and particle sizes of broccoli powder (25 – 53 µm, 53 – 125 µm, > 125 µm and "NS" (not sieved)). Filled bars correspond to the broccoli powder produced in-house (HMBP) and the unfilled bars correspond to the commercial broccoli powder (CBP).

Since there is such a low amount of GLs in CBP, this powder was not used further in the study, and the pasta or noodles were only enriched with HMBP. A possible reason for the large difference in the amount of GLs present in both powders could be the result of the type of broccoli (dependent on cultivar and cultivation conditions), the storage time of the powder (in the case of CBP) or the production process (myrosinase might not have been properly inactivated in the CBP production) [23].

3.2. Effect of BP particle sizes on the retention of GLs in Pasta/Noodles

Even though there is no difference in GLs content in the different particle sizes of HMBP (as seen in figure 5.2), the effect of the particle size on the microstructure of the pasta and noodles may still lead to differences in the loss of the glucosinolates during the cooking process. Therefore, pasta and noodles were produced with powders with the different particle sizes. Figure 5.3 shows the amount of glucosinolates present in fresh pasta and noodles prepared with 20% BP with different particle sizes. In chapter 2 we have shown that, in starch systems, at this volume fraction, the particles are densely packed and influence the microstructure to great extent. From figure 5.3 it is clear that at this volume fraction, the particle size does not influence the amount of glucosinolates extracted from these uncooked matrices.

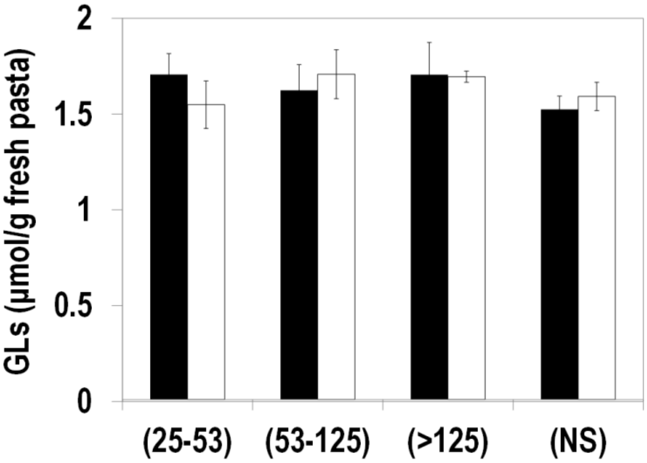

Figure 5.3 Glucosinolates content in fresh pasta and noodles with 20% HMBP with different particle sizes (25 – 53 µm, 53 – 125 µm, > 125 µm and "NS" (not sieved)). Filled bars correspond to durum wheat semolina pasta samples and the unfilled bars correspond to sweet potato starch noodle samples.

As the particles have the ability to swell and the starch granules change upon heating, the changing in the microstructure upon cooking might affect the leaching rate of the GLs from the matrices. Therefore, fresh and dried pasta and noodles containing 20% HMBP were also analyzed for GLs after cooking. No differences were found in these cooked samples (data not shown), indicating that the particle size has no influence. Therefore, the "not sieved" (NS) broccoli powder was used in the remaining experiments.

3.3. Effect of BP concentration on the retention of GLs in cooked Pasta/Noodles

DWS pasta and SPS noodles were enriched with 10, 20 and 30% BP (V/V). For the GLs quantification, both pasta and noodles were analyzed in the fresh state, before and after cooking to see the effect of the cooking step. Also the effect of drying was investigated by analyzing the samples in the dried state, before and after cooking. The results can be seen in figure 5.4.

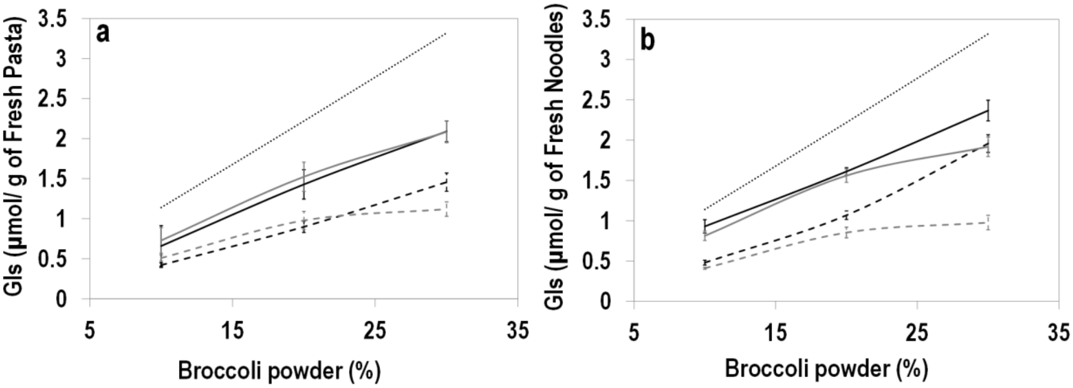

Figure 5.4 – Glucosinolates content in DWS pasta (a) and SPS noodles (b) enriched with 10, 20 and 30% BP. The dotted line corresponds to the expected amount of GLs. The black full line and the black dashed line correspond to the fresh samples before and after cooking, respectively. The grey full line and the grey dashed line correspond to the dried samples before and after cooking, respectively.

The results are normalized to an original 1 gram of fresh pasta (taking into account the drying and swelling parameters during the drying and cooking step). The fresh and dried pasta (figure 5.4a) and noodles (figure 5.4b) show an expected increase in their content of GLs before cooking with increasing volume fractions of HMBP. However, for all the different broccoli concentrations tested, the amount of GLs extracted from both the pasta and noodles was always lower than the GLs added. Since degradation of GLs is not expected during the low temperature preparation of the fresh products, a possible explanation for the lower amounts of GLs might be the entrapment of a certain fraction of these components in the matrix, making the extraction more difficult [14]. As figure 5.4 shows, the drying step does not influence the amount of GLs extracted from these matrices as dried samples showed similar GLs content as the fresh samples. After cooking, all products show a reduction in GLs content. This reduction can be explained by the fact that water soluble GLs leach from the pasta and noodles into the cooking water [24]. As previously discussed, this is an effect of the microstructure of the dough. For the fresh samples, uncooked and cooked, we see that the GLs content increases linearly with the concentration of the broccoli added. This indicates that up to 30% of vegetables, the microstructure is able to prevent the vegetable particles from leaching, and as a result the GLs are retained. However, when the samples were dried before cooking, we see that less GLs were detected than expected; the amount of GLs levels off at higher volume fractions. For the 30% BP pasta and noodles, the content of GLs in the cooked products is not significantly different from the dried cooked pasta and noodles enriched with 20% BP. This indicates that these samples most likely have a higher cooking loss and therefore also a higher leakage of the nutrients. This is likely to be a result of the microstructural changes as a result of the drying procedure in combination with a longer cooking time than needed for fresh pasta and noodles. Apparently, the drying of the pasta affects the structural organization of the matrix, which leads to a decrease in the ability to retain the vegetable particles. However, it is not clear which changes exactly occur during this drying step. When high amounts of vegetables are added, the system changes from a dispersed system to a more close-packed system. This results in a weaker matrix that can easily fall partly apart, which will ease the release of the vegetable particles from the matrix. This was indeed observed, since the cooking water turned greener indicating that more vegetables were present. In the range of volume fractions studied, 20% is the maximum amount of broccoli that the noodles/pasta can retain. For higher concentrations, the microstructure changes to such an extent that the vegetable particles (and the subsequent glucosinolates) are not included in the cooked product anymore. Therefore, from a nutritional point a view, there is no advantage to add more than 20% of BP. In general, the type of matrix does not show a large difference in the content of GLs for the same preparation conditions. This was unexpected, since the starch matrices already showed a more close-packed structure and higher cooking losses as discussed previously, and were expected to show a higher loss in glucosinolates. However, the cooking losses could also have existed from the starch instead of the vegetable particles. Apparently, the microstructure during cooking also changes and affects the release of the glucosinolates. So even though large differences can be seen in the microstructure, the difference in matrices does not seem to have a large effect on the loss of the glucosinolates from the vegetable particles. Only for 30%, the change in microstructure seems to be detrimental, but only for dried samples. Only a slight difference was observed for the fresh cooked 30% BP samples. In this case, the 30% BP fresh cooked noodles contained 15% more glucosinolates than the fresh cooked 30% BP pasta. This could be related with the cooking time, since the cooking time of pasta is twice the cooking time of noodles. A longer cooking time will probably increase the cooking losses, as well as thermal degradation explaining why more GLs are available in noodles than in pasta. The dried cooked samples, which have the same cooking time, show the same amount of GLs in pasta and noodles.

3.4. Sensory evaluation

From the analysis of the glucosinolates content, we observe that the matrix (durum wheat semolina, starch) does not have a large effect on the amount of glucosinolates maintained in the fresh cooked pasta, and could therefore both be considered as good candidates to produce vegetable-enriched pasta-like products. However, when looking at the textural properties, we have observed major differences. Since vegetables in starch systems have the ability to swell, the microstructure changes into a close packed system, which leads to higher values in strength, stiffness, and lower extensibility [18]. Durum wheat semolina pasta, on the other hand, does not show particle swelling, which leads to a texture similar to the one without added particles, as seen in chapter 4. Therefore, we believe that durum wheat semolina is the best candidate for the incorporation of vegetable particles. For the sensory evaluation, we have focused on the pasta prepared with durum wheat semolina.

In sensory evaluations, quantitative methods are used to measure either preference or acceptance of products. In this study, two different quantitative methods were used to access the acceptability of the pasta enriched with broccoli powder. The two different tests used were an acceptance test and an attribute diagnostics. Acceptance tests give an indication how much people like a specific product and can be very helpful in the evaluation of improved formulations. Attribute diagnostics gives more detailed information, such as how the perception of a specific sensory attribute can relate to the liking or disliking of a product [20]. Sensory evaluation of enriched broccoli pasta (with volume fraction of 0 (blank), 10, 20 and 30%) is shown in figures 5.5 and 5.6. Regarding the acceptance test (figure 5.5), four parameters were investigated: overall liking (a), color (b), texture (c) and taste (d) and the results show that all the parameters were above the minimum limit for acceptability (4.5). Overall, the parameters “Overall liking”, “liking of color” and “liking of taste” did not show significant differences between the blank samples and the enriched samples (10, 20 and 30% BP). However, we do see significant changes between the samples with 20 and 30% BP. In general we see that the 30% BP samples were rated slightly lower than the 20% for all parameters. For the parameter “liking of texture”, we also observe a significant difference with the blank.

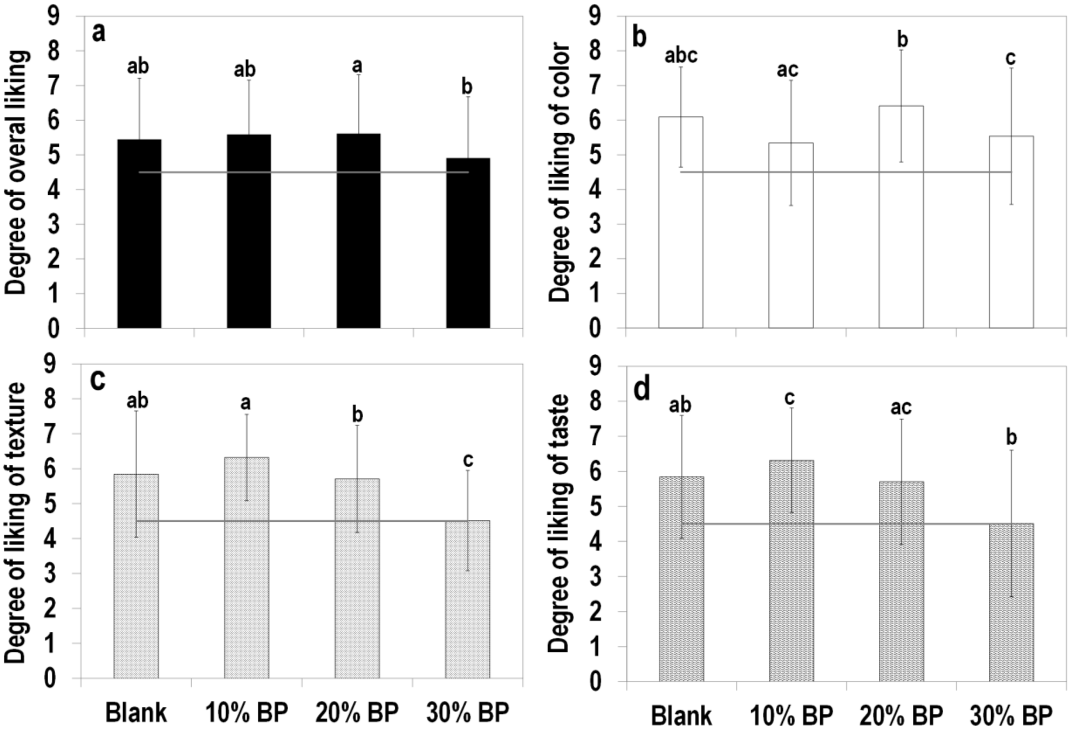

Figure 5.5 Acceptance test, overall liking (A), liking of color (B), liking of texture (C), and liking of taste (D) of pasta with 0, 10, 20 and 30% BP. The same letter above the bar indicates no significant difference between samples (P < 0.05).

Regarding the attribute diagnostics test (figure 5.6) clear differences were observed between samples and the tested parameters. For this test, a commercial sample (*Tagliatelle Verdi*, from Grand'Italia with 2% spinach powder) was also included. For the parameter "Firmness" (figure 5.6a), the blank and the commercial sample were perceived as the firmer samples, with the blank sample being slightly firmer than the commercial sample. The broccoli-enriched samples had a lower firmness than the blank and the commercial sample and this parameter decreased as the concentration of BP incorporated increased. Regarding "Stickiness" (figure 5.6b) there is no difference between samples, with the exception of the commercial *tagliatelle* which is significant lower than the other samples. In figure 5.6c the results of the parameter "vegetable flavor" are shown. For this parameter, the perception of vegetable flavor increased with increasing concentration of BP added. As the blank sample was not marked as zero for the vegetable flavor it still scored a slight positive value. The commercial sample, which contains only 2% spinach powder, had a perceived vegetable flavor lower than our sample with the lowest vegetable concentration (10% BP), as expected.

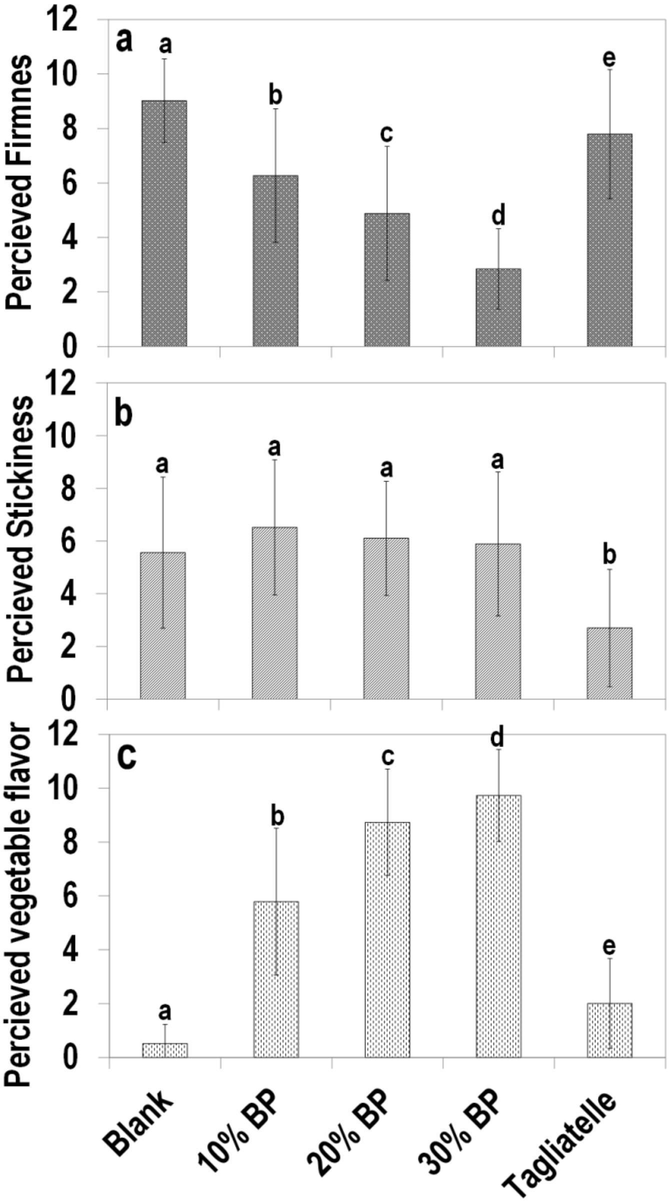

Figure 5.6 Attribute diagnostics test, Firmness (a), Stickiness (b) and Vegetable flavor (c) of blank and commercial pasta (Tagliatelle Verdi, 2% spinach powder) and the pasta with 10, 20 and 30% BP. The same letter above the bar indicates no significant difference between samples (P < 0.05).

Combining the results of the acceptance test and the attribute diagnostics, it is possible to link the sense of liking with the different attributes. The decrease in “Firmness” with increasing concentration of BP added might explain why the “liking of texture” decreased, as good quality pasta should be somewhat firm [21]. In the same way, the decrease in “linking of taste” is likely to be the result of the increasing vegetable flavor as the concentration of BP added increased.

In summary, we can say that despite all samples (0 – 30% BP) are considered acceptable, the sample with 30% BP is clearly less liked than the other samples, caused by the very low firmness and very strong vegetable flavor. However, up to 20% BP, the acceptability of the pasta did not decrease significantly, which shows that the pasta could be incorporated with 20% of vegetables, much higher that the concentrations commonly available on the commercial market.

3.5. Texture Analysis

The texture of the samples prepared in the lab extruder (for the sensory evaluation) was analyzed in the texture analyzer, measuring the parameters “Strength”, “Extensibility” and “Stiffness”. Texture analysis was previously performed with samples prepared in a commercial sheeting/cutting machine, discussed in chapter 4. Comparing the results between the two preparation methods, we see that up to a concentration of 20% BP (V/V), the texture analysis leads to similar results, so the method of preparation does not lead to great differences in the texture. Regarding the “strength” of the samples prepared in the lab extruder (figure 5.7a), there is an increase in this parameter when 10 and 20% BP is added, as a result of the increased volume fraction of particles in the system. When 30% BP is added, the strength decreases to values similar to that of the blank. This decrease in strength for highly enriched samples show that the texture is changed significantly and is an indication that the system cannot cope with high volume fractions of particles added, making the structure weaker as already discussed before. The extensibility of these samples can be seen in figure 5.7b. Extensibility decreases with an increase in the concentration of BP added, to values half of that of the blank. Figure 5.7c shows the stiffness of the samples. The stiffness of the pasta increases when only 10% BP is added and decreases again for higher concentrations of BP, resulting in a stiffness for 30% BP sample similar to the blank sample.

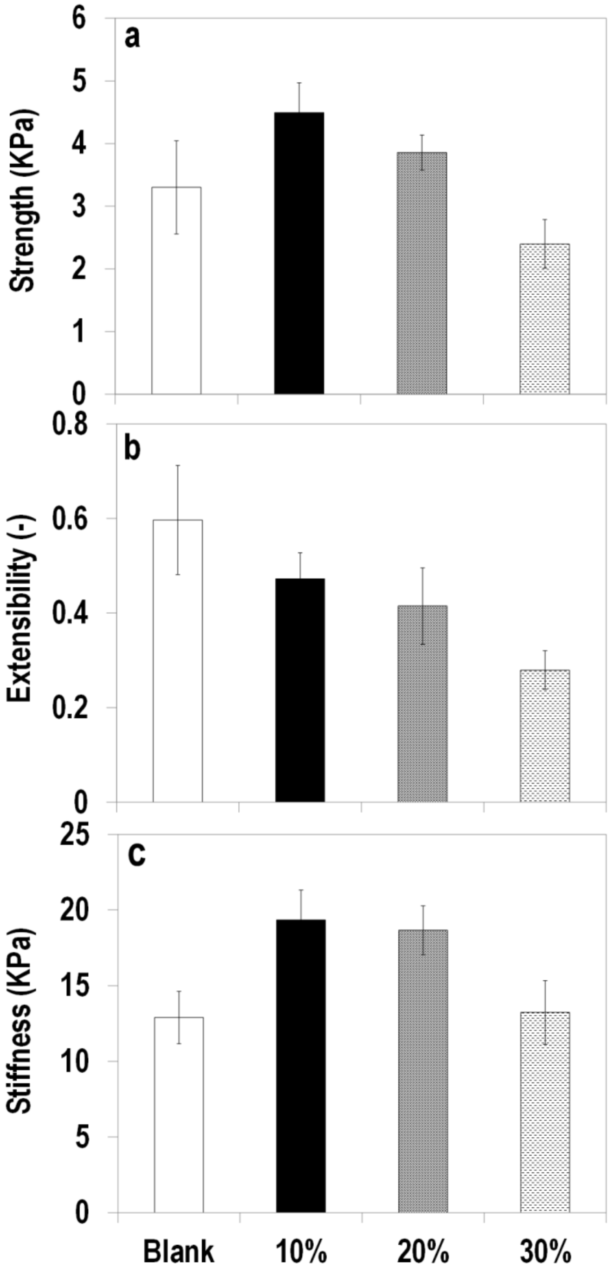

Figure 5.7 Texture parameters, strength (a), extensibility (b) and stiffness (c) of DWS pasta with 0, 10, 20 and 30% BP incorporated.

These results are in agreement with those of the parameter strength, which indicates again that the samples become weaker and cannot cope with high concentrations of flour being replaced by broccoli powder.

Overall, we see that the largest change in the textural property appears between 20 and 30%. This can be related to the microstructural properties of the pasta, for which we saw that at these high loadings, the system becomes close-packed, leading to a weak structure (lack of a firm continuous matrix) and high losses of glucosinolates. This is in agreement with the sensory evaluation that also shows that the liking of texture and taste changes largely between 20 and 30%. Therefore, we can assume that the microstructure, textural properties and the sensory acceptance are related. Since the relation between sensory attributes as stickiness and firmness to textural parameters are not yet clearly correlated and reported in literature, it is difficult to relate them directly. From our results, we see that the mechanical properties of pasta with BP (measured in the texture analyzer) are largely in agreement with the perception of “Firmness” described in the sensory evaluation. With the exception of the blank, the decrease in stiffness and strength corresponds to a decrease in firmness upon addition of BP.

In summary, we can conclude that we can enrich pasta and noodles with a volume fraction up to 20% of broccoli. At this concentration, the microstructure of the pasta is able to retain the glucosinolates and to remain acceptable for its texture.

4. Conclusions

Sweet potato starch noodles and durum wheat semolina pasta were enriched with concentrations of broccoli powder up to 30% (V/V). This work focused on the nutritional and sensorial characterization of these highly-enriched products. As previously shown, these high loadings strongly affect the rheological and textural properties of these products, and were expected to influence the nutritional and sensorial properties. The processing of the pasta and noodles showed to have an effect on the retained GLs, as cooking slightly decreased the amount of glucosinolates present in the pasta-like products due to thermal degradation and leaking of the broccoli during the cooking step. Drying the samples before cooking leads to a further decrease in retained GLs, which can be explained by a possible change in structure and an increase in the cooking time. In general, the glucosinolates content increased with increasing broccoli content. However, for samples that where first dried and then cooked, the glucosinolates content increased only up to 20% broccoli powder and then leveled off to a constant value for GLs when 30% broccoli powder was added. Sensory evaluation revealed that all samples were considered acceptable, and up to 20% of broccoli no negative effects on acceptability were observed. At higher loadings, of 30% broccoli powder, we see that the sample was clearly less liked than the others. It also suggested that there is a relation between the decrease in "liking of texture" and the decrease in "Firmness" with increasing concentration of broccoli powder. In the same line of thought, the decrease in "liking of taste" could be related with the increase in the "vegetable flavor" as the broccoli powder concentration increased. Therefore, combining the nutritional and sensorial results, we can conclude that we are able to incorporate 20% broccoli powder to these types of matrices. Higher loadings do not lead to additional nutritional health benefits. The concentration of 20% of broccoli powder is much higher than the concentrations found in commercially available products.

Acknowledgments

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CHAPTER 6

GENERAL DISCUSSION

1. Introduction

In the last two to three decades people have adopted more and more a lifestyle that combines poor food choices with very low or no physical activity, resulting in the development of diseases such as obesity [1-7]. Obesity has become a global epidemic [8-12], and has been linked to the development of other chronic diseases such as type II diabetes, hypertension, coronary heart disease and several types of cancer [7, 13-16]. One of the most effective strategies to fight obesity combines physical activity and the consumption of low energy-dense foods, such as vegetables, starting at an early age [17-19]. Implementing this strategy already at an early age is important since there are strong indications that childhood obesity will persist into adulthood [20-24]. A problem for this strategy is the fact that children dislike vegetables [25, 26]. One of the possible solutions to increase vegetable intake by children is to incorporate vegetables in a food matrix they like. This strategy has also been used to treat dietary imbalance [27]. Several studies have shown that pasta is very appreciated by children [26, 28, 29]. Besides being liked by children, pasta is also regarded by the WHO and FDA as a suitable matrix to be enriched with other ingredients/nutrients [30-33]. The enrichment of pasta products is an old practice, dating back more than five decades [34]. At that time, soy flour was added to macaroni to increase the products' protein content and since then the interest in this topic has increased, as evidenced by the number of publications on this topic. Over the years, proteins [35-37], dietary fiber [38-41], and different types of legume/vegetable flours [30, 33, 42-50] have been used for enrichment of pasta-like products with the objective of using local raw materials, using cereal by-products, produce gluten-free pasta or develop products with additional health benefits [51]. However, the enrichment of pasta still represents challenges since the incorporation of particles will dilute the matrix affecting the quality of these products [43, 52]. Most common problems of high enrichment levels are high cooking losses [42, 43, 53-55], deterioration of texture (such as decreased firmness and increased stickiness) [42, 43, 45, 52, 54, 55] and change in taste [42, 45], as well as difficulties during processing [52, 54, 56]. Pasta is considered a healthy component of the diet because it contains complex carbohydrates, has a low content of salt and fat, and has a low glycemic response [32, 43, 57]. But pasta also has a low content of the essential amino acids lysine and threonine [30, 45, 54]. Therefore, nutritional improvement is pointed out in literature as the main reason for the enrichment [31, 33, 39]. Since the enrichment of pasta products can serve different purposes, the type of ingredients added varies from soluble and insoluble polysaccharides [35, 38-41, 58-69], to fruit [57, 70, 71], legume/vegetable flour [30-34, 36, 43-47, 49, 50, 52-54, 72-81], proteins [35-37, 48, 55, 61, 82], seeds [31, 43, 46, 49, 53, 68, 83, 84], and cereals other than wheat [31, 43, 50, 56, 78, 79, 85-89]. In this thesis, broccoli powder was used to enrich pasta-like products. Broccoli is very popular for its health benefits, related to Glucosinolates (GLs) [90-92]. Despite the profitable changes concerning health, the enrichment of pasta-like products with high concentrations of unusual ingredients usually results in the dilution of the structuring matrix, leading to increased cooking losses and decreased sensory and textural properties [33, 54, 93]. A possible way to overcome these negative effects is by using hydrocolloids, as they have been extensively used in the food industry as texture enhancers. With respect to matrix type, a lot of work has been done on the enrichment of durum wheat semolina pasta, whereas enrichment of starch noodles is not a common practice and only a few studies can be found [36, 37, 39]. Vegetable-enriched pasta is already commercially available, but vegetable concentrations are often low, usually around 2 – 3%. This is the most significant difference in comparison to our work, in which we incorporated broccoli powder in concentrations up to 30% (V/V) to either starch– or wheat–based matrices. These systems were characterized in terms of shear rheology, microstructure, texture, and nutritional and sensory properties (figure 6.1). In the first part of this thesis we have described a more fundamental study of the rheological behavior of the enriched systems, in which the swelling was found to be a determining factor (figure 6.1, first image on the far left side of the figure). In the remaining chapters we have explored different matrices (sweet potato starch in combination with hydrocolloids and durum wheat semolina) and investigated their effect on the microstructure and rheological behavior (figure 6.1, second image), and the textural, sensorial and nutritional properties (figure 6.1, image on the far right side of the figure). This chapter discusses the interconnections between the chapters and the future directions for further exploration of the strategy to provide pasta products with high levels of vegetables.

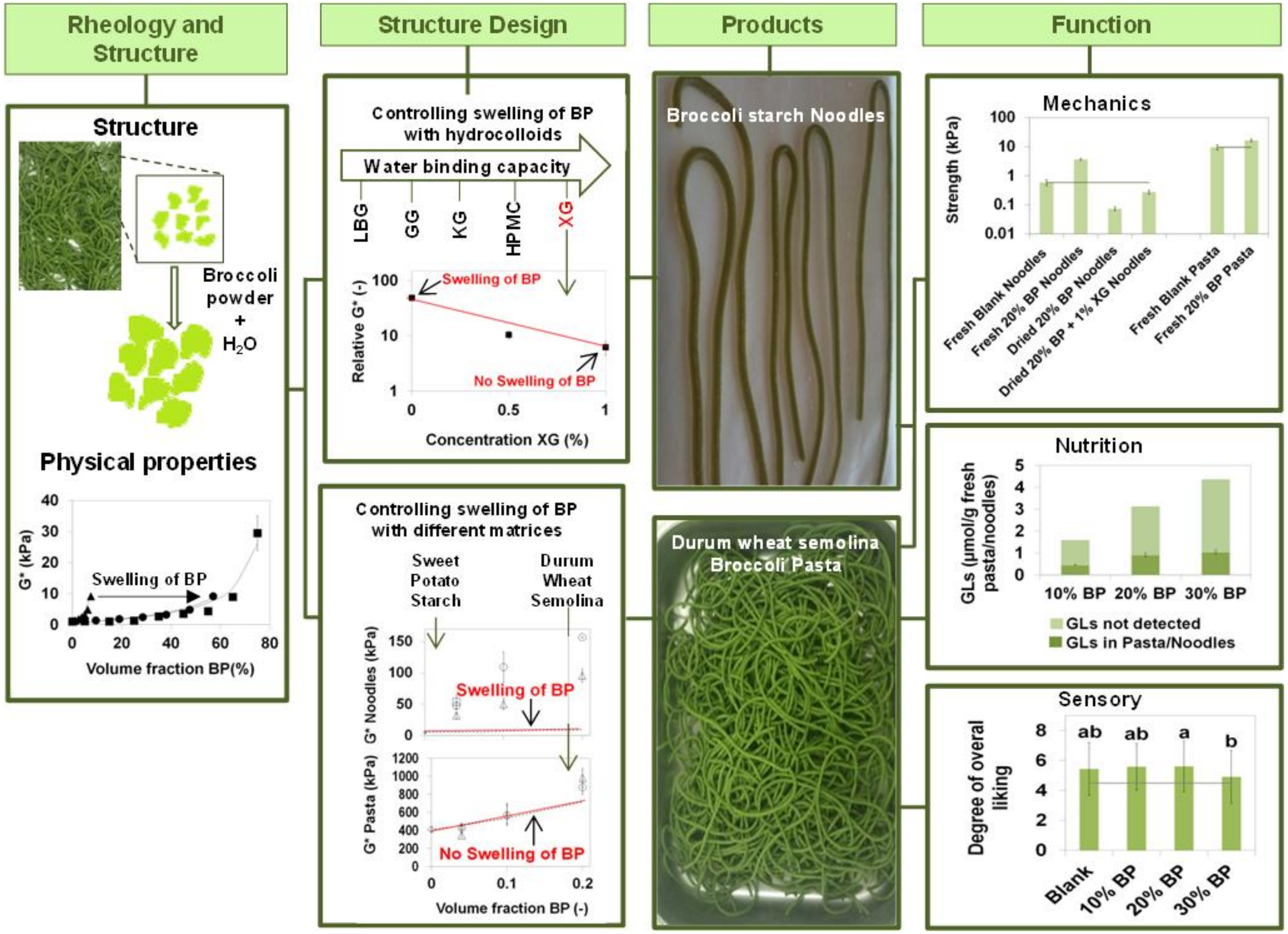

Figure 6.1 Schematic overview of the thesis research approach.

2. Rheological properties versus structure

Food systems such as dough exhibit a very complex rheological behavior [94]. A small difference in the raw materials (such as particles' size, shape and properties) can induce large differences in the rheological behavior of the system [95]. Rheological measurements provide valuable information on the structure and texture of the systems that in turn can be used in the design development and processing of new products [96-98]. These are the reasons why rheology has been a large part of our studies.

2.1. Enriched sweet potato starch noodles

In pure starch products, a continuous visco–elastic matrix is formed by pre-gelatinization of part of the starch [35, 99]. When starch granules are heated in excess water, they absorb water and swell many times their original size. As a consequence of this swelling, the granules burst open and amylose leaches out. This results in an increase of the viscosity of the suspension and finally a continuous gel phase is formed by amylose (i.e. gelatinization has occurred). In this continuous phase, amylopectin and swollen starch granules are embedded and it is this matrix that will be responsible for the noodle structure [99-107]. Since pre-gelatinized starch has such importance in the production of noodles and on its final texture [108], the effect of different concentrations of pre-gelatinized starch with different amounts of broccoli powder was investigated in this thesis (chapter 2). The rheological characterization of the blank starch dough (with no broccoli powder added) showed an increase in the complex modulus as the concentration of pre-gelatinized starch increased from 10 to 30%, making the dough tougher (figure 2.2a). This increase can be explained by the fact that blank systems (not enriched) can be considered dispersions, for which the modulus is mostly determined by the modulus of the continuous matrix. A schematic overview of this type of systems can be seen in figure 6.2a. Therefore, when the pre-gelatinized starch concentration increases from 10 to 30%, the continuous matrix becomes stronger, and is responsible for the increase of the modulus. However, when the dough is enriched with 20% broccoli powder, a decrease in the complex modulus is observed when the concentration of pre-gelatinized starch increased from 10 to 30% (figure 6.2c). The explanation for this decrease is related to the swelling of the particles. During sample preparation, the dry broccoli powder particles absorbed water and swell, increasing the effective volume fraction of the particles. Swelling tests performed on the broccoli particles have shown that the broccoli particles (produced in-house) can swell to up to 7.6 times their original volume. This means that dough with an original 20% of dry broccoli powder would already be above the maximum packing fraction, taking into account maximum swelling. Considering this, the enriched dough is not a dispersion of particles in a matrix, but a cellular material, in which broccoli particles and starch granules are closely packed and glued together by the amylose matrix. In such cases, the modulus of the system is not determined by the continuous matrix, but strongly dependent on the total volume fraction of particles. Therefore, when the concentration of pre-gelatinized starch increases from 10 to 30%, more starch granules are gelatinized, decreasing the total volume fraction of particles (starch granules and broccoli particles) and consequently the modulus. A schematic overview of this type of systems can be seen in figure 6.2b.

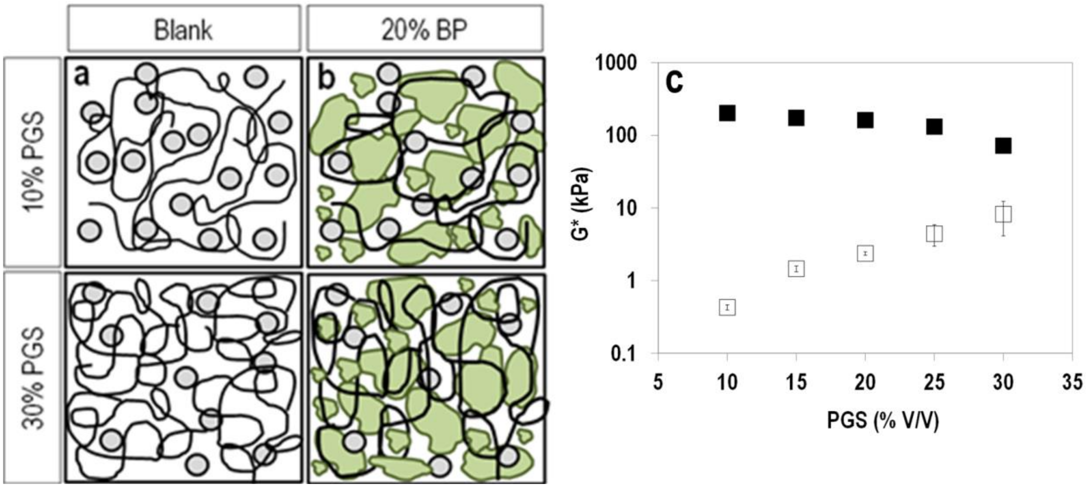

Figure 6.2 Schematic overview of the two different starch systems; dispersion of particles (a) and closely packed system (b) and a comparison of the rheological response between blank (□) and 20% broccoli powder samples (■) as a function of the PGS concentration (c).

The incorporation of 20% broccoli powder increased the complex modulus roughly between 1 and 2 orders of magnitude, for the same level of pre-gelatinized starch, which is an effect of the addition of particles and their swelling capacity (figure 2.2). The increase in the modulus upon the incorporation of particles has been described theoretically. Several theories, such as the one of Krieger and Dougherty (1959) [109] for concentrated dispersions of hard sphere particles, predict an increase in the modulus of the system in a non-linear fashion to infinity when the volume of particles approaches its maximum packing fraction. However, the predicted increase is far smaller than what our experimental data reveal (figure 2.5). The mismatch between that model and the experimental data can be explained by the capacity of the vegetable particles to swell, which was not taken into account when describing the experimental data. The large swelling capacity of the broccoli particles increases the effective volume fraction of particles. When the swelling capacity, and therefore the increase in volume fraction, is taken into account, the rheological response of the enriched dough becomes similar to that of the model. Upon particle swelling, a system with 20% broccoli powder is not a dispersion of particles in a gelled matrix, but a closely packed system, that can be considered a cellular material. This explains the large increase in the complex modulus upon the addition of 20% broccoli powder.

Despite the accepted importance of the pre-gelatinized starch on the production and final quality of starch noodles, still no studies relating the rheological properties and the amount of pre-gelatinized starch could be found in literature. Only few works reported on the enrichment of sweet potato starch noodles, but these did not include the shear rheological properties of these products. Instead, focus was put on the final products' quality and nutritional, structural and textural properties of the product [36, 37, 39]. Bhattacharya and co-workers [108] state that some level of pre-gelatinization is needed to form a matrix that will provide structure to the noodles and that high amounts of pre-gelatinized starch have negative effects on the production of starch noodles, such as high extrusion pressures. An optimal level of gelatinization is therefore needed. Research has shown that pre-gelatinized starch concentrations between 7 – 15% resulted in good quality noodles [108].

An important conclusion of our work on rheological properties of starch noodles' dough enriched with broccoli powder is that there are two important factors affecting these properties. The first one is the amount of pre-gelatinized starch used for the formation of the matrix and the second factor is the swelling capacity of the broccoli particles. At relatively high broccoli concentrations, both factors become important in determining the rheological properties of the system. As the amount of pre-gelatinized starch should be enough to form a continuous matrix sufficiently strong to hold the additional vegetable particles, further studies were conducted on samples prepared with 10% pre-gelatinized starch. To control the rheological properties of the dough, the effective volume fraction of the particles needs to be limited, and particle swelling should be avoided as much as possible. The swelling capacity of the broccoli particles cannot be limited by the starch dough itself, therefore other ingredients/raw-materials are needed to overcome this problem. In order to do so, the use of hydrocolloids or the use of a different type of matrix was considered to be a suitable approach to limit the swelling of the particles.

2.2. Effect of hydrocolloids in starch noodles

The effect of the incorporation of broccoli powder into sweet potato starch noodles is largely determined by the swelling capacity of the broccoli particles, that extracts water from the matrix. To prevent the particles from swelling, the water should be maintained in the dough. The ability to hold the water is related to the water binding capacity of the matrix ingredients. Hydrocolloids were added to increase the water holding capacity of the starch dough and thereby limiting the swelling of the particles (chapter 3). Hydrocolloids have long been used in combination with starch [104]. Due to their large water binding capacity, some hydrocolloids have been used to control the gelatinization of starch granules [110]. In our study, we have used several hydrocolloids that varied from low to high water binding capacities to investigate their ability to limit the swelling of the particles. Hydrocolloids with low water binding capacity (locust bean gum and guar gum, figure 3.1 and 3.2, respectively) and medium water binding capacity (konjac glucomannan, figure 3.3) were not able to control the swelling of the broccoli particles. This was seen in the rheological response of the samples, for which the systems with and without hydrocolloids show very similar behavior, indicating the same effective volume fraction of the dispersed phase. If the hydrocolloids would have been able to limit the swelling of the particles, the effective volume fraction would have decreased, and hence the modulus of the system would have decreased. The hydrocolloids with high water binding capacity (hydroxypropyl methylcellulose and xanthan gum, figure 3.4 and 3.5, respectively) were the ones that could prevent the particles from swelling. Limiting the particle swelling resulted in a decrease in the relative complex modulus of the systems with hydrocolloids in comparison with the systems that did not contain hydrocolloids [65]. The complex modulus of the enriched noodles dough with xanthan gum was lower than the modulus of the system with hydroxypropyl methylcellulose, related to the higher water binding capacity of xanthan (1.3 times higher than hydroxypropyl methylcellulose). Since this decrease was seen in the relative complex modulus, which only takes the addition of broccoli particles into account, these results suggest that hydroxypropyl methylcellulose and xanthan gum are actually able to prevent the swelling of the broccoli particles to some extent.

We can conclude that the degree of swelling of vegetable particles can be limited by the addition of hydrocolloids. Therefore, the addition of hydrocolloids makes it possible to obtain higher volume fractions of vegetable particles in pasta-like products, in comparison with products without hydrocolloids.

2.3. Enriched durum wheat semolina pasta

In the production of pasta from durum wheat semolina, gluten plays a pivotal role. It is responsible for the development of a network that will confer consistency to the pasta strands, for determining the rheological properties of the dough, cooking quality, and the quality of the end product [111-113]. In literature it is found that when durum wheat semolina is replaced by non-gluten flour, the network will become weaker [43, 54]. In terms of rheological properties of durum wheat semolina dough enriched with broccoli powder, our observations in chapter 4 show a small increase in the complex modulus when 4 and 10% dry broccoli powder is used (figure 4.2b). The addition of 20% broccoli powder to durum wheat semolina dough had a somewhat larger increase in the complex modulus than the lower broccoli powder concentrations, but even this increase is not very significant (figure 4.2b). In conjunction with our findings on particle swelling in sweet potato starch noodles, these results suggest that the gluten proteins present in durum wheat semolina form a very strong network that embeds the broccoli particles, preventing them from swelling. The comparison of experimental data with a Batchelor model [114] proved that, when embedded in a durum wheat semolina network, the broccoli particles do not swell (figure 4.2b). The theoretical curve was constructed by using the volume fraction of the dry broccoli particles as the assumed volume fraction of particles. As the experimental data were in agreement with these theoretical predictions, it means that when broccoli particles are incorporated in durum wheat semolina dough, their volume fraction is the same as when they are in the dried state, meaning that no swelling occurs. This is confirmed by the analysis of the microstructure described in the next section. Therefore, addition of hydrocolloids and a gluten matrix both can limit the degree of particle swelling, enabling incorporation of high broccoli particle volume fractions in pasta-like products.

2.4. Microstructure and vegetable particles

The structure of food products results from physical and chemical interactions between the different food components, their type and concentration, as well as temperature and energy input during formation [96]. Structure itself has a large influence on product appearance, smell, taste and texture [115]. Besides providing clues on how to control the behavior of food products during processing, understanding the microstructure as a function of composition, temperature and energy input can be very useful in formulation of new food products [116, 117]. Confocal Laser Scanning Microscopy (CLSM) and Scanning Electron Microscopy (SEM) have been widely used in the investigation of the microstructure of food products [115-118]. Enriched pasta-like products are more often analyzed with SEM. For example, Gopalakrishnan and co-workers have analyzed the microstructure of sweet potato noodles enriched with whey protein concentrate, defatted soy flour and fish powder using SEM [37]. With this type of microscopy, they were not able to visualize the protein and starch separately, which they attributed to the fact that the starch granules were embedded in a strong protein starch network. This protein is not gluten, but just protein present in the sweet potato flour. From the analysis of the microstructure they were able to conclude that the noodles enriched with 10 and 20% whey protein concentrate formed a strong interpenetrating starch-protein network. With the incorporation of whey protein concentrate they observed the formation of sheet-like structures, which became thicker as the concentration of whey protein concentrate increased. Enrichment of noodles with defatted soy flour produced a slightly loosened starch protein network as was visible around the edges. The incorporation of fish powder resulted in a weak protein starch network, with the starch granules fully swollen. From this we can conclude that this type of microscopy is very valuable to understand the microstructure of these products.

In our work, CLSM was used to study the effect of incorporation of broccoli powder particles on the structure of pasta-like products. Regarding the microstructure of sweet potato starch noodles dough, two different concentrations of pre-gelatinized starch were investigated, 10 and 30%, either without broccoli powder added (blank) or with broccoli powder added at 4 or 20%. When broccoli powder was incorporated in the matrix, the starch was replaced by an equal volume of broccoli powder. In the blank sample with 10% pre-gelatinized starch, the microstructure of this system is characterized by a small amount of continuous matrix, completely filled with starch granules (figure 2.7a). Pre-gelatinizing 30% of the starch resulted in a higher volume fraction of the continuous matrix and a lower amount of dispersed starch granules in the matrix (figure 2.7d). Addition of 4% broccoli powder did not have a large effect on the microstructure of the samples with 10 and 30% pre-gelatinized starch (figure 2.7b and 2.7e, respectively). For samples with 10 or 30% pre-gelatinized starch containing 20% broccoli powder (figure 2.7c and2.7f, respectively), different microstructures were visible. The sample with 20% broccoli powder and 10% pre-gelatinized starch is a closely packed system of broccoli particles and starch granules, glued together by the matrix (figure 2.7c), while the sample with 20% broccoli powder and 30% pre-gelatinized starch is a starch gel filled with broccoli particles and fewer starch granules (figure 2.7f). With the amount of pre-gelatinized starch increasing, the total volume fraction of particles (starch granules and the broccoli particles) decreased, leading to a weaker system (observed in the rheological properties). In samples containing 20% broccoli powder and 10% pre-gelatinized starch, black regions were present in the matrix, indicating that the samples are inhomogeneous. The sample with the same amount of broccoli particles and 30% pre-gelatinized starch was more homogeneous due to the presence of a larger amount of continuous matrix. This shows that at these high particle loadings it is difficult to mix the starch dough and the vegetable powder to obtain a homogeneous system.

Another set of sweet potato starch noodles and noodles dough with 10% pre-gelatinized starch was investigated by CLSM, as a function of the type and concentration of broccoli powder (chapter 4). The different types of broccoli powder used were broccoli pulp (fresh broccoli blended), commercially available broccoli powder (produced by freeze-drying) and in-house produced broccoli powder (also produced by freeze-drying). The microstructure of the noodle dough containing 4% broccoli pulp is characterized by the presence of a few very large broccoli particles and more very small particles (figure 4.3b). Noodle dough with 4% commercial broccoli powder (figure 4.3c) and 4% in-house produced broccoli powder (figure 4.3d) presented comparable microstructures, with a few small broccoli particles evenly dispersed in the matrix. Despite having the same concentration of broccoli powder, the noodle dough with 10% in-house produced broccoli powder (figure 4.3f) seemed to have more and larger particles than the sample with 10% commercial broccoli powder (figure 4.3e). This is the result of the swelling capacity of the broccoli powders, as it was found by us that broccoli powder produced in-house can swell almost 3 times more than commercial broccoli powder. Since both broccoli powders are processed by freeze-drying, a possible explanation for the difference in the swelling capacity might be related with the parts of the broccoli that were used to make the powder. To make the powder, we have only used the broccoli florets, but we believe that this procedure might not be followed by a food company, but instead the whole broccoli is probably used to produce the commercial broccoli powder. The noodle dough with 20% broccoli powder showed the same trend as the samples with 10% broccoli powder; the dough with 20% in-house produced broccoli powder (figure 4.3h) seemed to have a larger volume fraction of broccoli than the dough with 20% commercial broccoli powder (figure 4.3g). Moreover, when comparing the noodle dough containing 20% commercial broccoli powder (figure 4.3g) and 10% in-house produced broccoli powder (figure 4.3f) the latter seemed to contain more broccoli than the former. This can be explained by the difference in swelling capacity of the particles, as for the in-house produced powder, 10% based on dry volume would correspond to 76% volume fraction upon complete swelling, whereas the 20% commercial broccoli powder would give a volume fraction of at most 52% upon complete swelling. This difference in structure is consistent with the difference observed in the rheological properties of the doughs. The microstructure of the cooked noodles (figure 4.4) showed the starch granules completely gelatinized and with respect to the broccoli powder it showed the same trend as the noodle dough: the samples containing broccoli powder produced in-house seemed to have a higher amount of broccoli particles than the samples containing the same concentration of commercial broccoli powder (as well as a higher modulus). These results show that the microstructure of these enriched systems strongly depends on the type of particles added.

Pasta prepared from durum wheat semolina was also evaluated for the effect of broccoli powder incorporation. The CLSM pictures of the microstructure of the blank pasta showed a gluten network with the starch granules dispersed within it (figure 4.5a). The same observations were obtained by Zweifel and co-workers [119], using the same type of microscopy (CLSM) and by Mercier and co-workers [48] using scanning electron microscopy. In our study, the effect of the broccoli particles in the network could not be observed, as the labeling technique did not allow to differentiate between the gluten network and the broccoli powder. This would have been possible with the use of gluten-specific labeling agents. The microstructure of pasta enriched with faba bean and split pea flour was investigated by Petitot and co-workers [54]. They also saw that the influence of the enrichment on the protein network depends on the characteristics of the particles, but they did not address the swelling of the particles in their work. They found that adding 35% faba bean flour did not have a significant impact on the network whereas the addition of 35% split pea flour induced some changes, such as thinner protein films [54]. This is in agreement with our findings; the microstructure of enriched products depends on the type of the particles added, as observed when broccoli pulp, broccoli powder commercially available and broccoli powder produced in-house were used.

2.5. Microstructure and hydrocolloids

The effect of hydrocolloids on the microstructure of the noodles' dough and cooked noodles was also investigated. Locust bean gum and xanthan gum were used as an example of hydrocolloids with a low and high water binding capacity, respectively. The addition of the different hydrocolloids did not result in visible changes in the microstructure of noodles dough with 0, 4 and 20% broccoli powder. However, when the noodles were cooked, significant changes were being observed in all the samples (0, 4 and 20% broccoli powder), but these changes were only visible regarding the starch granules. When no hydrocolloid was added, the starch granules remained intact in the dough, and were completely swollen in the cooked noodles (figure 3.6). This was also observed when 1% locust bean gum (low water holding capacity) was added to the dough (figure 3.7). Xanthan gum (high water holding capacity) showed a large effect on the microstructure of the cooked noodles (figure 3.8). In the cooked noodles with xanthan gum (figure 3.8b), the starch granules were still intact, suggesting that the xanthan gum prevented granule swelling and subsequent starch gelatinization, by reducing the amount of water available to the granules [104]. Despite that the limited broccoli particle swelling could not be seen in the CLSM pictures, rheological results show that the xanthan gum can limit the swelling of the broccoli powder. In the literature, no studies documenting the effect of hydrocolloids on enriched pasta-like products (produced with durum wheat semolina) could be found. Hydrocolloids have only been extensively used in combination with starch, to modify starch gelatinization and retrogradation behavior, and to improve water-holding capacity and freeze-thaw stability of aqueous starch system [120]. The effect of hydrocolloids on starch seems to be dependent on the specific combination of starch-hydrocolloid, the ratio between the two, the preparation methods, and the conditions during measurement [104].

3. Textural properties

Texture has a fundamental role on consumers' acceptance of a product. The liking of texture is one of the primary pre-requisites that makes a consumer buy a certain product [96]. Textural properties vary from product to product and sometimes, even the same type of products have different textures; cheese forming a good example. Optimal textural properties are essentially the same for both starch noodles and durum wheat semolina pasta. When it comes to texture, good quality pasta and noodles should be firm, non-sticky and should have some elasticity [99, 121]. Mung bean starch and durum wheat semolina are referred to as the best raw-materials for the production of noodles and pasta, respectively, producing firm, chewy and non-sticky strands [99]. The use of other raw-materials, such as potato, sweet potato and cassava, produces noodles with a high degree of stickiness which hampers the measurement of the quality attributes [99]. For the same type of starch, growing location and conditions can also lead to differences in the quality of the noodles produced [101, 104].

Throughout this thesis, the textural properties of the enriched products were studied based on the parameters strength, extensibility and stiffness. Regarding sweet potato starch noodles, their textural properties were measured in both dried and fresh conditions. In the dried conditions they were compared to a commercial vegetable pasta sample, containing 2% spinach powder, since this was the only similar enriched product that was commercially available. Even though the commercial sample can provide some information on the texture of these products, comparisons between these two samples need to be done carefully because the gluten proteins present in commercial sample will confer a much stronger network than just the starch matrix. The parameters strength and stiffness of the starch noodles with 10% pre-gelatinized starch containing 4 and 20% broccoli powder were lower than the commercial available sample. In the sample with 4% broccoli powder (figure 3.11), the addition of 0.5 and 1% Konjac glucomannan and 1% xanthan gum increase the values of strength and stiffness to values very similar to the commercial sample. The extensibility of the starch noodles with 4% broccoli powder was higher than the commercial sample and all the hydrocolloids used (locust bean gum, guar gum, konjac glucomannan, hydroxypropyl methylcellulose and xanthan gum), at a concentration of 1%, lowered the extensibility to values similar to the commercial sample. The strength of samples with 20% broccoli powder only reached values close to the commercial sample when 1% xanthan gum was added. All the hydrocolloids and concentrations tested, with the exception of xanthan, were able to increase the stiffness of the samples containing 20% broccoli powder (figure 3.12) to values similar to the commercial sample. The extensibility of noodles with 20% broccoli powder was lower than the commercial sample and none of the hydrocolloids was able to increase it. This can be explained by the fact that this parameter is the most dependent on the visco–elastic properties of the matrix, and at 20% broccoli powder the matrix is strongly disrupted by the particles added.

Concerning the fresh starch noodles, no comparison with a commercial sample was possible since enriched fresh noodle products are not commercially available. Therefore, the only comparison could be made with a blank sample. For the production of these samples, three different broccoli particles were used; the broccoli powder produced in-house (that was also used in the production of the dried noodles), the broccoli pulp (fresh broccoli blended), and commercially available broccoli powder. The comparison between the textural properties of fresh and dried starch noodles after cooking showed that the drying process has a significant effect on these properties. The strength and stiffness of dried cooked starch noodles have considerably higher values than the fresh starch noodles and the extensibility of dried starch cooked noodles is significantly lower than the fresh noodles. According to Tan and co-workers, drying (between 25 – 60 ºC) should not have a negative effect on textural properties of starch noodles [99]. Drying is known to be beneficial for the textural properties of the noodles, as it reduces the size of the air cells in the noodles, improving the cooking quality by slowing down water penetration and absorption [100].

In comparison with the blank sample, textural properties of starch noodles enriched with 4% broccoli showed that this enrichment does not have a large effect on strength, extensibility and stiffness of starch noodles enriched with the different types of broccoli powder, or pulp (figure 4.7). Addition of 10% broccoli powder (both commercial and in-house produced) also did not affect the strength and extensibility of the starch noodles, but it did show a small increase in the stiffness of these systems. Addition of 20% broccoli caused an increase in the strength and stiffness and decreased the extensibility of the enriched starch noodles, with a larger difference for the samples prepared with broccoli powder produced in-house compared to the samples with commercial broccoli powder (figure 4.7). This outcome resulted from a difference in the swelling capacity of both powders (2.6 for the commercial broccoli powder compared to 7.6 for the broccoli produced in house). A higher swelling capacity results in a higher volume fraction of particles that will toughen the samples, as observable from the increased strength and stiffness of the samples containing the in-house produced broccoli powder.

Regarding the durum wheat semolina pasta, only the fresh samples were tested, either produced in a sheeting/cutting machine or in a lab-extruder. Producing the samples with these two different methods made it possible to see the effect of processing in the samples. It was shown that up to a concentration of 20% broccoli, the preparation method does not seem to influence the texture of the enriched durum wheat semolina pasta. The values of the texture parameters of samples produced under both processing methods were very similar. In general, the texture parameters of the samples did not show to be greatly influenced by the addition of broccoli powder (figure 4.8).

The lab-extruder allowed the preparation of samples with a higher broccoli powder concentration, up to 30%, which had not been possible in the sheeting/cutting machine. For the preparation of these samples, only broccoli powder produced in-house (10, 20 and 30%) was used and a comparison was done with a blank sample. The strength and stiffness of the samples with 10 and 20% broccoli powder was a little bit higher than the blank sample, whereas the extensibility was a little bit lower (figure 5.7). The sample with 30% broccoli powder had a significantly lower strength, extensibility and stiffness than all the other enriched samples. The stiffness of the sample with 30% broccoli powder was the same as the blank sample. These results suggest that at enrichment concentrations of 30% broccoli powder the network of these systems is essentially disrupted. This is a consequence of a very closely packed system that behaves like a cellular material in which the structure is weakened, not being able to retain all the particles together.

4. Nutritional properties

A very large number of published studies on the enrichment of pasta products refer to nutritional improvement as their goal [56]. The reason for nutritional improvement is based on the fact that pasta, despite being considered a nutritious food, lacks two of the eight essential amino acids, namely lysine and threonine [81]. Even though pasta is not the main source of protein in human diet, the fact that pasta is largely consumed worldwide is a good justification for the nutritional improvement of this type of product. The enrichment of pasta products with vegetable/legume flour can compensate for this deficiency as they are known to be rich in these amino acids [37, 49, 54]. Broccoli is very popular for its health benefits, related to Glucosinolates (GLs), its secondary metabolites [90-92]. Glucosinolates are by themselves primarily inactive but upon consumption or processing, these phytochemicals are hydrolyzed by an endogenous enzyme, myrosinase, or by enzymes in the human gut flora, thereby forming breakdown products. These breakdown products include isothiocyanates that are associated with anti-carcinogenic activity [122-125]. Because of its high nutritional value, broccoli is an appropriate ingredient for the enrichment of pasta-like products.

Before broccoli powder was incorporated into the pasta-like products, both broccoli powder commercially available and broccoli powder produced in-house, were analyzed for their GLs content (figure 5.2). The broccoli powder produced in-house showed a much higher GLs content than the commercial broccoli powder, 10.98 ± 0.38 and 0.44 ± 0.18 µmol/g of dry weight, respectively. Possible explanations for this large difference could be the result of the parts of the broccoli that were used to prepare the powder, the type of broccoli (dependent on cultivar and cultivation conditions), the storage time of the powder (in the case of commercial broccoli powder) and/or the production process (myrosinase might not have been properly inactivated in the production of the commercial broccoli powder) [126]. Broccoli powder with different particle sizes did not show significant differences in the amount of GLs present in the powders, and neither did the pasta and noodles enriched with broccoli powder with different particle sizes (figure 5.3). This shows that the particle size does not influence the amount of GLs extracted from these matrices. For the GLs quantification, durum wheat semolina pasta and sweet potato starch noodles were enriched with 10, 20 and 30% broccoli powder (V/V). Both pasta and noodles were analyzed in their fresh, dried, and cooked state, to investigate the effect of the drying and cooking step (figure 5.4). The fresh and dried pasta and noodles (before cooking) showed the expected increase in their content of GLs, with increasing volume fractions of broccoli powder. For all the different broccoli concentrations tested it was noted that the amount of GLs recovered from both the pasta and noodles was always lower than the amount of GLs added. In literature, the amount of GLs lost during cooking of broccoli florets is referred to be between 10 and 75% [125]. In our study, the observed percentage of GLs loss was 31 – 66% in pasta products and 18 – 70% in noodles. A possible explanation for the difference between the amount of GLs expected and the amount of GLs found in the samples might be the entrapment of a certain fraction of these components in the matrix, making the extraction step in the analysis method for GLs less efficient [127]. Drying was shown not to influence the amount of GLs extracted from these matrices. For both uncooked and cooked fresh samples, the GLs content increased linearly with the concentration of the broccoli powder. This suggests that the microstructure of the fresh pasta does not have a large influence on the leaching or loss of GLs during cooking. However, when the samples were dried and afterwards cooked, the microstructure does seem to have an influence on the entrapment of the GLs in the matrix; the amount of GLs levels off at higher volume fractions of broccoli powder. After cooking, all products show a reduction in GLs content. This reduction can be explained by the fact that water soluble GLs leach out from the pasta and noodles into the cooking water [125]. For the dried pasta and noodles with 30% broccoli powder the content of GLs in the cooked products is not significantly different from the pasta and noodles enriched with 20% broccoli powder. This suggests that the 30% broccoli powder samples most likely have a higher cooking loss and therefore also a higher loss of the nutrients. This could be attributed to a more disrupted structure as a result of the drying procedure, in combination with a longer cooking time than for fresh pasta and noodles. The different matrices, durum wheat semolina and sweet potato starch did not show a large difference in the content of GLs remaining in the cooked product. With regard to the amount of broccoli powder added to the different matrices, the only significant difference in the amount of GLs present in the samples was noted in the fresh noodles with 30% broccoli powder. This sample contained 15% more glucosinolates than the semolina pasta containing the same amount of broccoli powder. An explanation for the higher Gls content in starch noodles could be attributed to the cooking time, since the cooking time of pasta is twice as long as the cooking time of noodles. A shorter cooking time will probably lead to smaller cooking losses, as well as a lower thermal degradation. The dried cooked samples, which have the same cooking time, show the same amount of GLs in pasta and noodles.

In literature, the incorporation of several non-common flours into pasta has been shown to improve the nutritional properties of these products. Wood [52] showed that the enrichment of pasta with 15 and 30% chickpea increased the lysine content by 64 and 182%, respectively. Rayas-Duarte and co-workers [43] also found an increased lysine content in enriched pastas with concentrations up to 30% of light buckwheat, dark buckwheat, amaranth and lupin. Pasta with lupin flour was the one with the highest content of lysine among all the flours. The replacement of 25, 35 and 50% of durum wheat semolina by soy flour doubled the amount of protein and more than quadrupled the amount of lysine at the highest substitution level [128]. Nielsen and co-workers [42] reported approximately 10% increased protein content with the incorporation of pea flour and pea protein concentrate. The incorporation of germinated pigeon peas seeds into pasta increased not only the protein content, but also the content of dietary fiber, micronutrients, such as calcium, sodium and potassium, and vitamins, such as B1, B2, C and E, in comparison with the control sample [83].

According to the Dutch *voedingscentrum,* a daily intake between 50 and 150 g of vegetables and (cooked) pasta for children younger than 9 years old is recommended. Considering that dry pasta has a swelling capacity of about 3 fold, 150 g of cooked pasta corresponds to 50 g of dry pasta. An intake of 150 g of cooked pasta enriched with 20% broccoli powder, results in the intake of 10 g of dry matter of "pure" broccoli, which corresponds to 100 g of broccoli, considering that broccoli has approximately 90% water. The amount of GLs detected in the pasta products proves that there is still a high amount of broccoli components retained in the pasta. This shows that for children that do not like to eat broccoli (or other vegetables) the consumption of this type of pasta would lead to a significant intake of nutrients. The consumption of this type of pasta would be a healthy alternative, providing a considerable amount of vegetables, just by the pasta alone.

5. Sensory properties

When it comes to new or reformulated food products, sensory evaluation is regarded as the best method to evaluate the acceptability of these products [129]. Several authors have totally or partially replaced durum wheat semolina in the production of pasta-like products and found that the acceptability of the enriched products is mostly the same as durum wheat semolina pasta. Most studies show that durum wheat semolina can be replaced without affecting to a large extent the sensory properties. For example, Wood [52] reported that the fortification with 15 and 30% chick pea flour was as acceptable as the control sample, even when 30% enriched pasta was considered much firmer than the other samples. Nielsen and co-workers even reported that enrichment lead to more liking; fortification with 33% of raw pea flour showed a higher overall preference than the control sample [42]. Also enrichment with germinated pigeon pea flour, in concentrations of 5, 8 and 10%, the products showed the same acceptability, color, flavor and texture as the control sample [83]. Even though these highly enriched pasta products are mostly considered acceptable, sensory evaluation shows that panelists can perceive the differences in the sensory parameters, such as texture and taste of enriched products. Petitot and co-workers [81] have found that replacing 35% of durum wheat semolina by split pea flour or faba bean resulted in acceptable products, but with significant changes in hardness, elasticity and fracture properties. Rayas-Duarte and co-workers also found that differences in the textural properties of pasta products can be perceived when they are enriched with light buckwheat, dark buckwheat, amaranth and lupin flours [43]. When the levels of enrichment are below 15%, sensory panelists do not notice differences in the sensory properties of these products [43]. However, significant changes were observed at enrichment levels above 25%. Also replacement of all durum wheat semolina by amaranth seeds flour did not show large effects in the sensory properties. Chillo and co-workers [31] used amaranth flour and carboxymethylcellulose sodium salt (CMC) as gluten replacer and enriched the pasta with approximately 10% flour from quinoa seeds, chick pea seeds or broad bean. They only found differences in stickiness, while all the other sensory properties tested (Bulkiness, Adhesiveness and Firmness) showed no differences with regard to the control pasta sample, made from 100% durum wheat semolina. Shogren and co-workers [128] did not see large difference in the sensory properties for replacement of durum wheat semolina by soy flour. Up to 35% enrichment, there were no significant differences in texture and taste, and only small differences were observed for replacements of 50% of durum wheat semolina.

In our study, we have also tested the acceptability of our enriched pasta products by performing an acceptance test and an attribute diagnostics test (chapter 5). These tests were performed to access the acceptability of the pasta enriched with different concentrations of broccoli powder up to 30%. For the acceptance test, four parameters were investigated: overall liking, color, texture and taste and the results show that all the parameters were above the minimum limit for acceptability (figure 5.5). The parameters ‘overall liking’, ‘liking of color’ and ‘liking of taste’ did not show significant differences between samples (0, 10, 20 and 30% broccoli powder). Only for the parameter ‘liking of texture’, the sample with 30% broccoli powder was rated slightly lower than the other samples and was found to be statistically significant. The decrease in ‘liking of texture’ is a consequence of an extremely high volume fraction of particles, that weakens the system and as a result it falls apart. This result suggests that, in general, even at high levels of enrichment, the incorporation of broccoli does not lead to undesirable taste, and only texture is slightly affected. Regarding the attribute diagnostics test (figure 5.6), clear differences were observed between samples and the tested parameters, firmness, stickiness and vegetable flavor. A comparison with a commercial sample (*Tagliatelle Verdi*, from Grand'Italia with 2% spinach powder) was also included. Regarding 'Firmness', the incorporation of broccoli powder lead to a decrease in this parameter with an increase in broccoli volume fraction, much lower than commercial sample. The parameter 'Stickiness' showed no difference between samples, with the exception of the commercial *tagliatelle* which was significantly lower than the other samples. For the parameter 'vegetable flavor', the perception of this parameter increased with increasing concentration of broccoli powder. As expected, the commercial sample, which contains 2% spinach powder only, had a perceived vegetable flavor lower than our sample with the lowest vegetable concentration (10% broccoli powder). Combining the results of the acceptance test and the attribute diagnostics, it is possible to link the sense of liking with the different attributes. The decrease in 'Firmness' with increasing concentration of broccoli powder might explain why the 'liking of texture' decreased, as good quality pasta should be somewhat firm [130]. In the same way, the decrease in 'liking of taste' is likely to be the result of the increasing vegetable flavor as the concentration of broccoli powder increased. In summary, all samples (0 – 30% broccoli powder) are considered acceptable. Only the sample with 30% broccoli powder was noticeably less liked than the other samples, possibly caused by the very low firmness and very strong vegetable flavor. Even though our pasta products could not be tested by children, the fact that adults found it to be acceptable, gives good perspectives for future research. In summary, enrichment of pasta with 20% broccoli powder results in products with a very similar acceptability to non-enriched products and this concentration is still much higher than those commonly found in commercially available pasta products.

6. Concluding remarks and outlook

The enrichment of pasta-like products has been shown to be a successful way to improve nutritional properties of this type of products. In this thesis we have successfully incorporated high concentrations of broccoli powder into pasta-like products that have acceptable characteristics and additional health components (GLs). Since pasta is very appreciated by children, unlike vegetables in general, the incorporation of vegetables in pasta is a healthy alternative to children that normally do not eat vegetables, and could be used as part of a strategy to fight obesity amongst children. We have demonstrated that enrichment of sweet potato starch noodles produced large changes in the structure of these products that were somehow compensated with the use of xanthan gum. Unlike sweet potato starch noodles, the textural properties of durum wheat semolina pasta did not show to be greatly affected by the incorporation of broccoli powder in concentrations up to 20% (V/V). However, the development of new or reformulated starch products should receive more attention since starch products can be a source of slowly digestible starch contributing to a healthier diet, and could be valuable as replacement for gluten-rich products for the increasing number of people suffering from celiac disease (gluten allergy) [131].

The high swelling capacity of broccoli powder was found to be the major factor affecting the rheological, microstructural and textural properties of enriched systems investigated. Hydrocolloids with high water binding capacity, such as hydroxypropyl methylcellulose and xanthan gum, were able to prevent the broccoli powder from swelling to some extent. The swelling is likely to be a limiting factor for the amount of particles that can be incorporated in this type of products. The fact that broccoli powder swells at the expenses of the water present in the matrix, the concentration of amylose in the matrix will increase. And since water distribution is such an important parameter in the production of these products, future research should look into variation in the amylose concentration.

Our observations show that durum wheat semolina pasta is not significantly affected by the incorporation of broccoli powder up to 20% (V/V) but above this concentration changes start to be noticeable. This shows that up to 20% (V/V) the gluten network is still strong enough to prevent the particles from swelling. But as more flour is replaced with broccoli powder, the gluten network becomes disrupted, the particles swell, and the structure changes. This probably explains why in literature, concentrations of particles (usually legume flour) do not go above 30 - 35%. In this thesis, we have not investigated whether the use of hydrocolloids in durum wheat semolina pasta has an effect on the particle swelling and the overall textural properties of the pasta. Further research should be conducted to investigate the effect of hydrocolloids for the possibility to increase the concentration of vegetable powder even further, while maintaining acceptable sensorial properties. Along with hydrocolloids, also the addition of extra gluten or the use of flour from a strong gluten variety could be investigated for their ability to control the swelling of particles, and therefore increase the volume fraction of vegetables in the systems.

We have demonstrated that the enrichment of pasta products with broccoli powder with acceptable sensorial properties is possible. The nutritional characterization of the enriched cooked samples showed that GLs content increased with increasing concentrations of broccoli powder up to 20% (V/V). Despite that only GLs were investigated, many other nutrients might be present as well, as broccoli is very rich in dietary fiber, Vit A and C. It was calculated that consumption of 150 g of cooked pasta with 20% broccoli powder corresponds to an intake of 100 g of vegetable. Literature shows that also other types of vegetables can be incorporated, and thus the type of vegetable can be changed. Controlling the microstructure of pasta products enriched with other types of vegetables with a different nutritional profile might lead to a large variety of products with different nutritional benefits. This also represents an opportunity to increase sustainability in the food industry, as some by-products have high nutritional value, especially in terms of fiber.

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SUMMARY

Even though the enrichment of pasta-like products is not a recent practice, the replacement of flour and/or starch by non-pasta ingredients can still be considered a technological challenge. The incorporation of particles will dilute the matrix and change its microstructure, thereby affecting the textural properties of these products. Especially for children, the consumption of vegetable-enriched pasta is advantageous, and could be used as a strategy to fight obesity amongst children. Vegetables are known to have a protective role on the onset of chronic diseases like obesity, but children tend to dislike vegetables and their consumption is therefore often lower than the recommended intake. On the other hand, pasta-like products are very appreciated by children and thus the incorporation of vegetables into this type of products could increase their vegetable intake (**chapter 1**). Therefore, the aim of this thesis was to understand how to produce vegetable enriched pasta-like products with acceptable texture and taste, while retaining nutritional components.

In **chapter 2** we incorporated dried broccoli powder (BP) into sweet potato starch (SPS) dough, varying the concentration of pre-gelatinized starch. The addition of 20% BP (V/V) to this type of matrix strongly affected its rheological properties, increasing the complex modulus by 1 – 2 orders of magnitude. The significant increase in the complex modulus (G*) was attributed to the swelling capacity of the broccoli particles, which was found to be 7.6 times its original volume, in dilute solutions. Besides the increase in the G* upon the addition of 20% BP, there was also a dependence on the concentration of pre-gelatinized starch (as G* decreased with increasing concentration of pre-gelatinized starch) suggesting that, at 20% BP, the system is not a dispersion of broccoli particles and starch granules in an amylose matrix but a closely packed system of particles glued together by the amylose. The importance of the swelling of the broccoli particles in the rheological properties of these systems was confirmed by dispersing BP into a simpler matrix consisting of fish gelatin. By doing so, the effect of the SPS matrix was also excluded. The rheological properties of the fish gelatin with BP added were compared with that of a model system consisting of fish gelatin with quartz beads incorporated. From this comparison, together with the expected theoretical values using the generalized Frankel and Acrivos model, we were able to conclude that the enriched SPS matrix is a closely packed system that can be considered a

cellular material. This was also confirmed by confocal laser scanning microscopy (CLSM) images.

Considering the importance of the swelling of the broccoli particles described in **chapters 2**, **3** and **4** we have focused on controlling the rheological properties of pasta-like products filled with high volume fractions of broccoli powder.

In **chapter 3** the influence of several hydrocolloids with different water binding capacities (WBC) on the shear rheology of SPS dough and on the texture of cooked noodles with 4 and 20% BP is described. To control the swelling of the BP, several hydrocolloids with distinct WBC were used, namely locust bean gum (LBG), guar gum (GG), konjacglucomannan (KG), hydroxypropyl methylcellulose (HPMC) and xanthan gum (XG). We found that the hydrocolloids with high WBC (HPMC and XG) were able to prevent the BP from swelling as there was a decrease in the relative complex modulus of the starch dough with 20% BP. The rheological properties of starch systems with 4% BP did not show to be influenced by the addition of the hydrocolloids as for these low volume fractions, the modulus is predominantly determined by the matrix. CLSM images of noodle dough and cooked noodles showed that hydrocolloids prevented the starch granules from swelling upon cooking. Despite being able to control the rheological properties of starch systems with high volume fractions of BP, the addition of hydrocolloids with high WBC has a significant effect on the textural properties of these products. These hydrocolloids significantly increased the strength and stiffness of the cooked noodles and decreased their extensibility.

In **chapter 4** we have controlled the rheological properties of the pasta-like products with high volume fractions of BP by using different matrices (durum wheat semolina (DWS) vs. sweet potato starch (SPS)) as well as different types of broccoli particles (broccoli powder produced in-house (HMBP), broccoli pulp and commercial broccoli powder (CBP)). The swelling capacity of the CBP was also tested and it was found to be 2.6 times its original volume, almost 3 times lower than the swelling capacity of HMBP (which we have determined in chapter 2). The addition of the different types of BP into SPS dough always led to an increase in the G*, similar to the systems with added HMBP (**chapter 2**). The dough with CBP showed a lower modulus than the dough with HMBP or broccoli pulp (in the sample with 4% BP), which is in accordance with the lower swelling capacity of these particles. The incorporation of the different types of BP to a DWS matrix did not show significant differences between particles, indicating that there is no swelling occurring and that the increase in the modulus is caused by the higher volume fraction of particles upon the incorporation of BP. The swelling of the BP in the different matrices was evaluated through the comparison between the experimental values of the moduli and expected values calculated according to the Batchelor model. In the SPS systems, the experimental and calculated values were not in agreement, meaning that the actual volume fractions are much higher than those based on dry volume, indicating swelling of the BP. For the DWS systems, the experimental values are fairly similar to the calculated ones, showing that no swelling occurs when BP is added to DWS. CLSM images also showed smaller BP particles in DWS than the BP present in SPS systems. The incorporation of high volume fractions of BP to DWS did not affect its textural properties as much as it affected the SPS systems; the stiffness of the highly enriched SPS noodles was 7 times larger than the blank noodles, whereas the stiffness of the highly enriched DWS pasta was only 2 times larger than the blank pasta.

In **chapter 5**, we focused on the nutritional and sensorial characterization of these pasta-like products. Since broccoli is regarded as a good source of glucosinolates (GLs, phytochemicals associated with health benefits) we have evaluated the presence of these components in the enriched products. CBP showed a much lower GLs content when compared with HMBP, and between DWS and SPS there were little differences in the amount of GLs present in these matrices enriched with HMBP. DWS pasta had a range of detected GLs between 34 and 69% of the amount initially incorporated, whereas SPS noodles had a range of detected GLs between 30 and 82%. We found that the amount of GLs present in fresh and cooked pasta and noodles increased linearly with the volume fraction of BP added (10-30%). However, when samples were dried and cooked, the amount of GLs still present did not increase linearly with volume fraction of BP, but leveled off to a constant value for volume fractions of BP above 20%. Since there was no difference in the amount of GLs present in the DWS pasta or SPS noodles, DWS pasta with different volume fractions of HMBP was used for the sensory evaluation. This consisted of two different tests, an “Acceptance test” and an “Attribute diagnostics”. Through the “Acceptance test” we found that all the samples tested (0 – 30% BP) were acceptable, but some evaluated parameters such as “texture” and “taste” were on the limit of acceptability for the sample containing 30% BP. In the “Attribute diagnostics” we saw that the perception of “firmness” decreased with increasing concentration of BP and the perceptions of “vegetable flavor” increased with increasing concentration of BP. Combining the results of the two sensory tests we can speculate that the low scores for “liking of texture” and “liking of taste” were caused, respectively, by the low firmness and high vegetable flavor of the samples with high concentrations of BP. From both a nutritional and sensorial point of view, 20% BP was regarded as the maximum volume fraction of BP to be added to this type of products. Incorporating volume fractions larger than 20% will not have additional health benefits and will decrease the acceptability of the enriched pasta by the consumers.

In **chapter 6** we discuss all the results presented in the previous chapters and put them into perspective with similar work from literature. Considering the added value of this type of enriched products, several suggestions are made for future research in order to increase the volume fraction of vegetable particles in these products and improve their nutritional and sensorial properties. We conclude that, enrichment of pasta with high volume fractions of broccoli particles is possible, and that from a nutritional and sensorial perspective, as much as 20% broccoli powder can be incorporated. The significant amount of broccoli powder present in an average portion of enriched pasta has the potential to increase vegetable intake of children. This work underlines the importance of the water distribution in this type of mixed systems, and shows how to control it in order to add high volume fractions of vegetables.

SAMENVATTING

Het verrijken van pasta-achtige producten is geen recente ontwikkeling. Desondanks wordt het vervangen van bloem en/of zetmeel door andere ingrediënten gezien als een technologische uitdaging. Het toevoegen van deeltjes zal de matrix verdunnen en zijn microstructuur veranderen. Hierdoor wordt de textuur van pasta beïnvloed. De consumptie van pasta verrijkt met groente heeft veel voordelen, in het bijzonder voor kinderen, en zou gebruikt kunnen worden als een strategie om obesitas onder kinderen te verminderen. Groenten staan erom bekend dat ze een rol spelen bij het voorkomen van chronische ziekten zoals obesitas, maar kinderen vinden groenten meestal niet lekker en hun consumptie ligt vaak lager dan de aanbevolen hoeveelheid. Aan de andere kant worden pasta-achtige producten vaak wel gewaardeerd door kinderen en zou de toevoeging van groenten in dit type product de inname van groenten kunnen verhogen (**hoofdstuk 1**). Daarom is het doel van dit proefschrift om te begrijpen hoe men met groente verrijkte pasta kan produceren met een acceptabele textuur en smaak, waarbij tegelijkertijd de voedingswaarde behouden blijft.

In **hoofdstuk 2** hebben we gedroogd broccolipoeder (BP) toegevoegd aan een deeg van zetmeel van zoete aardappel (ZAZ), waarbij de concentratie van voorgegeleerd zetmeel werd gevarieerd. Het toevoegen van 20% BP (V/V) aan dit type matrix had een grote invloed op de reologische eigenschappen. De complexe modulus nam toe met 1 – 2 orden van grootte. De significante toename in de complexe modulus (G*) was toe te schrijven aan het vermogen van de broccolideeltjes om op te zwellen. Dit vermogen was 7.6 keer het originele volume, in verdunde oplossingen. Naast de toename in G* bij het toevoegen van 20% BP, hing G* ook af van de concentratie voorgegeleerd zetmeel (G* nam af met toenemende concentratie voorgegeleerd zetmeel). Dit wees er op dat, bij 20% BP, het systeem niet langer een dispersie was van broccolideeltjes en zetmeelgranules in een amylose matrix, maar een op elkaar gepakt systeem van deeltjes samengelijmd door de amylose. De invloed van het zwellen van de broccolideeltjes op de reologische eigenschappen van deze systemen werd bevestigd door het dispergeren van BP in een simpelere matrix, bestaande uit visgelatine. Door deze aanpak werd het effect van de ZAZ matrix uitgesloten. De reologische eigenschappen van de visgelatine met BP werden vergeleken met die van een modelsysteem bestaande uit visgelatine en kwartsparels. Uit deze vergelijking konden we, gecombineerd met de verwachte theoretische waarden uit het algemene Frankel en Acrivos

model, concluderen dat de verrijkte ZAZ matrix een op elkaar gepakt systeem is dat gezien kan worden als cellulair materiaal. Dit werd ook bevestigd door confocale laser scan microscopie (CLSM).

Gelet op het belang van het zwellen van de broccolideeltjes zoals beschreven in **hoofdstukken 2, 3 en 4** hebben we ons gericht op het controleren van de reologische eigenschappen van pasta-achtige producten gevuld met een hoge volumefractie broccolipoeder.

In **hoofdstuk 3** is de invloed beschreven van een aantal hydrocolloïden met verschillen in het waterbindend vermogen (WBV) op de afschuifmodulus van een ZAZ deeg en op de textuur van gekookte noedels met 4 en 20% BP. Om het opzwellen van het BP te controleren, zijn verschillende hydrocolloïden met een duidelijk verschil in WBV gebruikt, namelijk johannesbroodpitmeel (JBP), guargom (GG), konjac glucomannan (KG), hydroxypropyl methylcellulose (HPMC) en xanthaangom (XG). De hydrocolloïden met een hoog WBV (HPMC en XG) konden het opzwellen van het BP voorkomen aangezien er een afname was in de relatieve complexe modulus van het zetmeeldeeg met 20% BP. De reologische eigenschappen van zetmeelsystemen met 4% BP werden niet beïnvloed door het toevoegen van hydrocolloïden omdat voor deze lage volumefractie de modulus voornamelijk wordt bepaald door de matrix. Door middel van CLSM van het noedeldeeg en de gekookte noedels konden we laten zien dat de hydrocolloïden voorkwamen dat de zetmeelgranules opzwollen tijdens het koken. Ondanks dat we de reologische eigenschappen van zetmeelsystemen met hoge volumefracties BP kunnen controleren, heeft de toevoeging van hydrocolloïden met een hoog WBV een significant effect op de textuur van deze producten. De hydrocolloïden zorgen voor gekookte noedels die sterker en stijver zijn en een verminderde uitrekbaarheid hebben.

In **hoofdstuk 4** hebben we de reologische eigenschappen gecontroleerd van pasta-achtige producten met een hoge volumefractie BP door het gebruik van verschillende matrices (griesmeel van harde tarwe (GHT) tegenover zetmeel van zoete aardappel (ZAZ)) en door het gebruik van verschillende typen broccolideeltjes (zelfgeproduceerd broccolipoeder (ZPBP), broccolipulp en commercieel broccolipoeder (CBP)). Het vermogen van CBP om op te zwellen werd getest en kwam uit op 2.6 keer het originele volume, bijna 3 keer lager dan het vermogen van ZPBP om op te zwellen (dat werd bepaald in hoofdstuk 2). Het toevoegen van verschillende types BP in een ZAZ deeg leidde altijd tot een toename in de G*, vergelijkbaar met de systemen met toegevoegd ZPBP (hoofdstuk 2). Het deeg met CBP had een lagere modulus dan het deeg met ZPBP of broccolipulp (bij 4% BP). Dit komt overeen met het lagere vermogen van deze deeltjes om op te zwellen. Het toevoegen van de verschillende types BP in een GHT matrix leidde niet tot significante verschillen tussen de deeltjes, wat aangeeft dat de deeltjes niet opzwollen en dat de toename in de modulus een gevolg is van de hogere volumefractie van deeltjes bij het toevoegen van BP. Het zwellen van het BP in de verschillende matrices werd geëvalueerd door middel van een vergelijking tussen de experimentele waarden van de moduli en de verwachte waardes berekend met behulp van het Batchelor model. In de systemen met ZAZ kwamen de experimentele waarden en de berekende waarden niet overeen, wat betekent dat de daadwerkelijke volumefractie veel hoger was dan die gebaseerd op hun droge volume, wat aangeeft dat het BP opzwol. Voor de GHT systemen waren de experimentele waarden redelijk vergelijkbaar met de berekende waarden, wat aangeeft dat er een zwelling optreedt als BP wordt toegevoegd aan GHT. CLSM liet zien dat er kleinere BP deeltjes aanwezig waren in een GHT systeem dan in een ZAZ systeem. Het toevoegen van hoge volumefracties BP in GHT had niet zo’n grote invloed op de textuur als bij ZAZ; de stijfheid van de hoog-verrijkte ZAZ noedels was 7 keer hoger dan die van de niet-verrijkte noedels, terwijl de stijfheid van de hoog-verrijkte GHT pasta 2 keer hoger was dan van de niet-verrijkte pasta.

In **hoofdstuk 5** hebben we ons gericht op de karakterisatie van deze pasta-achtige producten wat betreft voedingswaarde en sensorische eigenschappen. Aangezien broccoli wordt gezien als een goede bron van glucosinolaten (GL, phytochemicaliën die worden geassocieerd met voordelen voor de gezondheid) hebben we de aanwezigheid van deze componenten in de verrijkte producten onderzocht. CBP had een veel lager gehalte aan GL dan ZPBP, en tussen GHT en ZAZ was er weinig verschil in de hoeveelheid GL in de matrices verrijkt met ZPBP. GHT-pasta bevatte tussen 34% en 69% van de hoeveelheid GL die er oorspronkelijk in werd verwerkt, terwijl dit bij ZAZ-noedels tussen de 30% en 82% lag. De hoeveelheid GL aanwezig in verse en gekookte pasta en noedels nam lineair toe met de volumefractie van BP die werd toegevoegd (10-30%). Als de monsters werden gedroogd en gekookt nam de hoeveelheid GL die aanwezig was niet meer lineair toe met de volumefractie van BP, maar bleef op een constante waarde voor volumefracties van BP hoger dan 20%. Er was geen verschil in de hoeveelheid GL tussen GHT-pasta of ZAZ-noedels, daarom werd GHT-pasta met verschillende hoeveelheden ZPBP gebruikt voor de test van sensorische eigenschappen. Deze analyse bestond uit twee verschillende tests, een “Acceptatie test” en een “Diagnostiek van attributen”. Door middel van de “Acceptatie test” kwamen we erachter dat alle monsters die getest werden (0-30% BP) acceptabel waren, maar sommige van de geëvalueerde parameters zoals “textuur” en “smaak” waren op de grens van acceptabel bij het monster dat 30% BP bevatte. Bij de “Diagnostiek van attributen” zagen we dat de perceptie van “stevigheid” afnam met toenemende concentratie BP en dat de perceptie van “groentesmaak” toenam met toenemende concentratie BP. Als we de resultaten van deze twee sensorische testen combineren kunnen we speculeren dat de lage scores voor “waarderen van de textuur” en “waarderen van de smaak” veroorzaakt werden door, respectievelijk, de lage stevigheid en de hoge groentesmaak van de monsters met een hoge concentratie BP. Zowel op basis van voedingswaarde als op basis van sensorische eigenschappen werd 20% BP gezien als de maximale volumefractie van BP die toegevoegd zou moeten worden aan dit type product. Als men een volumefractie hoger dan 20% hanteert zijn er geen extra gezondheidsvoordelen en zal de acceptatie van deze verrijkte pasta door consumenten afnemen.

In **hoofdstuk 6** bespreken we alle resultaten die in voorgaande hoofdstukken gepresenteerd werden en gebruiken we vergelijkbaar werk uit de literatuur om deze resultaten in perspectief te plaatsen. Gelet op de toegevoegde waarde van dit type verrijkte producten, worden er enkele suggesties gedaan voor toekomstig onderzoek om de volumefractie van groentedeeltjes in deze producten te verhogen en om de voedingswaarde en sensorische eigenschappen te verbeteren. We concluderen dat de verrijking van pasta met hoge volumefracties van broccolideeltjes mogelijk is, en dat gezien de voedingswaarde en sensorische eigenschappen maximaal 20% broccolipoeder kan worden toegevoegd. De significante hoeveelheid broccolipoeder aanwezig in een gemiddelde portie van verrijkte pasta zou potentieel de inname van groente door kinderen kunnen laten toenemen. Dit werk onderstreept het belang van de waterverdeling in dit type gemengde systemen, en laat zien hoe het te controleren, om een hoge volumefractie groentedeeltjes te kunnen toevoegen.

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Silva, E., van der Linden, E., Sagis, L.M.C. (2011). Engineering functional foods with high vegetable content. Proceedings of the 11th International Congress on Engineering and Food, Athens, Greece.

CURRICULUM VITAE

Elisabete Silva was born in Portimão, Portugal, on October 28th, 1984. After attending elementary school in São Bartolomeu de Messines, she attended secondary school in Silves where she graduated in 2003. In the same year she started her studies in Food Engineering at Universidade do Algarve. During her studies she did two internships (in Portugal and Spain), both based on chemical analysis for quality control. After obtaining her degree in Food Engineering in 2008, she started her PhD project in the lab of Physics and Physical Chemistry of Foods at Wageningen University. The result of her PhD research is presented in this thesis.

OVERVIEW OF COMPLETED TRAINING ACTIVITIES

Discipline specific activities

Courses

Physical Chemistry School (Han-sur-Lesse, BE, 2009)
European School on Rheology (Leuven, BE, 2009)
Food Hydrocolloids (Wageningen, NL, 2009)

Conferences and Meetings

Liquids & Interfaces, NOW meeting (Lunteren, NL, 2009)
5th International Symposium on Food Rheology and Structure (Zurich, CH, 2009)
13th Food Colloids (Granada, ES, 2010)
3rd International Symposium on Delivery of Functionality in Complex Food Systems (Wageningen, NL, 2010)
Rheology and Fracture symposium (Wageningen, NL, 2010)
11th International Congress on Engineering and Food (Athens, GR, 2010)
13th European Student Colloid Conference (Falkenberg, SE, 2011)
6th International Symposium on Food Rheology and Structure (Zurich, CH, 2012)
16th International Congress on Rheology (Lisbon, PT, 2012)
11th International Hydrocolloids Conference (Purdue, USA, 2012)

General Courses

Teaching and Supervising Thesis Students (Wageningen, NL, 2009)
PhD competence assessment (Wageningen, NL, 2009)
“Creative problem solving” workshop (Wildhuis, CH, 2009)
VLAG PhD week (Maastricht, NL, 2009)
Techniques for Writing and Presenting a Scientific Paper (Wageningen, NL, 2010)
Project and Time management (Wageningen, NL, 2010)
Career Perspectives (Wageningen, NL, 2012)

Optional courses and Activities

PhD research proposal
Organized and participated in PhD trip (Japan, 2010)
Science Meetings (Wageninge, NL, 2008-2012)
IPOP Project Meetings (Wageninge, NL, 2009-2011)

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