Water retention in mushroom during sustainable processing

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This research was conducted under the auspices of the graduate school VLAG (Advanced studies in Food Technology, Agrobiotechnology, Nutrition and Health Sciences)

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Thesis

submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus
Prof. Dr A.P.J. Mol
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Tuesday, 8th Dec 2015
at 11 a.m. in the Aula.

Ekaraj Paudel

Water Retention in Mushroom during Sustainable Processing
148 pages.

PhD thesis, Wageningen University, Wageningen, NL (2015)

With references, with summary in English

ISBN 978-94-6257-596-7

For my Mother

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CHAPTER 1

General Introduction

1.1 Water holding capacity of food materials

The water holding capacity (WHC) is a widely used term in food science as this is one of the properties that determines quality parameters associated with foods. It is linked with sensorial and nutritional attributes of food products, and the economic and sustainability aspects of their processing.

Generally, the mechanical properties of food products are closely linked to their WHC. The more water a product contains, the more flexible or deformable it becomes. Furthermore, the ease by which water can be squeezed out influences their sensorial properties. For instance, fruits and vegetables containing more water will be more juicy, and the juiciness of meat and meat products is attributed to the WHC [1].

Similarly, the lower the water content of a product, the higher will be the content of proteins, polysaccharides and fats or oils, and hence it becomes denser in energy. In case of cheese, increasing the WHC while keeping the mechanical and sensorial properties the same, has been identified as an opportunity to convert cheese to a healthier product with less calorie intake per serving [2].

The water holding capacity of foods usually changes significantly during their preparation. Vegetables lose their turgor during heating, and solutes and water are released out from their vacuoles. Simultaneously, their cell walls soften due to thermal hydrolysis of pectin. Also high-protein foods like meat loose a part of their water during thermal treatment. This water loss is due to the conformational changes of the proteins, via which they expose their hydrophobic residues to the water. This makes denatured proteins to repel water. The lost juices often contain important compounds that contribute to nutritional and sensorial properties. The loss of these compound and water during food processing also have an impact on the sustainability. The products are often heated during processing – which results in the loss of a part of its water (and the heat it contains) by the product. The loss of juices, containing water and dry matter, means loss of matter, that has been drawn into the produce during their growth stage. Often this product loss has higher impact on sustainability than the loss of heat during processing – because of the large amount of energy and water used during the growth stage. In addition, such product loss

also has an impact on the commercial value of the produce for growers and food manufacturers.

Despite having its proven broad significance for food quality and food production, WHC is still far from understood. Moreover, in scientific literature there is still no consensus on the exact definition of the WHC [3]. In general, WHC is understood as the water held by the matrix of a food material at a defined mechanical load. For water holding it is required that a part of the food matrix is a biopolymer gel. Furthermore, there is a lack of a well-defined experimental technique for the quantification of the WHC. Hence, the reported values of the WHC obtained from different methods or different equipment settings vary largely. For meat, one of the most widely studied food material regarding WHC, the employed experimental techniques are:

The filter paper press technique [4, 5]: In this technique, samples are pressed against a filter paper, where the pressing releases juice from the sample. The WHC determination involves estimation of area formed by the released juices.
The drip loss technique [6, 7]: In this technique, samples are held in a plastic net bag or a perforated support and samples are allowed to drip under gravity for fixed number of days. The change in weight of the sample is used to calculate its WHC.
The centrifugation technique [8]: It involves application of centrifugal force to a known weight of sample. Known weighs of samples are given a centrifugal force which releases the juice from it. The weight difference relative to initial weight of sample is used as an indicator for WHC. Again, there is no agreement on speed or time of centrifugation.

We regard WHC as a thermodynamic property of food, that can be described with theories from the physics of polymer gels [3]. In this study, We have successfully employed the Flory-Rehner theory to describe WHC of meat [9, 10] and vegetables [11, 12].This theory describes the swelling behaviour of polymers, crosslinked to a gel. Total swelling of a polymeric gel has three independent contributions: 1) the osmotic binding of water by the biopolymers, 2) the electrostatic interaction by salts and possible charged polymers (named polyelectrolytes), and 3) the elastic deformation of the gel, imparted by the crosslinks between the polymers. The swelling is characterized by two parameters: the interaction between biopolymer and

water, as indicated by the Flory-Huggins interaction parameter, and the cross link density of the biopolymer network.

In this thesis we focus on the understanding of the water holding capacity of mushrooms, which lose significant amount of water during heating. We intend to describe these changes in WHC in terms of thermodynamics similar to the Flory-Rehner theory. In our experimental investigations we will apply different mechanical loads, which render different swelling equilibria, and from these data we will estimate model parameters. From a scientific perspective mushrooms are interesting, because they have a very distinct open pore space, in contrast to vegetables – which have small and closed pores. While inherently important from a scientific perspective, the understanding of the WHC will also offer insight on how one can minimize the loss of water during industrial processing of mushrooms.

1.2 White button mushroom

White button mushroom (*Agaricus bisporus*) is the most commonly grown mushroom, which falls under the kingdom *fungi.* Fungi are a group of organisms that are non-photosynthetic, and have multi-nucleated cells, with nuclei dispersed inside a cell wall [13]. Structure-wise, this mushroom has a cap (pilus), spore-forming parts (sprorophore or gills), a stipe (stem, stalk) and mycelium (hyphae) (Figure 1.1). The mycelium network of hyphae grows below the surface of compost. The fruiting body of mushroom grows above the compost, and can be divided into stipe, caps and gills. The fruiting body is used for consumption, which is the subject of study in this thesis.

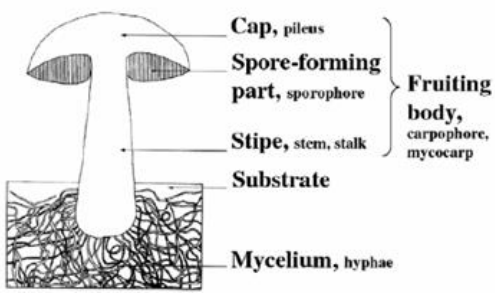

Fig 1.1: A sketch of mushroom illustrating its major parts. The picture is taken from Kalač [32]

Mushroom production is important since it uses waste materials (compost) as their raw material and converts part of it into fruiting bodies, which are assumed to be rich in high quality protein. Mushroom production and mushroom products are

traditional representatives of the current trend towards closure of the food chain; even though the conversion is only partial, and the spent raw materials still have to be discarded or distributed over the land.

Therefore the study of the water holding behaviour of mushrooms, in relation to its production is important: how the yield (the amount canned product obtained from given amount of fresh mushroom) of high-quality product be maximised from a given amount of raw materials. As there is a clear link between WHC, economics and sustainability of the processing; the impacts of processing on WHC and sustainability will also be included. Often in food production, sustainability and food quality have to be traded off.

1.3 Composition and water holding capacity in mushroom compared to meat and vegetables

As a first hypothesis we have assumed that the loss of WHC during heating of mushrooms has the same cause as in meat: the denaturation of proteins. This assumption is based on the facts that 1) both meat and mushrooms are rich in proteins, and 2) that experimental data on the loss of WHC during heating [14] follows a similar sigmoidal relation with temperature as found with meat [15]. Despite the claim of high protein content, we note here that the reported protein content in mushroom is not consistent and varies as widely as 7-38% on dry weight basis [16–19]. Many of the reports are based on the estimation of nitrogen in the tissue and convert it to protein content which makes the exact estimation complicated because of the presence of nitrogen containing compounds other than proteins.

There are certain dissimilarities between mushrooms and meat which have significant impact on the WHC of mushrooms. Fresh, harvested mushrooms still have their cell membranes intact. Thereby, the solutes in the vacuole create an osmotic pressure, which will attract water, expand the cell and the surrounding cell wall – until the turgor pressure generated by the stretched cell wall balances the osmotic pressure. In the case of meat, the cell membrane integrity is already lost after *rigor mortis,* which occurs soon after slaughter [20]. This loss of cell membrane integrity allows the intracellular fluid to leach out of the cells and create extracellular pockets

of fluid, the so-called drip channels. The water in the drip channels is held by capillary and it can easily be squeezed out (by gravity for example). In freshly harvested mushrooms, the pores are still filled with air [11], which are intentionally filled with liquid during processing [21–23].

In fresh plants or mushrooms water is present in three compartments, which are separated by the membranes. Water is contained in 1) the vacuole, 2) the cytoplasm, and 3) the cell wall. A fourth compartment is the pore space, which can be filled with water after processing. The water in the vacuole, we will name as the *intracellular water*. In our thermodynamic description we will assume that in fresh products all solutes are in the vacuole. All these solutes contribute to the osmotic pressure. As both the cell wall and cytoplasm contain mostly gel-like material, we will take them as one single compartment in our thermodynamic description of WHC. The water contained in both the cell wall and cytoplasm will be denoted as *gel water.* As said, in fresh produce the pore phase outside the cells is normally filled with air. However during processing of mushrooms or vegetables this air may get replaced with water – either of external origin via vacuum impregnation, or it is released from inside the cell due to loss of cell membrane integrity as during heating. The water in this compartment we will name *capillary water.* In our discussion on WHC of mushrooms we will distinguish thus three compartments: 1) the intracellular water in the vacuole, 2) the gel water held in cell wall and cytoplasm, and 3) the capillary water in the pore phase.

While mushroom tissue has similarities with that of vegetables, it differs in the composition of the cell wall. For instance, chitin is present in mushroom cell walls as fibrous material, while in vegetables this role is taken by cellulose. In the cell wall of vegetables water is largely bound to pectin, while in fungi (and mushrooms) this role is taken by beta-glucans. For both vegetables and fungi hold that protein are in minority in their cell wall material [24].

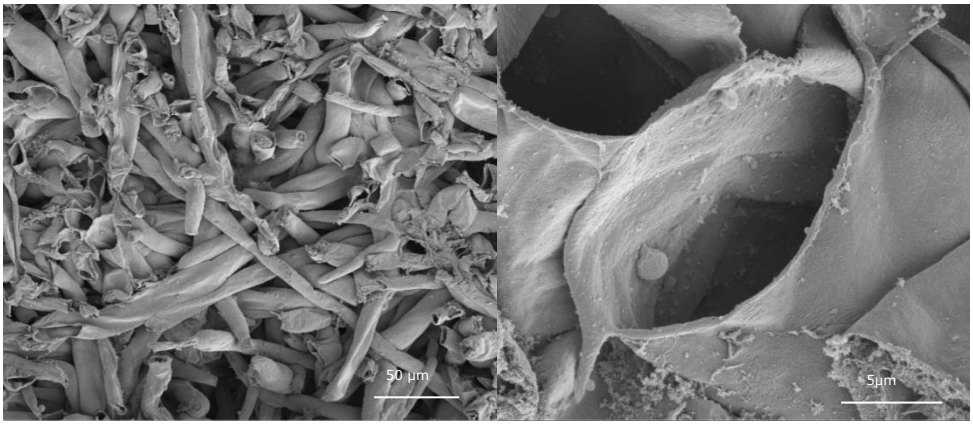

Figure 1.2: Scanning electron microscope picture of mushroom demonstrating the cellular structure of mushroom. ***Left*** *picture shows the hyphal structure and associated capillary space, the* ***right*** *picture shows the intracellular space*

1.4 Mushroom canning: WHC and sustainability

Like several other fresh vegetables with high water content and low acidity, mushroom is a highly perishable vegetable. A freshly harvested mushroom contains around 92% water [25] and has a pH value near neutrality, i.e. 6.3. As the market for fresh produce cannot absorb all the mushrooms produced, a significant part of the harvested mushrooms are processed for preservation over longer times by several methods such as canning, drying, blanching, or freezing.

Canning is the most widely used technique: more than half of the global mushroom production ends up being canned [26]. While canning of vegetables is a good means to preserve the vegetables for a longer period of time, the intensive heating in the sterilization process brings several changes in mushroom tissue such as changes in texture as indicated by an increase in puncture force and decreases the toughness of the mushrooms [27], and also a loss of water. The canning operation is intensive in energy and other utilities (e.g., water). The precise conditions during processing have great influence on the quality of the mushrooms, of which the temperature is a

major parameter. But, the water loss also determines the resource use efficiency (sustainability) of the preservation process. We take the current industrial processing conditions in this thesis as starting point, and then consider the optimization of both aspects: the loss of water holding capacity and the sustainability (resource use efficiency) of the production.

In figure 1.3, we show a scheme depicting the current industrial processing of canned mushrooms. As a first step the mushrooms are hydrated via vacuum impregnation with cold tap water. The intent of the vacuum impregnation is to fill the pore space with water. After storage at cold conditions, the hydrated mushrooms are blanched for 15 minutes at 90°C. The used blanching water is generally a waste product. After blanching the mushrooms are sliced, and filled into cans or jars, while the space between the mushroom slices is filled with some fluid (called the brine, which contains some salts and has a defined pH). The cans or jars are closed and sterilized for 15 minutes at 126°C, and finally cooled to room temperature.

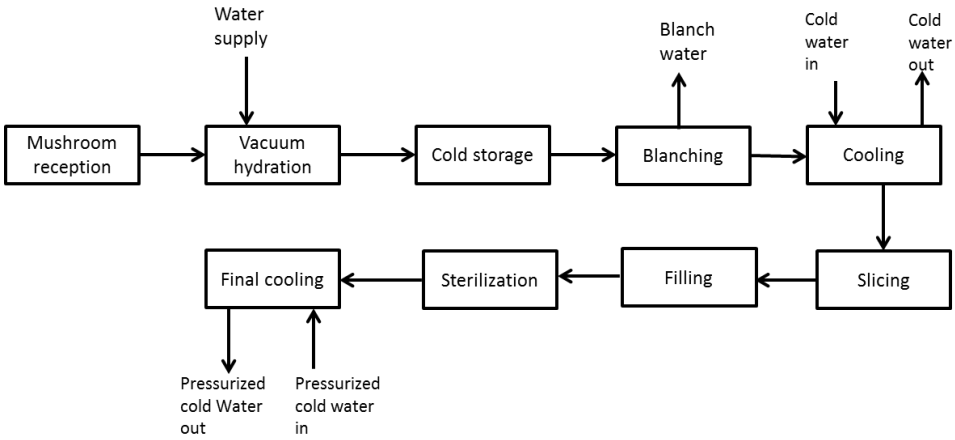

Fig 1.3: The block diagram of mushroom flow in canned mushroom production

Figure 1.4 shows the mass balance of the current mushroom canning process by means of a Sankey diagram. Mushrooms lose up to 40% of their original weight during the canning operation, because of the heat induced water loss [28]. This has multiple effects. First, it makes the amount of dry matter in mushrooms per kg of

canned mushrooms larger. Second, water soluble nutrients which are leached out of mushroom tissue during blanching end up in the blanching water rather than in the final canned product. This also holds true for the brine included in the can, which is drained before consuming, and thus must be considered as waste. Third, the canning process is a net producer of water, since more water is removed from the mushrooms, than is used in the process.

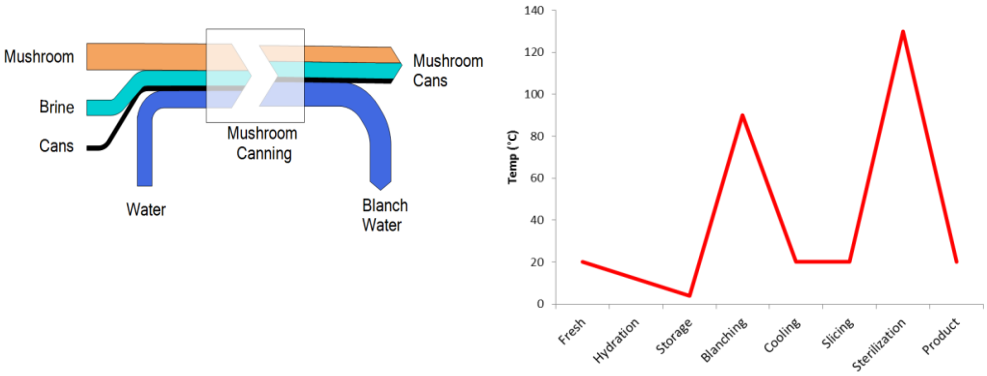

*Fig 1.4: **Left**: Mass balance of canned mushroom production shown with Sankey diagram. **Right**: Temperature change in mushroom at various unit operations.*

Figure 1.4 also shows the temperatures the mushrooms experience during processing. There are 2 subsequent heating and cooling stages, corresponding with the blanching and sterilization operations during processing. These heating cooling cycles increase the demand of energy and also the demand of cooling water in the production of canned mushroom. Water, apart from being used as a cooling utility, is also an ingredient in the production of canned mushroom. However, more water is removed from the mushrooms, than is used in the process. In spite of this, a significant amount of water is taken in into the process as well. This implies that this extra water also needs to be heated and cooled. This shows that there are possibilities for improving the sustainability of current mushroom production, while retaining the quality(such as their WHC) of mushrooms.

To identify these possibilities, we follow the guidelines of conceptual process design [29, 30]. First the objective or multiple objectives are defined. Then, the inputs and

outputs of the system are defined. The specification of the raw materials and the products are defined as precisely as possible [31]. The subsequent steps are *process identification* and *synthesizing unit operation* for such conversion.

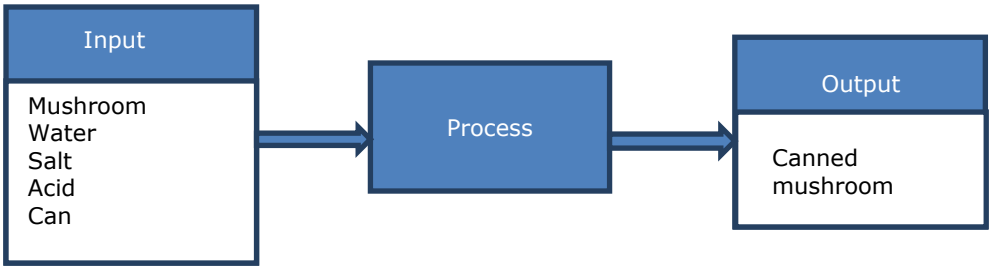

Fig 1.5: Input-output diagram for mushroom processing

Defining mushroom as a raw material is not trivial, as it is a highly structured food with natural variation in its microstructure. There are several unit operations involved in the conversion of mushroom to the canned product, each affecting its microstructure. This means that changing the process will probably change the quality of the product. This implies that good insight in the effects of the processing conditions is essential to maintain the quality of the mushrooms. Because of the above challenge of quality vs sustainability it is advantageous to approach this question at two different levels. We analyse the resource utilization efficiency in *mesoscale* at unit operation and production level. Here, we have included ideas from process intensifications, where unit operations have been combined. Water holding capacity is analyzed in *microscale* at molecular level as interactions and material properties of the mushroom. Here, interaction of water present in various cellular constituents in mushroom has been studied in relation to the structure of mushroom.

1.5 Aim and general outline of thesis

This thesis aims to obtain insight in the effects of process design decisions on 1) the improvement of the quality of the mushrooms, and 2) the efficiency at which resources are used. The first dimension is simplified by taking the WHC as a measure of the quality of the mushroom during processing. The second dimension includes

the efficient use of the raw materials, and maintenance of the full weight of the mushrooms during processing. In addition, it will also include the use of energy and water in the process.

Rather than using empirical correlations, we aim to obtain mechanistic insight into the effects of processing on the of mushroom tissue. Our hypothesis is that heating irreversibly changes the properties of the materials in the mushroom matrix. We intend to use a thermodynamic approach to describe the effects of these changes on WHC. From the obtained understanding, one can deduce the best ways to improve the process, while maintaining or even improving the water holding capacity of the mushrooms.

1.6 Thesis Outline

In this thesis we focus on the three different compartments in mushrooms that are holding water. In **Chapter 2** we start with the proper fundamental understanding of water holding capacity in the gel phase of mushroom. The water holding capacity in heat treated mushroom is described with the Flory-Rehner theory. The composition of mushroom is considered in describing its WHC. A simplified approach is taken, assuming that mushroom acts as a homogenous (bio) polymeric mixture, disregarding its cellular structure. The change in water holding capacity in mushroom with the thermal treatment is described in terms of three independent parameters, the Flory-Huggins interaction, the crosslink density and the polymer volume fraction at the relaxed (unswollen) state.

Chapter 2 simplifies mushroom as a homogeneous polymeric mixture, though in reality mushroom is a complex structure. **Chapter 3** and **chapter 4** study WHC in relation to the structure of mushrooms. In **chapter 3** the role of the water in the capillaries in the overall hydration of mushroom has been described. Effects of ageing, degree of hydration, interaction with temperature are also described in this chapter.

In **chapter 4,** we focus on the cellular structure where cell membrane integrity is of importance. Furthermore, cell wall is highly structured, with different components. Cell membrane integrity is important in retaining water and cytoplasm inside the cell.

Various processing conditions destroy the membrane integrity and thus release at least part of this water and its dissolved components. We therefore study the WHC in relation to the cell membrane integrity. The water bound to the structural components in the cell wall is studied by partial hydrolysis of those individual components with enzymes.

The sustainability, interpreted here as the efficiency with which resources (raw materials, energy, water) are used, is investigated in **Chapter 5**. We visualize the current production system with Sankey diagrams and analyse the system with second law efficiency, using exergy. This gives the assessment of the sustainability status of unit operations involved in the current production process of canned mushroom. Using ideas from Process Intensification three alternative production routes are proposed for the production of the canned mushroom. Pinch analysis is used to determine the minimum heating and cooling utilities required for those scenarios. The alternatives are evaluated and compared based on the production yield, quality and exergy requirement to find the route that deliver the best combination of product quality and resource use efficiency for the production of canned mushroom.

Chapter 6 concludes with the general discussion of all the results presented in this thesis. The main findings described in each chapter are summarized, and some general conclusions are drawn. The chapter concludes with a discussion about the perspectives for conceptual process design for canned mushroom production.

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CHAPTER 2

Change in Water Holding Capacity in Mushroom with Temperature Analyzed by Flory Rehner Theory

This chapter is published as *Paudel E., Boom R.M., van der Sman R.G.M. (2015). "Change in Water Holding Capacity in Mushroom with Temperature: Analyzed by Flory-Rehner Theory", Food and Bioprocess Technology (8) 960-970*

Abstract:

The change in water holding capacity of mushroom with the temperature was interpreted using the Flory-Rehner theory for swelling of polymeric networks, extended with the Debye-Hückel theory for electrolytic interactions. The validity of these theories has been verified with independent sorption measurements. The change in water holding capacity with temperature could be quantified as changes in three model parameters: the Flory-Huggins interaction parameter between the biopolymers and the aqueous solvent phase, the crosslink density of the network, and the biopolymer volume fraction at the relaxed state. The elastic pressure is analysed using the Flory-Rehner theory, which is traditionally used to describe the swelling of polymers. From the explicit knowledge of the elastic pressure, we can determine how model parameters, such as the crosslink density, depend on temperature. We argue that the dependencies of model parameters can be understood as a consequence of protein denaturation. Having knowledge how all contributions to the swelling pressure depend on temperature, and composition, we can compute the water holding capacity of mushroom for a wide range of temperatures and mechanical loads.

Keywords: water holding capacity, thermodynamics, gel compression, mushroom processing

2.1 Introduction

Protein-rich foods are well known for the change in their capacity to hold water after thermal processing, and change of pH or ionic strength. Meat is a good example of a protein-rich food, which has been studied extensively in the past concerning its water holding capacity (WHC) [1–7]. The WHC has been defined and measured differently by various authors [7–11]. Since all those methods use different definitions of WHC, the comparison of the results is difficult. It is commonly agreed, that the WHC of foods refers to the amount of water that is held within the three-dimensional biopolymeric network of the food. However, true understanding of the WHC in terms of the fundamental material property is still lacking [12]. In previous papers [4, 6], we described the change of the WHC of meat induced by temperature change, and analyzed it using the Flory-Rehner theory, which is commonly applied to swelling of synthetic polymer gels [13]. The theory was earlier used successfully also to describe skinned muscle fiber relaxation [14], and the WHC of carrots and mushrooms [15], and can be expected to extend further for example, to describe WHC of novel protein food [16].

The WHC of carrots was shown to be independent of temperature. The WHC of mushrooms have only been analyzed after short blanching at 90°C. Like meat, mushroom is a protein-rich food, and consequently we expect a change of the WHC with temperature. Hence, the objective of this paper is to investigate in detail how the WHC of mushroom changes with temperature, and how this can be explained by the Flory-Rehner theory.

As in our previous paper [15], we regard mushroom as a mixture of biopolymers, carbohydrates, salts and water. In mushrooms the biopolymer network is composed of polysaccharides and proteins, and the liquid phase is composed of water, with mannitol and potassium phosphate as main solutes. Fresh mushrooms have a cellular structure which forms a random web of hyphae. The pore space in between the hyphae is filled with air. During industrial processing the pore space of mushrooms is filled with water via vacuum impregnation. We take this into account in this paper. In order to reduce the complexity of the system, we have chosen for filling the pore space with an isotonic fluid instead of pure water, with the same composition as the liquid phase of fresh mushrooms. Furthermore, to reduce the

complexity in our analysis, we assume the mushroom to be a homogeneous biopolymer gel, and disregard its cellular structure. This is especially permissible at temperature above 40°C, at which the hyphae lose the integrity of their cell membranes – and thus the liquid phase mixes with the biopolymer network itself [17].

We describe the thermodynamic state of the mixture of the biopolymer network and the liquid phase with the swelling pressure, which can be decomposed in three independent contributions. The first contribution is the osmotic pressure due to mixing of biopolymer, water, and carbohydrates, described by the Flory-Huggins theory [6, 17–21]. When the system incorporated electrolytes, a second contribution is the osmotic pressure due to the mixing of water and ions, described by the Debye-Hückel theory. The last contribution is the elastic pressure, that counter-acts the action of osmotic pressure, and is due to stretching of the biopolymer chains in the gel network. The elastic pressure is described as in Flory-Rehner theory, taking the crosslink density and the biopolymer density in the relaxed state as model parameters.

The experimental methodology follows that of our previous paper [15]. Via centrifugation, we apply a range of mechanical loads, which induces an extra pressure that counteracts the swelling pressure. Cylindrical samples are taken from pre-heated mushrooms and put in centrifugation tubes having a permeable bottom, which allows the liquid phase to be expelled to another tube. Since we use an isotonic fluid in the pores of the mushroom, the expelled liquid always has the same composition. Knowing the initial composition of the fresh mushrooms and the amount of expelled liquid, we can estimate the osmotic pressures following the Flory-Huggins and Debye- Hückel theory. Earlier we have shown that these theories can be applied to several food materials to describe moisture sorption (water activity) from their composition [15, 17, 20, 22]. Via separate sorption experiments we verify the validity of these theories for the impregnated mushrooms. In our analysis, we assume a homogeneous mixture, i.e. that the pore space is absent. Above a certain threshold of the centrifugation speed, we assume that all pores have collapsed. This assumption is verified experimentally via electron-microscopy.

From the water activity we find the total osmotic pressure [6, 22]; by subtracting this pressure from the swelling pressure, the elastic pressure is obtained as a function of the mechanical load and the temperature. Analysis of this relation with the Flory-Rehner theory shows how the crosslink density and the relaxed state of the biopolymer network changes with temperature. We will interpret the changes in these model parameters with protein denaturation. The work described in this paper shows that our earlier assumption of temperature independency of model parameters in the elastic pressure [6] needs a reconsideration. The study captures the most interesting temperature range of 40-60°C where sudden change in WHC of mushroom takes place. The study supports the idea of universality of Flory- Rehners' theory on describing change in WHC of various vegetables depending on their composition.

2.2 Theory

2.2.1 Flory-Rehner theory

The thermodynamic state of a polymer gel is characterized by the swelling pressure (Π_{swell}) on the fluid in the polymeric network. At equilibrium, this pressure is equal to the external pressure applied (p_{ext}).

$$\Pi_{swell} = p_{ext} \qquad [2.1]$$

The Flory-Rehner theory assumes that the swelling pressure is a linear superposition of independent contributions due to mixing of polymer and solvent (Π_{mix}), the ionic interactions (Π_{ion}) and the elastic deformation (Π_{elas}) of the network:

$$\Pi_{swell} = \Pi_{mix} + \Pi_{ion} + \Pi_{elas} \qquad [2.2]$$

Mixing pressure

We describe the mixing pressure, Π_{mix}, with the Free-Volume-Flory Huggins (FVFH) theory, which we used previously to describe the sorption behavior of several food materials [6, 17].

The mixing pressure is given by:

$$\frac{v_w \Pi_{mix}}{RT} = ln(\phi_w) + \left(1 - {}^{1}/_{N_{eff}}\right)(1 - \phi_w) + \chi_{eff}(1 - \phi_w)^2 + F_{FV} \quad [2.3]$$

Here R ($J\,K^{-1}\,mol^{-1}$) is the universal gas constant and T (K) is the temperature at which the external pressure was applied to the samples, v_w is the molar volume of water, ϕ_w is the volume fraction of water, N_{eff} is the effective ratio of molar volume of water versus solutes (sugars and polymers), χ_{eff} Is the volume averaged Flory-Huggins interaction parameter between water and solutes. F_{FV} is the free volume contribution due to the absence of relaxation in the glassy state. The water activity is related with the mixing pressure Π_{mix} by the relation: $v_w \Pi_{mix} = RTln(a_w)$ [20].

The experiments are carried out at or above room temperature. The glass transition temperature (T_g) of freeze dried mushroom is known to be lower than room temperature [23]; hence, the contribution of F_{FV} on the mixing pressure can be considered to be zero. The values of the effective parameters are computed as follows [15, 24]:

$$\frac{1}{N_{eff}} = \frac{\sum_{i \neq w} \phi_i / N_i}{\sum_{i \neq w} \phi_i} \quad [2.4]$$

$$\chi_{eff} = \frac{\sum_{i \neq w} \phi_i \chi_{iw}}{\sum_{i \neq w} \phi_i} \quad [2.5]$$

in this, ϕ_i is the volume fraction occupied by solute i, N_i is the ratio of molar volumes of water and solute; χ_{iw} is the Flory-Huggin interaction parameter of the pure solute with water (the contributions of the mutual interaction between solutes is neglected since they are all present at low concentrations). For high molecular weight polymers like polysaccharides and proteins we can safely assume, $\frac{1}{N_i} = 0$. The values of N_i and χ_{iw} for the mushroom components are given in Table 2.1.

Table 2.1: The N_i and χ_{iw} for mushroom components

Components	χ_{iw}	$\frac{1}{N_i}$	References
Mannitol	0.27	0.160	[15]
Trehalose	0.53	0.084	[15]
Polysaccharides	0.8	0	[6, 15, 25]
Proteins	0.8 - 1.4	0	[6, 17]

The interaction parameter between the biopolymer ($i = P$) and water is composition dependent which is described by the following relation [6, 17]:

$$\chi_{Pw} = \chi_{Pw,o} + \left(\chi_{Pw,1} - \chi_{Pw,o}\right)(1 - \tilde{\phi}_{w,eff})^2 \qquad [2.6]$$

Where, $\chi_{Pw,1}$ is the interaction parameter of the dry polymer with a vanishing amount of water, and $\chi_{Pw,o}$=0.5 (the interaction parameter at very low polymer concentration that remains independent of temperature). The composition dependency of the biopolymer Flory-Huggins interaction parameters accounts for the effects of hydrogen bonding.

The FVFH theory is an approximation of the more fundamental Pincus-deGennes-Tanaka cluster theory, as discussed previously [19]. The composition dependency of the interaction parameter is an empirical relation, for which there is no fundamental justification; often, a quadratic relation is taken. The precise values of parameters is difficult to obtain for food materials, as literature data often deals with moisture sorption at water activities below 0.95, while for WHC the range of a_w > 0.95 is of interest. The precise form of this relation needs further investigation, but the current form serves our purpose well.

Recently, we found that sugars and carbohydrates act together with water as a single effective plasticizer. Their plasticizing (solvent) effect can be characterized by the number of hydroxyl (OH) groups per molecule, which accounts for the number of possible hydrogen bonds per molecule. Consequently, we account for this effect in

$\tilde{\phi}_{w,eff}$, which is a measure of the density of hydrogen bonds of the plasticizers present in the system, which bind to the biopolymer:

$$\tilde{\phi}_{w,eff} = \phi_w \left(1 + \frac{N_{OH}^w/M_w + N_{OH}^s/M_s}{y_s/M_w + y_s/M_s}\right) \quad [2.7]$$

Here, N_{OH}^i, is the effective number of OH groups per molecule, with $i = s, w$ for solutes and water respectively, while M_i is the molecular weight. The values of N_{OH}^i for water, mannitol and trehalose are 2, 3.56 and 7.72 respectively [24].

The $\chi_{Pw,1}$ value for the proteins are temperature dependent because their thermal denaturation results in different interaction with the solvent mixture [6]. We assume that the $\chi_{Pw,1}$ parameter for protein follows the same temperature dependency as is followed by the WHC of mushroom at zero external mechanical load. The $\chi_{Pw,1}$ parameter for proteins is estimated with following logistic (sigmoid) function:

$$\chi_{pw,1}(T) = \chi_{p1,0} + \frac{\chi_{p1,\infty} - \chi_{p1,0}}{1 + a \cdot \exp\big(b(T - T_e)\big)} \quad [2.8]$$

In which $\chi_{p1,0}$ and $\chi_{p1,\infty}$ are the values attained by $\chi_{pw,1}$ when the proteins are their native state, and fully denatured states respectively. T is the temperature at which protein is subjected to the heat treatment and T_e is the mid-point of the sigmoid curve. This temperature dependency of the protein interaction parameter with temperature was used previously to describe the change of WHC of meat with temperature [6]. The values of $\chi_{p1,0}$ and $\chi_{p1,\infty}$ are known to be 0.8 and 1.4 respectively for meat [6], broccoli [17], mushrooms and carrots [15]. The values of T_e and the constants *a* and *b* are obtained via curve fitting of the WHC to the above expression. It should be borne in mind that the change is irreversible: a lowering of the temperature does not result in a return to the original $\chi_{p1,0}$ and $\chi_{p1,\infty}$ values.

Ionic pressure

The osmotic pressure due to the presence of ions is computed with the extended Debye-Hückel equation [26], which applies for dilute solution having molar ionic concentration less than 0.1 [27], as is given in equation 2.9.

$$\frac{v_w \Pi_{ion}}{RT} = 1 - M.\exp\left(-\frac{1.17|z_+ z_-|\sqrt{I}}{1+\sqrt{I}} + 0.2I\right) \quad [2.9]$$

Where, M is the molality of the electrolyte solution, I is the ionic strength, and z_+, z_- are the positive and negative charges, with values of 1 and -3 respectively for potasium (K^+) and phosphate (PO_4^{3-}),. The ionic strength can be computed as:

$$I = \frac{(z_+{}^2 c_+ + z_-{}^2 c_-)}{2} \quad [2.10]$$

c_+ and c_- are the molarity of the ions in the solution.

Elastic pressure

The elastic pressure follows from the Flory-Rehner theory, which is based on the assumption that the polymers form a Gaussian polymer network:

$$\frac{v_w \Pi_{el}}{RT} = -N_c \phi_o \left[\frac{1}{2}\frac{\phi}{\phi_o} - \left(\frac{\phi}{\phi_o}\right)^{1/3}\right] \quad [2.11]$$

in which ϕ is the volume fraction of polymer in the solution, ϕ_o is the volume fraction of the polymer at their relaxed state [14], and N_c is the cross link density [28].

2.3 Methods and materials

2.3.1 Experimental

Mushrooms

We used white button mushroom (*Agaricus bisporus*) with a cap diameter of 4-5 cm for the experiment. The mushrooms were received within 2 hours of their harvest.

We assumed the following dry matter composition of fresh mushroom: 63% carbohydrate, 27% protein, and 10% ash (all in dry weight). The carbohydrates consist of mannitol (43%), trehalose (3.2%) and polysaccharides such as beta-glucans (55%), as follows from literature [29–32] (once more in dry weight). The moisture content in the fresh mushroom cap was measured by drying approximately

5 g of finely chopped mushrooms in a hot air oven maintained at 105 °C for 24 h. The loss of weight of the sample relative to the weight after drying was used as the moisture content of the fresh mushroom, and was determined to be 92.1%.

An isotonic mushroom solution was formulated with the same ratio (R_i) of solutes to water as is present in fresh mushroom. Preliminary experiments showed that the mushroom samples swelled in water and shrunk in a hypertonic solution ($R_{i,hypo} = 2R_{i,iso}$), but did not swell or shrink in the isotonic solution. The composition of the fresh mushrooms and the isotonic solution is shown in Table 2.2.

Table 2.2: Composition of the fresh mushroom and the isotonic solution (% are in wet weight):

components	Fresh mushroom (%)	Isotonic solution(%)	Ratio with water (R_i)
Water	92.1	97.0	1
Mannitol	2.13	2.25	0.023
Trehalose	0.16	0. 16	0.001
Protein	2.13	Na	0
Polysachharides	2.61	Na	0
Minerals (K3PO4)	0. 79	0.83	0.008
pH	6.3*	6.3	Na

*pH of the mushroom liquid as obtained by centrifuging the fresh mushroom in centrifugal filtration tubes.

Mushroom hydration

The vacuum impregnation of the pore space of mushroom hydration was carried out with the isotonic solution, with its composition shown in Table 2.2 The isotonic solution was adjusted to a pH value of 6.3 by adding a 2M HCl solution. This pH value is the natural pH of mushroom, as measured from the fluid expelled from centrifuged mushrooms. With the use of isotonic solution, the solute composition of the expelled fluid and in the mushroom remains constant throughout the experiment, in contrast to our previous investigation [15].

Fresh mushroom caps (with weight W_0) were submerged in the isotonic solution and then subjected to a vacuum of 20 mbar at room temperature for 10 min. The vacuum was then released and the caps were kept in the isotonic solution for another 5 min to allow a full impregnation of the solution into the pores. The vacuum treatment time and the soaking time were determined from previous experiments in the laboratory as the time at which the mushroom reach their maximum swelling in terms of the weight gain. The caps were then removed from the solution and then, were stored at 4 °C for 24h before the heat treatment.

Heat treatment

The hydrated mushrooms were immersed in a temperature controlled bath of the isotonic solution (1:5 weight ratio of hydrated mushroom to the isotonic solution). The mushrooms remained in the bath until equilibrium had been reached. The time required to reach equilibrium was determined via preliminary experiments. This equilibration time is temperature dependent, and varies from 15 minutes (at 90 °C), to 60 minutes (at 30 °C). After the heat treatment, the mushroom caps were taken out of the bath and cooled to room temperature in isotonic solution at room temperature, which is required for obtaining a rigid sample that allows centrifugation.

Mushroom centrifugation

A cylindrical sample was taken from the heat treated mushroom cap with a biopsy punch, having a height of 5 mm and a diameter of 7 mm. The cylinder was placed with the cylindrical bottom down, in a centrifugal filtration tube that consisted of two compartments separated by a perforated filter that allows the passage of a fluid (Pall Centrifugal devices, 0.2 microns). The water expelled from the sample permeated through the filter and was collected in the lower compartment of the tubes so that the expelled fluid did not come in contact anymore with the sample. The cylindrical sample was subjected to centrifugation force of 200, 1000, 4000, 8000 and 15000 *g* applied for 80 minutes till equilibrium which was found from preliminary experiments. The experiment was carried out with eight replicates.

Sorption analysis

Water vapour sorption measurements were performed on (1) fresh mushroom caps without any prior vacuum impregnation or heat treatment, on (2) mushroom caps that were vacuum impregnated and on (3) mushroom caps that heated at 60 °C and 90 °C. The temperatures were selected to observe changes in the sorption behavior due to protein denaturation. The heat treated samples were vacuum hydrated in the isotonic solution. The samples were, then heated till equilibrium in the isotonic solution as was described above. All three samples were freeze dried and subsequently inserted into a differential water vapor sorption analyzer (DVS, Elevated Temperature). The sample pan was cleaned carefully, and dried completely. Approximately 10 mg finely ground sample was placed on it. The pan along with the sample was hung on the wire connected to the microbalance. The nitrogen with a known relative humidity was passed over the sample at 200 sscm (standard cubic centimeter per minute) at 25 °C. At the beginning of the experiment the sample is kept in the DVS at 0% RH for 24 h; after this, the RH was increased stepwise, and then again reduced stepwise. Following this, equilibrium was considered to be established at each step when the mass change over 30 minutes was less than 0.0005%/min.

Scanning electron microscopy

All the microscopic observations shown in this paper were carried out with a thin slice of mushroom in a table top scanning electron microscope (Phenom). The observations with the microscope were made to examine the collapse of the pore space of mushroom samples, subject to the heat treatment and the centrifugation.

2.3.2 Calculations and data analysis

Mushroom composition calculations after various treatments

The composition of the mushroom dry matter components (y_i) after each of the treatments (hydration, heat treatment and centrifugation were calculated with the following equation:

$$y_i = \frac{W_o}{W_1} y_{i,o} + \frac{(W_1 - W_o)}{W_1} \frac{R_i}{(1 + \sum R_i)} \quad [2.12]$$

Where, W_o is the weight of sample before the treatment, W_1 is the weight of the sample after the treatment, $y_{i,o}$ represent the composition of the components before the treatment. For the first treatment, the vacuum impregnation, the dry matter composition of fresh mushrooms are used, as listed in table 2.2. It is important to notice that the expelled fluid, and liquid phase inside the mushroom always have the same composition due to the use of isotonic fluid in our experiment.

Volume fraction of the polymer network

The mass fractions of the components ($y_{i,ct}$) were converted into their volume fractions $)\phi_i$ (by using the density (ρ_i) of the components with Equation 2.13.

$$\phi_{i,ct} = \frac{y_{i,ct}/\rho_i}{\sum\left(y_{i,ct}/\rho_i\right)} \quad [2.13]$$

in which ρ_i represents the density of the individual components, with values are taken from Van der Sman [33].

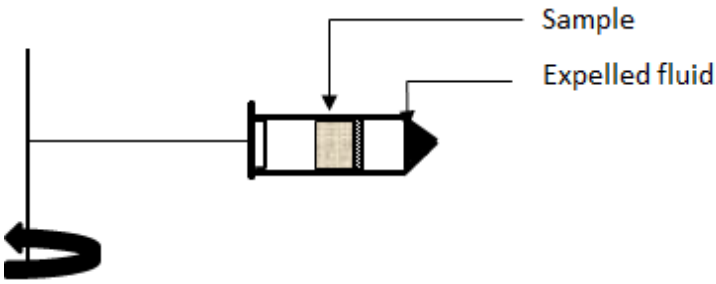


Fig 2.1: An illustration of application of centrifugal force in the mushroom sample.

Centrifugation force

Since the mushroom samples are not at the bottom of the outer centrifugation tube (as shown in figure 2.1), the actual g-force experienced by the bottom of the mushroom tissue (g_{actual}) is less than the set value (g_{set}); thus a correction is needed:

$$g_{actual} = g_{set} * \frac{R_{sample}}{R_{bottom}} \quad [2.14]$$

Where, R_{sample} and R_{bottom} represents the distance of the sample and the bottom of the centrifugal filtration tube from the axis of rotation.

Estimation of the swelling pressure

When the external centrifugal force is applied, water and solutes leach out of the mushroom tissue, leaving the cross linked polymer and associated bound liquid in the sample behind. The loss of water and associated solutes has two consequences: the size (height) of the sample is reduced and there is a change in osmotic pressure. This effect has to be taken into account for the calculation of external pressure p_{ext} and the osmotic pressure Π_{mix}.

The centrifugal force depends on the mass of material that creates a gradient in the applied external pressure over the height of the sample. Following Buscall and White [34], we adopted the mean approximation method to reduce the complexity of the model:

$$p_{ext}(H) \approx \frac{1}{2}\Omega^2 R \sum_i \rho_i \bar{\phi}_i H = \frac{1}{2}\bar{\rho} g_{actual} H \quad [2.15]$$

With g_{actual} the effective gravity imposed by the centrifugation force.

During centrifugation, the height of sample decreases. Therefore and material (solids) centered co-ordinates are more convenient to use [35]. Initially, the height of the sample is H_o and the polymer volume fraction is $\phi_{p,o}$. Because the amount of polymers remains constant during centrifugation, the product of sample height and average polymer volume fraction remains constant:

$$\phi_{p,o} H_o = \bar{\phi}_p H \quad [2.16]$$

Prediction of sorption isotherm

For a range of water volume fraction ϕ_w values, composition of mushroom was calculated as volume fractions of sugars, protein, polymers and mineral; and corresponding values of χ_{eff} and N_{eff} were computed according to equation 2.4 and 2.5. The values were substituted to equation 2.3 to calculate the value of $\frac{v_w \Pi_{mix}}{RT}$, and subsequently water activity (a_w) of sample. The latter was then plotted against mass fraction of water.

Curve fitting

The Flory-Rehner expression for the elastic pressure was fitted to our experimental data using non-linear regressing with Matlab using the *lsqcurvefit* function. This was carried out for each temperature independently. Hence, we obtained the temperature dependency of the model parameters, i.e. N_c and ϕ_o.

2.4 Results and discussion

2.4.1 Change in WHC in mushroom as a function of temperature at zero load

First, we present the results on the WHC of mushroom at zero mechanical load, which is used to estimate the change of the Flory-Huggins interaction parameter of the proteins. We assume that the interaction parameter for proteins follow the same temperature dependency as WHC at zero mechanical load. This assumption was used previously to successfully describe the water holding capacity loss upon heating of meat [5, 6]. The results are shown in figure 2.2, where the WHC is expressed as the water content of the heat treated mushrooms.

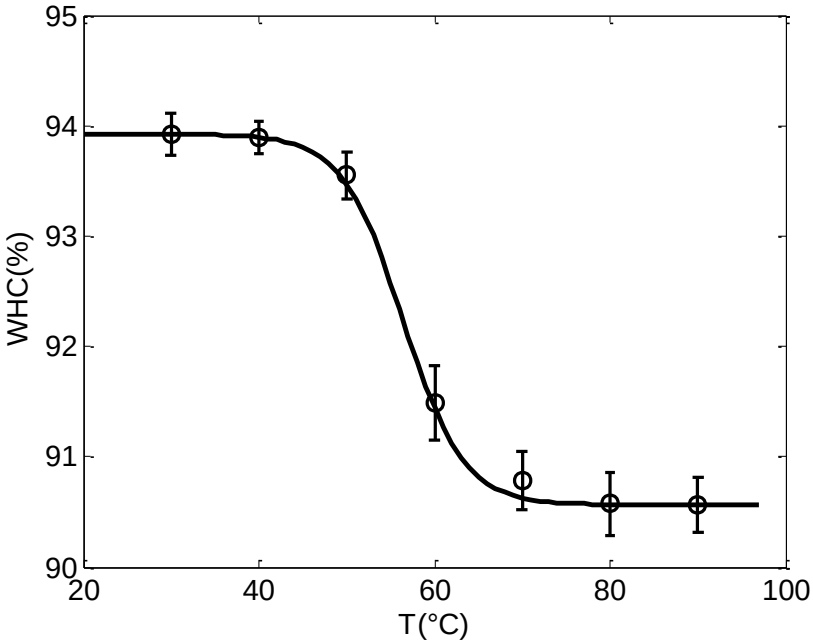


Fig 2.2: WHC, expressed as the moisture content in the heat treated mushroom cap (WHC=y), as a function of the temperature. The moisture content was computed from the weight loss data of thirty individual mushrooms after the heat treatment. The open circles show the experimental measurements, the bars show the standard error and the line show the fitted relation.

A logistic function is fitted to the WHC data.

$$y = y_2 - \frac{y_2 - y_1}{1 + a \cdot \exp\big(b(T - T_e)\big)} \qquad [2.17]$$

which yields the following values: y_2 =93.92%, y_1 =84.48%, T_e =54.3°C, *a* = (1.72±0.5) and *b* = -0.28±0.06 °C^{-1} .

The WHC of meat shows similar behavior: in a previous paper [4] the value of *b* and T_e in meat were found to be -0.25 and 52°C respectively. Thus, mushroom and meat behave similarly in their WHC around the mid-point of the sigmoid curve. The value

of a was 30 for meat, which is much higher than the value in mushroom. The lower value of a in mushroom indicates a lower onset temperature for the collapse; however, the shape of the curve is relatively insensitive to the value of a.

We attribute the loss of WHC with temperature to the denaturation of the protein. This hypothesis is based on two reasoning. First, the water loss in mushroom is quite similar to meat when heated, as described in the previous paragraphs. Second, the value of T_e is in the range where most of the proteins of vegetable origin denature. Our dynamic scanning calorimetric analysis (DSC) of mushroom showed that the protein denaturation temperature is 50-60 °C (result not shown in this paper).

The values of a, b and T_e are used in equation 2.8 for the Flory-Huggins interaction parameter. The resulting dependency of $\chi_{pw,1}$ with temperature is shown in the left pane of figure 2.3, while the right pane shows the change of χ_{pw} with composition, for two temperatures (30 and 90 °C)

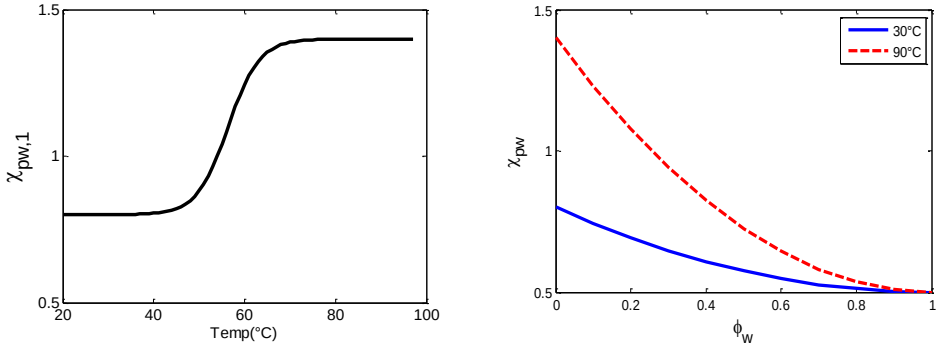


Fig 2.3: The χ_{pw} parameter for mushroom proteins. The left diagram shows the temperature dependency of χ parameter and the right diagram shows the composition dependency at T=30 and 90 ^{o}C which follows equation 2.6.

2.4.2 Water vapour sorption

To check the validity of the temperature dependency of χ_{pw}, we performed water vapour adsorption measurements for fresh mushroom samples that did not receive vacuum impregnation or heat treatment, and heat treated samples which were vacuum impregnated and then heat treated T=60 and T=90 °C. Subsequently, the

samples were freeze dried. Results are shown in figure 2.4. In our previous paper [15], we showed the prediction of the sorption isotherm of fresh and blanched (for 1 minute) mushrooms with the FVFH theory. Figure 2.4 reconfirms the validity of this theory. In the same figure we also show the sorption data for fresh mushroom as obtained by Shi [23]. We recall that figure 2.4 is a prediction of the FVFH theory, Eq. 2.3, and that we have not applied any fitting to the sorption data.

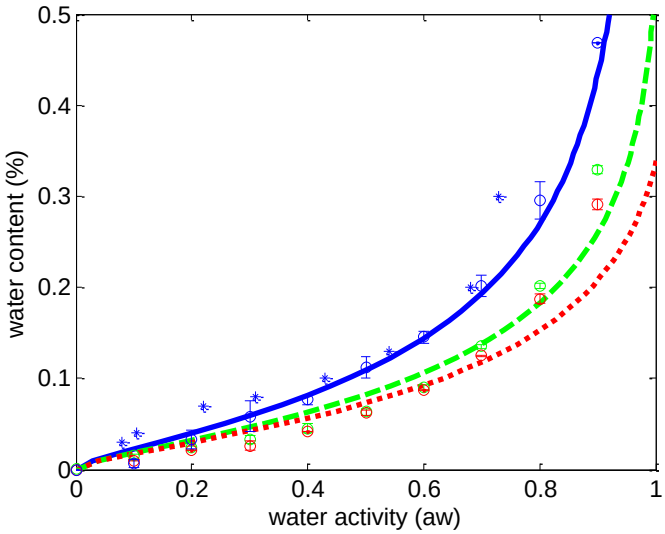


Fig 2.4: Prediction of the sorption curve of fresh (blue-solid) mushroom and heat treated mushrooms at T=60°C (dashed-green) and T=90°C (dotted-red). The open circle show the average of duplicate measurement and the lines show the prediction of the sorption curve by FVFH theory. The blue steric represent the data from Shi et al, 2012 [23] *for fresh mushroom. Water content is expressed in the dry basis.*

The predictions shown in in figure 2.4 compare favorably with the experimental data. The RMSE values of our measurement with the prediction were determined to be 0.0144, 0.0212 and 0.0293 for fresh mushroom sample and samples heat treated at 60 and 90 °C.

For heat treated samples, the experimental data point shows a deviation at higher water activities. This is probably due to the elastic contribution to the water activity,

which is not accounted for in the FVFH theory. The Flory-Rehner theory does take this into account, but at the moment the model parameters are not known, which in fact is the objective of the work presented below. In our previous paper we showed that the elastic contribution is indeed significant in this range of a_w, and that with it, the prediction is improved over that of the FVFH theory. At low water activity, $a_w <$ 0.8, the elastic contribution is negligible, and the sorption data allows for a good comparison of the osmotic pressure, Equation 2.3.

2.4.3 WHC at various mechanical loads imposed by centrifugation

Via centrifugation we applied a range of mechanical loads to the heat treated mushrooms. The applied centrifugation force sets the swelling pressure, as follows from Eq. 2.15. For comparison with the Flory-Rehner theory, the WHC is now expressed in terms of the polymer volume fraction. The change in polymer volume fraction with swelling pressure (i.e. external pressure) is shown in figure 2.5 for all temperatures used in the heat treatment. The figure clearly shows that there is a transition of the WHC of mushroom with temperature.

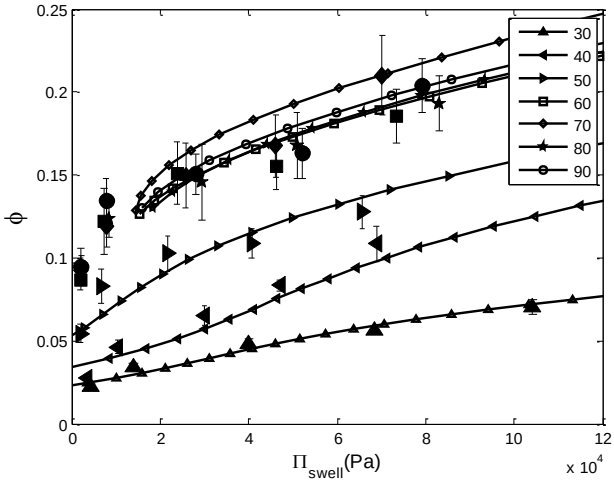


Fig 2.5: The WHC of heat treated mushroom samples as predicted by Flory-Rehner's theory. The solid symbols show the average of eight measurements and the bar along with the symbols show the standard deviation of measurements.

For equal swelling pressure, the polymer volume fraction of the sample is significantly increased for temperatures above 60°C. However, beyond that temperature the WHC does not change anymore. This is an indication that the interaction between biopolymers and water levels off beyond 60 °C.

Electron microscopy shows that the mushroom capillaries had collapsed both during the heat treatment and during the application of the centrifugal force, as evident from figure 2.6. The capillary collapse is particularly evident for samples that were heated above 60°C.

Figure 2.5 shows a rapid increase in the polymer fraction with swelling pressure at temperatures of 60 °C and above. We assume this rapid increase is due to pore collapse. The fact that above 60 °C there is no large change in polymer volume fraction anymore, is in line with this – all pores have already collapsed. Electron microscopy shows that at low swelling pressure values, the pores in mushroom are indeed still intact, and hold some (capillary) water. At swelling pressures larger than 10 kPa, the pores have indeed collapsed. We note that our theoretical description does not yet account for the contribution of capillary water to the WHC. Consequently, the data points at low swelling pressure are excluded from the fitting procedure.

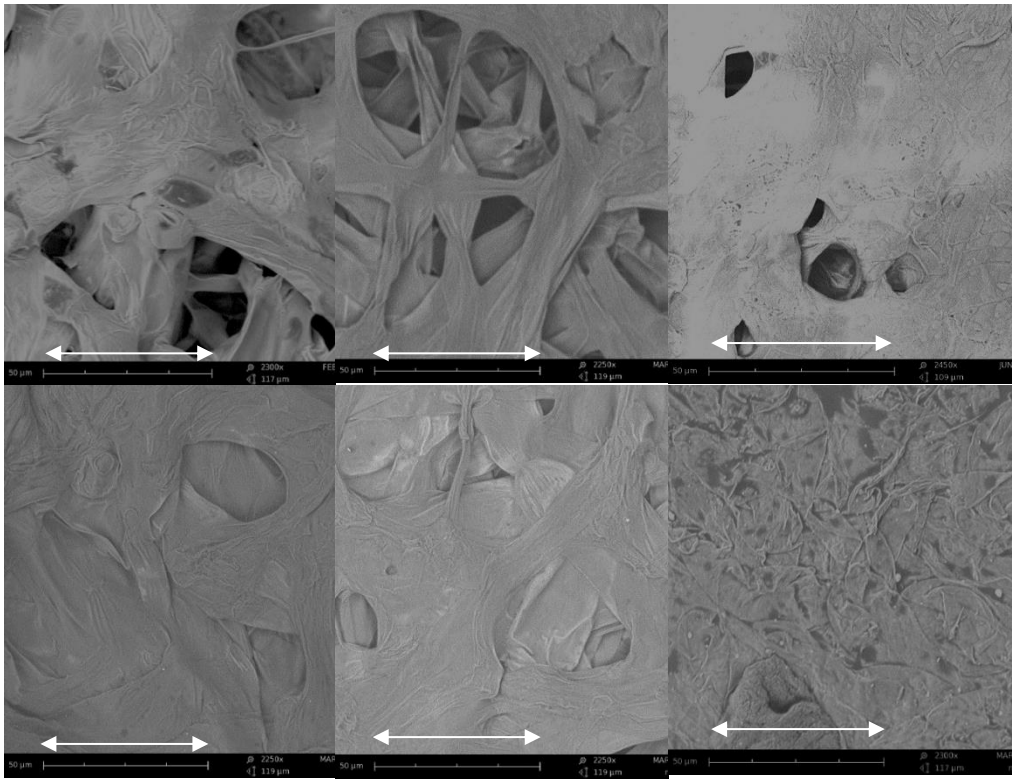


Fig 2.6. Top: Fresh hydrated mushroom(Left), heat treated at 40°C (middle) and heat treated at 90°C (right). Bottom: Corresponding samples centrifuged at 8000g. The horizontal line represents length scale of 50 µm.

4.4 Temperature dependency of model parameters in elastic pressure

Via non-linear regression we fitted Eq. 2.2 to the WHC data in figure 2.5, shown as solid lines in the figure. The Flory-Rehner theory fits quite well to the WHC data at higher swelling pressure values. Via the fitting procedure performed for each temperature separately, we obtain the relation of the model parameters in the elastic pressure, Nc and ϕ_o, with temperature. The results are shown in figure 2.7.

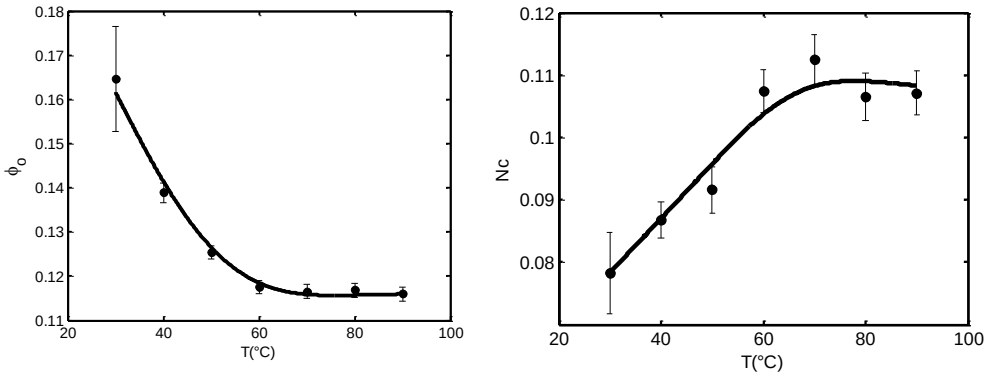
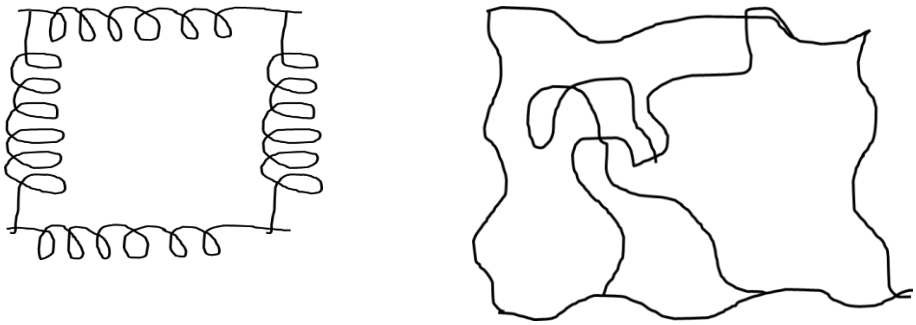


Fig 2.7: The temperature dependency of the fit parameters Nc and ϕ_o in the elastic pressure. The bars along with the fit parameters represent the error values in their estimation. The smoothened lines serve to guide the eyes.

Figure 2.7 clearly shows a decrease in the value of ϕ_o with increasing temperature whereas a reverse trend is seen in *Nc*. At temperatures above 60°C the values do not change anymore. Previously, we assumed that the model parameters ϕ_o and *Nc* are independent of the temperature [36]; the current data shows that we have to recall that hypothesis.



A B

Fig 2.8: A conceptual diagram of the mushroom polymers before (A) and after (B) the heat treatment.

The parameter ϕ_0 is the volume fraction of the polymer in the relaxed state (i.e., without any elastic force). The fact that the value is reduced with higher temperatures, indicates that the chains between the crosslinks have become longer (i.e., are more swollen), possibly due to the loss of the original conformation of the proteins. The larger values of *Nc*, the crosslink density, indicates that in fact more crosslinks have been forms, by aggregation of the proteins. The fact that the model parameters do not change any more above 60 °C indicate that the changes are likely due to protein denaturation. Denaturation brings with it a lot of changes in physical and chemical properties of the material that includes uncoiling of the proteins, and aggregation, i.e. the formation of new bonds. The changes in the biopolymer network due to protein denaturation are sketched schematically in figure 2.8.

We here applied extensive heating to ensure equilibrium, since the WHC is a thermodynamic quantity. Recent research showed that protein denaturation has some dynamics in the order of minutes, [37, 38], which is in the range of industrial processing times – as used during blanching. If the Flory-Rehner theory can be linked to a kinetic model of protein denaturation, one can design the mushroom processing for optimal WHC after treatment.

2.5 Conclusion

In this work we described the changes in water holding capacity in mushroom as a function of temperature. The changes of the WHC with temperature could be quantified as changes in three model parameters: the Flory-Huggins interaction parameter between the proteins and the aqueous solvent phase, the crosslink density of the network, and the biopolymer density at the relaxed state. The change of all three parameters can be explained by protein denaturation.

While most of the parameter values are similar to those for meat, the onset of the collapse was much quicker. Indeed one can see that at lower temperatures (< 50 °C) already some collapse takes place.

Part of the collapse of the structure seen at higher temperatures can be ascribed to the collapse of the pores between the hyphae. The current theory cannot yet describe this; however it is an important effect which explains the large reduction in water content when going from 50 and 60 °C.

Even though there are differences between various types of food matrices such as meat, carrot and mushrooms, the Flory-Rehner theory is shown to apply to all of them. Given the diversity of the materials, we expect that it can be applied to other foods as well. The control in WHC in foods becomes increasingly important to predict and control sensory attributes such as juiciness and digestive properties in new food products.

Acknowledgements

We are grateful to C4C hodings B.V., The Netherlands for the financial support.

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CHAPTER 3

Effects of Porosity and Thermal Treatment on Hydration of Mushrooms

This chapter is *accepted* for publication *by Food and Bioprocess Technology* as *Paudel E., Boom R.M., van der Sman R.G.M.(2015) Effects of Porosity and Thermal Treatment on Hydration of Mushrooms*

Abstract:

Many porous food materials compose of a solid, gelling phase and a gas-filled pore phase. However, the biphasic character of these materials is hardly acknowledged with respect to the hydration of the matter. In this study we consider mushrooms as such a porous food material. Its solid phase consists of intertwined hyphae, having cell walls with a swellable polymeric matrix, and a pore phase made up by the space in between the hyphae. We have investigated the hydration of mushrooms as function of initial porosity and thermal treatment. Variation in porosity is induced by the natural variation in the growth of mushroom. Porosity is measured by the weight gain during vacuum infiltration of mushroom caps with the mushroom isotonic solution. The hydration of the heat treated mushroom shows a linear increase with the porosity of mushroom. The hydration after the thermal treatment is also linearly related with the fraction of pores that is filled during the vacuum infiltration process. Storage of mushroom increases the porosity with the number of storage days, which has also been confirmed with XRT measurements. Finally, we show that the hydration of thermal treated mushroom has two independent contributions, from porosity and temperature of the heat treatment. Current theories deal only with the latter contribution to the hydration of foods, and need to be extended for accounting porosity of the food.

Keywords: Mushroom processing, Porosity, Degree of hydration, Water holding capacity

3.1 Introduction

In the last recent years we have been researching a mechanistic explanation for the water holding capacity (WHC) of food materials using the Flory-Rehner (FR) theory. We have applied that theory to meat [1], freeze-dried vegetables [2] and mushrooms [3]. Theoretically, the FR theory only applies to gel-like materials. Some foods like freeze-dried vegetables clearly have both a gel-phase and a pore phase. The gel phase consists mainly of cell wall material which swells upon hydration. In the pore phase, the water is held by capillary forces. In our previous research [2], we have not been able to separate these two different contributions to WHC, because of the leaching of solutes from the gel phase to the pore phase.

In this paper, we present the first experimental results on the effect of these two contributions to the WHC of mushrooms. Fresh mushrooms have clearly a distinct pore space, filled with air. The gel phase consists of intertwined hyphae. Its dry matter consists mainly of cell wall material and carbohydrates in the vacuole. In our previous paper [3], we have investigated the WHC of vacuum infiltrated mushrooms via centrifugation. Via vacuum infiltration we have filled the pore space with an isotonic liquid, having the same composition as the intracellular fluid. At sufficient high centrifugation speeds, the pore phase has collapsed. Furthermore, the swelling of the gel phase due to difference in osmotic pressure between gel and pore phase is prevented via vacuum impregnation of the pore phase with an isotonic fluid. By these two measures we have been able to determine the WHC of only the gel phase.

Other systems having both a gel phase and a pore phase are freeze-dried fruits and vegetables and coarse gels. Properties of the freeze-dried systems are already discussed [2]. Coarse, particulate gels can be viewed as a network of aggregated microgel particles with a liquid filled pore phase [4, 5]. This is in contrast to fine stranded gels, where the biopolymers form a space filling network. The water holding of these fine stranded gels can be fully explained by Flory-Rehner theory, and is linear with their gel strength [6]. Water holding of coarse gels is little explained by gel strength, but rather by the size of the pore [7]. Theoretical investigation of the contribution of the pore space to the water holding of particulate gels is hampered by the fact that pore size is often in the submicron range, where both the van der Waals

forces and the capillary forces contribute to the water binding in the pore space [8, 9].

In mushrooms the pore size measures several micrometers, and the van der Waals forces can safely be assumed negligible compared to the capillary forces. Hence, we view mushroom as a good model system for a detailed investigation of the contributions of the gel phase and the pore phase to the water holding capacity. The pore space is well open to the environment and can easily be filled with liquid via vacuum impregnation. The natural variability in porosity of mushrooms and its maturation render a large variation in porosity, namely in the range of 0.25 to 0.50, which gives us a means to vary the contribution of the pore phase. On the other hand, temperature treatment gives us a means to alter the contribution of gel phase on the water holding capacity. At high temperatures, proteins present in the cell wall materials denature, which lowers the water binding of the gel phase. During storage mushrooms are still physiological active, and mature. Prolonged maturation has shown an increase in their hydration after a thermal treatment [10–12]. While the reason for such an increase is attributed to the proteolytic changes in protein [10, 13], this paper gives the insight that maturation influences the hydration properties via porosity changes.

3.2 Methods and materials

3.2.1 General methodology

An important measure for the contribution of the pore space to the WHC is its volume fraction, i.e. the porosity. The global value of porosity has been measured via weight gain following vacuum impregnation with an isotonic fluid. As reported in our previous paper [3], fluid with the composition equal to cellular fluid does not lead to swelling of the gel phase. Hence the isotonic fluid just fills the pore while displacing air during the vacuum impregnation. Next to the global volume fraction of the pore phase, we have investigated on its local variation via x-ray tomography (XRT). Mushrooms with different global porosity values were obtained from their biological variation and after their maturation. To enhance their porosity via maturation the mushrooms are stored at temperature of 4°C and 95% RH prior to the vacuum impregnation. The contribution of gel phase in WHC changes with the

thermal treatment as a result of protein denaturation. This change in the contribution with the temperature is studied by giving a thermal treatment to the mushrooms. Porosity is determined via vacuum infiltration for every mushroom. Water loss is measured at different temperatures of thermal treatment. Collapse phenomenon of the pore phase as a result of thermal treatment is studied by giving the thermal treatment to mushrooms in which pores were previously partially filled with the isotonic solution. We have used white button mushroom (*Agaricus bisporus*) with a cap diameter of 4–5 cm for all our experiment. The mushrooms were received within 2 h of their harvest.

3.2.2 Formulation of isotonic solution

The mushroom isotonic solution contained 2.2% mannitol, 0.2% trehalose and 0.8% potassium phosphate, dissolved in deionized water, and was adjusted to a native pH value 6.3 of fresh mushrooms, as suggested in our previous paper [3]. The solution was used both for measuring porosity of fresh mushroom, and as a heat transfer medium providing the thermal treatment to the mushroom samples.

3.2.3 Estimation of porosity of mushroom with isotonic solution

Porosity of mushroom is computed by the weight gain of mushroom after its vacuum impregnation with the isotonic solution. Caps of mushrooms are submerged in the isotonic solution and then subjected to a set vacuum of 20 mbar at room temperature for 10 minutes. The vacuum is then released and the caps were kept in the isotonic solution for another 5 min to allow full impregnation of the solution into the pores. The porosity was calculated following equation 3.1:

$$\epsilon = \frac{(w_H - w_F)/\rho_{is}}{(w_H - w_F)/\rho_{is} - w_F/\rho_{th}} \qquad [3.1]$$

Here, w_F and w_H are the weight of mushroom before and after the vacuum impregnation, ρ_{is} is the density of isotonic solution, and ρ_{th} is the theoretical density of the gel phase of mushroom calculated from its composition [3]: 92.1% water ($\rho_w = 1000$), 2.3% sugar (ρ_s=1550), 2.6% polysaccharides (ρ_c=1550), 2.1% protein

(ρ_p =1330), and 0.8% minerals (ρ_m =2440). The theoretical density was then calculated from the individual densities following the relation [14]:

$$\frac{1}{\rho_{th}} = \sum_i \frac{y_i}{\rho_i} \qquad [3.2]$$

3.2.4 WHC measurements

The vacuum impregnated mushrooms are stored at 4°C for 24h and are subjected to a thermal treatment with a temperature controlled bath holding the isotonic solution (1:5 weight ratio of hydrated mushroom to the isotonic solution). After the thermal treatment, mushrooms are taken out of the bath and cooled in a bath of the isotonic solution held at the room temperature. The cooled mushrooms are allowed to drain surface attached water, and their surface are dried with a tissue. Subsequently, their weight was measured. Water holding capacity is expressed in terms of relative degree of hydration (Y_H) as the ratio of the weight of the heat treated sample (w_T) compared to their fresh weight (w_F) before vacuum impregnation.

$$Y_H = \frac{w_T}{w_F} \qquad [3.3]$$

For storage study, mushrooms were stored without vacuum impregnation for three consecutive days. The stored mushrooms were given the vacuum impregnation and the thermal treatment without any storage in between.

3.2.5 Different degrees of air trapping

The pore phase in mushroom is filled with the isotonic solution to various degrees and the effect on the hydration behaviour after thermal treatment is studied. Partial infiltration of mushrooms was achieved via setting the suction pressures to 1000, 500, 300, 100 and 20 mbar. The fraction of the pores filled is obtained from the weight gain during the infiltration. Afterwards, mushrooms are provided a thermal treatment at 90°C for 15 minutes and WHC was measured as described above.

3.2.6 XRT imaging

XRT is used as a measure for the local porosity change during storage. Two mushrooms are analysed with XRT for three consecutive days. During storage the mushrooms are kept in a refrigerator at 4°C. For imaging, the mushroom are taken out of the refrigerator, and are put back after imaging for half an hour. The samples are imaged using the General Electrics Phoenix X-ray microfocus CT system. A power setting of 70 KV voltage and 350 µA currents are used. The transmission of the conical X-ray beam through the sample is recorded by a CCD camera. The sample is rotated with a step size of 0.18 degrees and the beam of x-ray is recorded by the camera for an acquisition time of 250 ms. The process is repeated 2000 times to get a full 360 degree rotation of the sample. The total readout time is 33 minutes. The 3D density map is obtained after tomographical reconstruction of images. Reconstructed images are analyzed for their (local) porosity values.

The reconstructed XRT image is analyzed in Avizo 8.1 image analysis software package. Among the series of reconstructed images, an image at the center of the mushroom is taken for the measurement of their greyscale value. A line probe is selected for calculating the greyscale value along the axis of the probe. The greyscale value in the probe is analyzed in a sequence of cylindrical discs with 2mm in radius and 50 μm in height along with the probe from the top of the mushroom to its bottom as shown in diagram. A greyscale value of 320 is assigned to the solid phase of the mushroom and 0 for the pore phase. The value of the solid phase is determined via preparing a paste, where all air is removed via vacuum treatment.

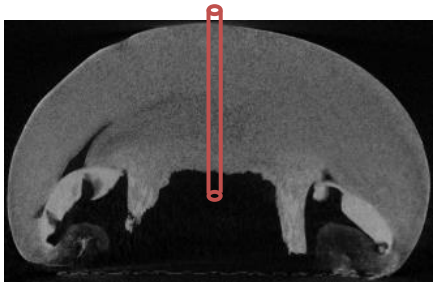


Fig 3.1: The schematic diagram of sampling of mushroom in Avizo-software.

3.3 Results and discussion

3.3.1 Porosity of fresh mushroom and the degree of hydration after thermal treatment

A large number of batches of mushrooms are examined to find a relation between the porosity and the water holding capacity after the thermal treatment. A higher degree of hydration after the thermal treatment is found for mushrooms with higher porosity, as indicated in figure 3.2. It shows the measurements for individual mushrooms in three different batches. Via linear regression, we obtain correlation coefficients R^2 value in the range of 0.5-0.7 for mushroom of various batches. Figure 3.3 shows the variation of porosity between various batches. The coefficient of linear regression values between the batches were not significantly different to each other. Ignoring the differences between batches and assuming the differences are due only to variation in the initial porosity, improved the correlation coefficient value to 0.90.

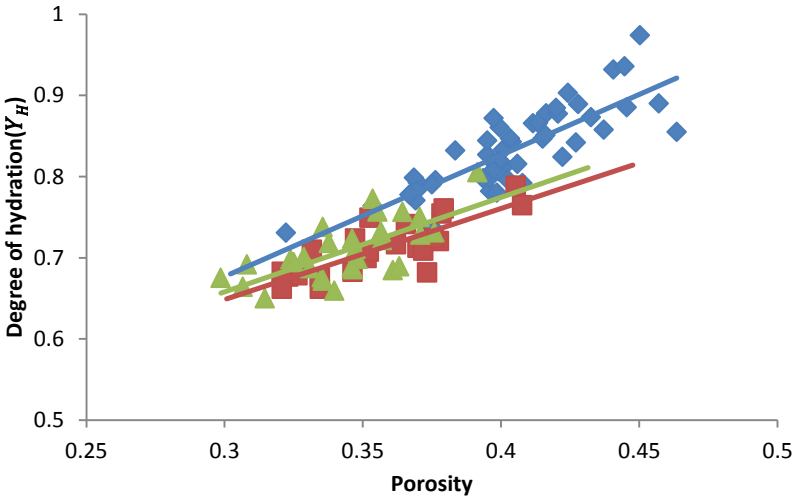


Fig 3.2: The relationship between porosity of mushrooms and degree of hydration after thermal treatment (The thermal treatment was provided at 90°C for 15 min).

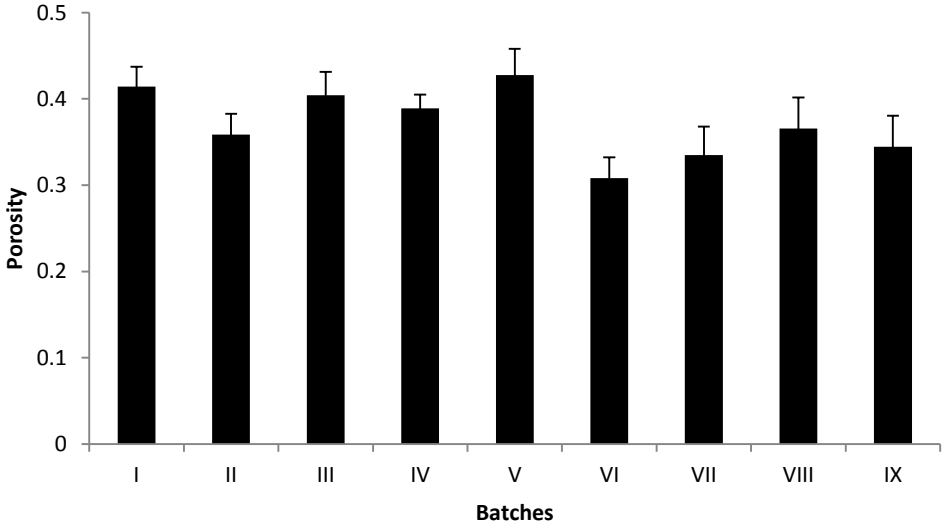


Fig 3.3: The variation of porosities between various batches examined

While porosities of individual mushroom seem to play an important role in determining their degree of hydration after the thermal treatment, there is a considerable variation in porosities of mushrooms within a batch as shown in figure 3.2 and between the batches as shown in figure 3.3. Though mushrooms among various batches used in the current study were on the same stage of physiological growth, the porosity value of mushroom ranged from 0.25 to 0.5.

3.3.2 Change in mushroom hydration with temperature

The change in the degree of hydration of mushroom as function of porosity for various temperatures is shown in figure 3.4. We observe that the degree of hydration of heat treated mushroom increases with porosity for all temperatures. At equal porosity, the degree of hydration decreases with the treatment temperature, as reported in several previous studies [2, 3, 15–17].

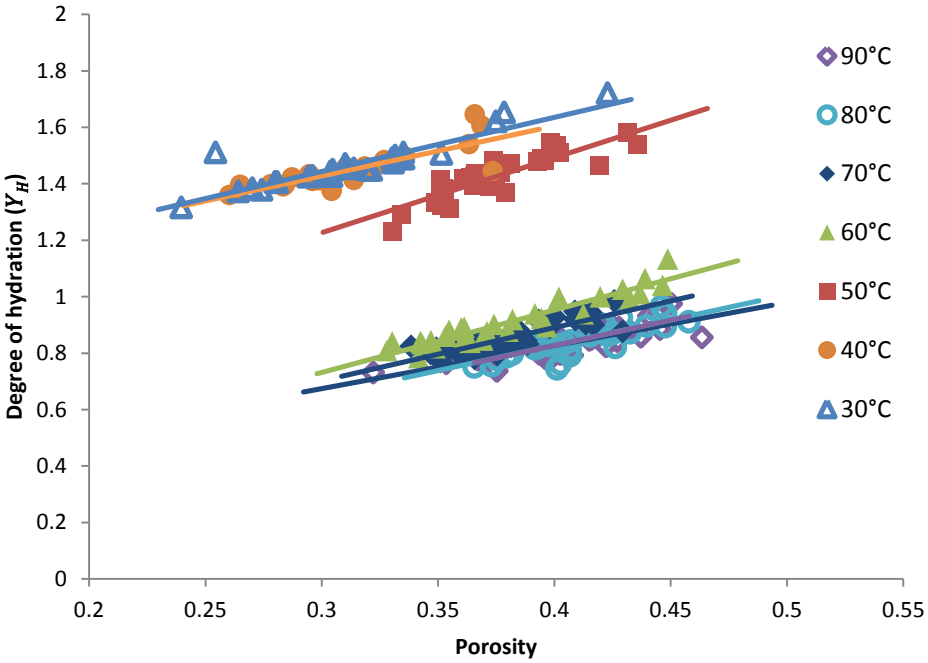


Fig 3.4: Yield of mushroom after the thermal treatment as a function of temperature.

We have performed linear regression to the data in figure 3.4, and have determined the slope and intercept of the fitted lines, including the error in their estimates. Their values are shown in figure 3.5. There are no significant differences in the slopes of the regression lines for different temperatures. However, the intercept of the line does decrease with temperature.

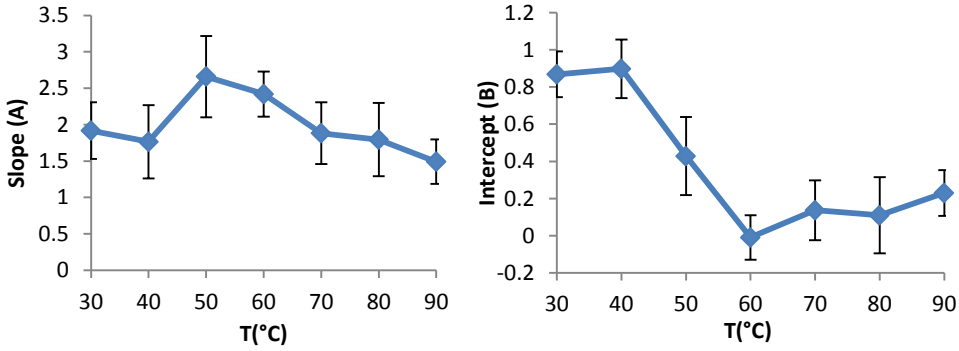


Fig 3.5: Slope (A) and intercept (B) of the straight lines that were obtained by linear regression model of first order between the yield against porosity at various temperatures. Lines are drawn to guide the eyes.

The data from figure 3.4 and 3.5 suggest that the degree of hydration of mushroom has two major, independent contributions: the temperature of the thermal treatment and the initial porosity of the mushrooms. The temperature dependency we attribute to the gel phase of the mushroom, which is shown to depend on temperature following a sigmoid relation [3]. The largest change in the degree of hydration happens in the temperature range of 50-60 °C. Figure 3.4 also shows the largest change in hydration in this temperature range. We assume that the hydration of whole mushroom follows the same sigmoid relation as the gel phase, and that it depends linearly on the porosity. This can be expressed as follows:

$$Y(T,\epsilon) = \frac{\rho_{is}}{\rho_{th}}\left(\frac{\epsilon}{1-\epsilon}\right) + \left(Y_{30}^{0} - \frac{Y_{30}^{0} - Y_{90}^{0}}{1 + exp(\mathrm{b}(T_0 - T))}\right) \qquad [3.4]$$

Where, Y_{30}^{0} and Y_{90}^{0} are the degree of hydrations of gel phase of mushroom at temperature 30 °C and 90 °C respectively. T_0 is the midpoint temperature of the sigmoid curve and b is the constant whose values are obtained from the curve fitting of the degree of hydration against the temperature for various mushroom porosities. The first and second term in equation 3.4 represents the weight ratio of fluid present in the capillaries and the gel phase compared to the weight before vacuum

impregnation respectively. The first term is obtained from the rearrangement of equation 3.1, the second term is adopted following our previous study [3].

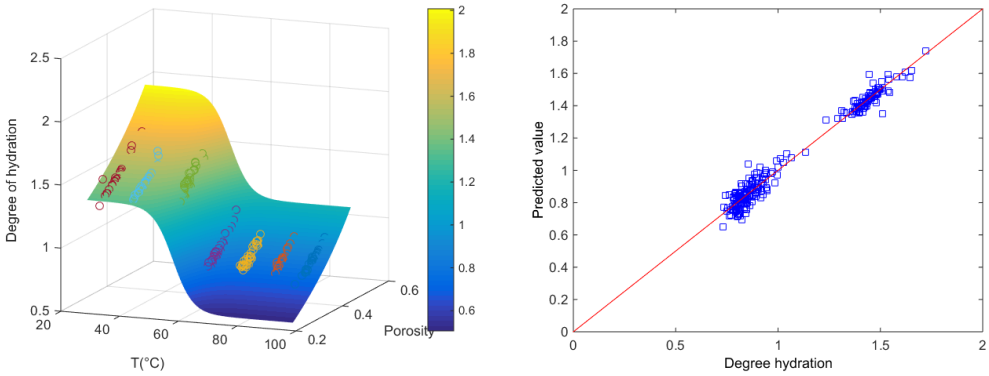


Fig 3.6: ***Left:*** *Prediction of the yield of heat treated mushroom at various temperatures. The lower and upper boundaries of the sigmoid curves show the prediction at 0.25 and 0.5 respectively. The open circles show the experimental data point used for the curve.* ***Right:*** *A plot of real yield value against the predicted values*

We have fitted Eq.(3.4) to the complete dataset as shown in figure 3.4 via non-linear regression, which is shown in fig 3.6. We have obtained the following values for the model parameters: Y_{30}^{0}=1.01±0.01; Y_{90}^{0}=0.17±0.01; b= 0.30±0.02; T_0=54.1±0.37 °C. The values of the constants b and T_0 agree with the values obtained in our previous paper [3]. The goodness of fit is computed via the RMSE, having a value of 0.0392. Hence, the data analysis confirms that the gel phase and the pore phase contribute independently to the hydration of mushrooms, with only the contribution of the gel phase being temperature dependent.

3.3.3 Change in mushroom porosity and degree of hydration upon storage

Figure 3.7 (left) shows the change in porosity in mushroom and the degree of hydration as function of storage days for two batches of mushroom.

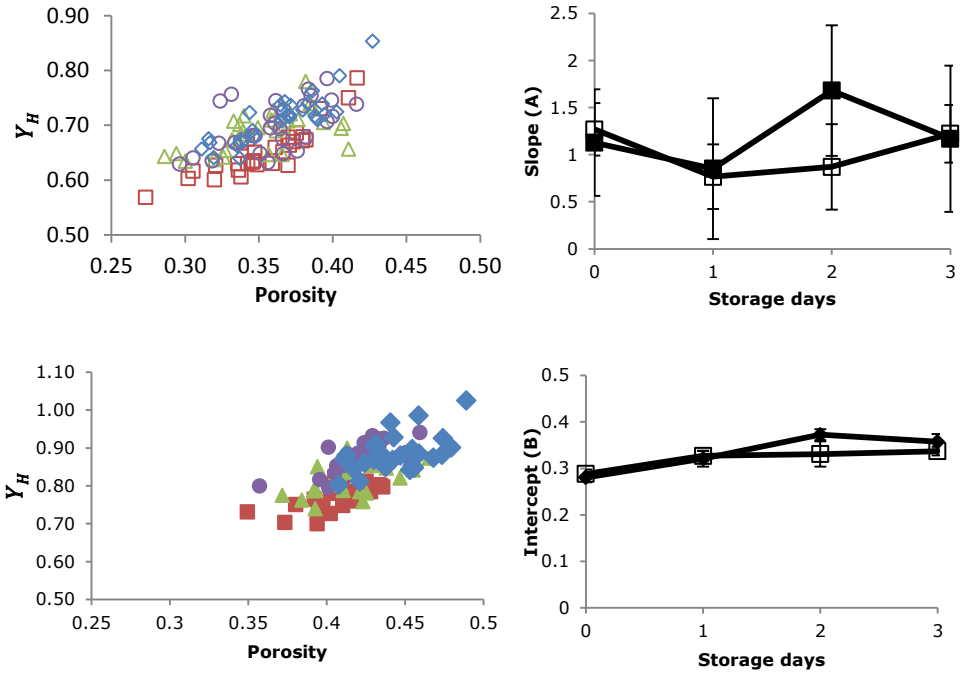


Fig 3.7: ***Left:*** *The degree of hydration of heat treated mushroom (90* °C/15 min*) plotted against their porosity for day zero (red square), one (green triangle), two (purple circle) and three (blue diamond).* ***Right****: Top, The slope (A) and, Bottom, the intercept (B) of the linear regression line of the degree of hydration of heat treated mushroom against porosity for various days. The open and closed symbol represent two different batches.*

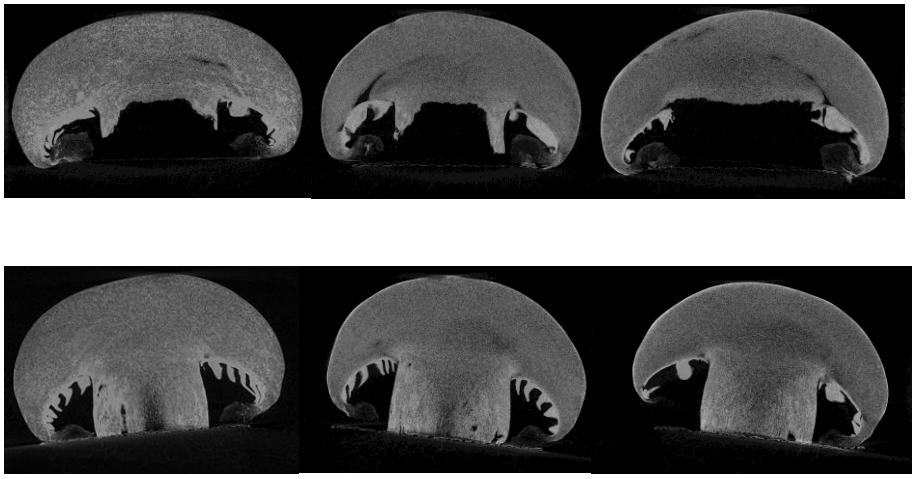
Despite the large natural variation in porosity of individual mushrooms, the values for both the porosity and degree of hydration increased with the storage days. An analysis of covariance (ANCOVA) test showed that the values of the degree of hydration for those lots increased significantly ($p<0.05$) till the second day of storage. For longer storage there are no significant changes. We have also performed linear regression on the data (hydration versus porosity). The top right of figure 3.7 shows the slope (A) for the different storage days. Due to the natural variation there are no significant differences in the slope of the regression lines. As expressed by Equation 3.4, we have not expected any difference in the slope. We

assume that storage only influences the intercept of the relation of hydration vs. porosity. With the assumption of a constant slope, we have performed the linear regression again, to obtain more accurate estimations of the intercepts. The data on the intercept is shown in the right bottom of figure 3.7.

The intercept (B) in two batches increased significantly ($p<0.05$) till day 1 and day 2 of storage respectively. The change in the intercept with storage indicates that storage not only induces change in porosity, but also induces other physiological changes in the gel phase of mushroom altering the hydration of stored mushroom. It is known in literature that the storage of mushroom brings about several biochemical changes in gel phase of mushroom, including a decreased level of mannitol, total and soluble protein content, glucan level and an increase in structural components chitin and cell wall proteins [12, 18–21]. In the fungal cell wall, chitin provides mechanical strength to the cell wall [12, 22]. With the storage, both the space within the hyphae and their mechanical strength increases because of increased chitin level. This increased space between the hyphae leads to an increased capacity of mushroom to hold more water. The higher mechanical strength keeps mushroom relatively firmer when it is subjected under the thermal treatment. The hydration of the gel phase might have increased due to increase in chitin and cell wall protein level with storage.

3.4 Change in porosity during storage as measured by XRT

The change in the local porosity of mushrooms as measured by X-ray tomograpy is shown in fig 3.8. We observe that, both of the scanned mushrooms show a decrease in the greyscale value with prolonged storage, indicating an increase of porosity in the stored mushroom.



Day 0 *Day 1* *Day 2*

Fig 3.8: The change in mushroom with the storage days as shown by XRT imaging. Lowering white intensity with the storage days indicate a lower density of mushroom as a result of increased pore spaces in the tissue

The change in the solid fraction expressed as the greyscale value is shown in figure 3.9. The greyscale value of mushroom tissue is lower than the value measured independently for evacuated mushroom paste, which shows a greyscale value of 320. There are various regions with localized density in mushroom [16], as is also indicated in figure 3.8. This variation we have quantified for the central axis of the mushroom. Results are shown in figure 3.9. The result shows a drop in greyscale value in both the mushrooms with storage days indicating an increased porosity in mushroom with storage days. The figure shows a higher density (lower porosity) at the top of the mushroom cap with storage. The cap surface is reported to be more porous having larger openings [21] with a lower density than the rest of the tissue [16]. The increased desity at the surface in the stored mushroom might be due to surface drying of mushroom with storage days.

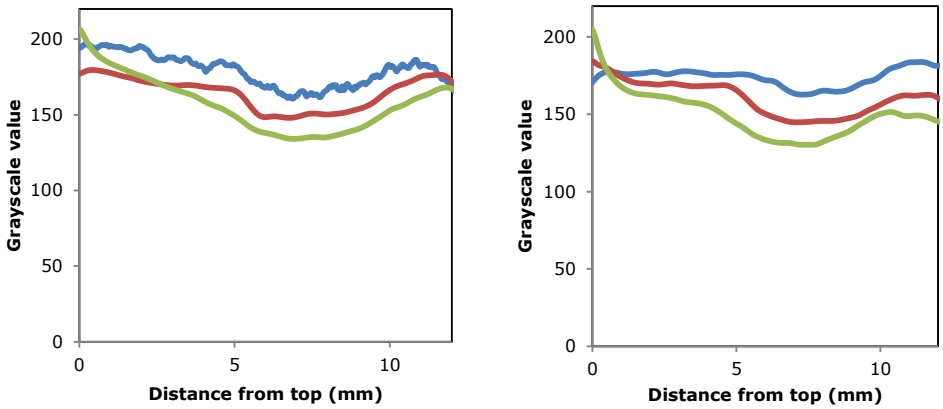


Fig 3.9: The greyscale values of two stored mushrooms after zero (blue), one (red) and two (green) days of storage.

3.3.5 Degree of hydration after thermal treatment in relation to the filling of mushroom pores

A plot of the fraction of pores filled against the degree of hydration after the thermal treatment of the vacuum infiltrated mushrooms is shown in figure 3.10. The degree of hydration increases linearly with the fractions of the pores filled as shown in the figure. Similar observation is reported in several previous studies [10, 23–25] which report a beneficial effect of infiltration of mushroom to the hydration after the thermal treatment.

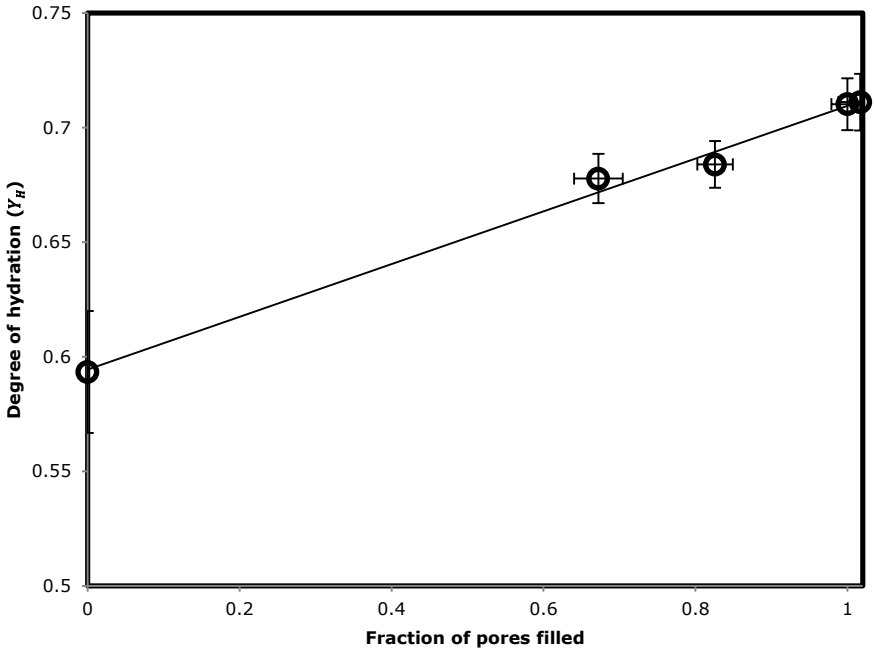


Fig 3.10: The relation between the fraction of pores filled with the degree of hydration of heat treated mushroom.

We argue that the decreased hydration in mushrooms with partially filled capillaries is because of pore collapse. Pore collapse occurs if the capillary forces in the partially filled capillaries exceeds the critical buckling stress of the wall matrix [26]. A schematic diagram of the phenomenon is shown in figure 3.11. Due to the limited amount of water, the capillary forces will be larger than in fully filled capillaries. The thermal treatment makes the wall less stiff because of protein aggregation which is evident by an increase in the cross link density of gel phase after the treatment [3]. The loss of stiffness makes the wall prone to collapse (Figure 3.11, middle) because of the capillary force. The thermal treatment also causes the gel phase to release water. After thermal treatment of the partially filled mushrooms, no porosity is present in the system which is checked with XRT. The capillaries will be fully filled (Figure 3.11, right), but the limited amount of water induces a higher curvature of

the water meniscus at the mushroom/air interface. This higher curvature induces higher capillary forces, which makes the mushroom shrink more, until it balances the elastic forces (due to entanglements of the hyphae).

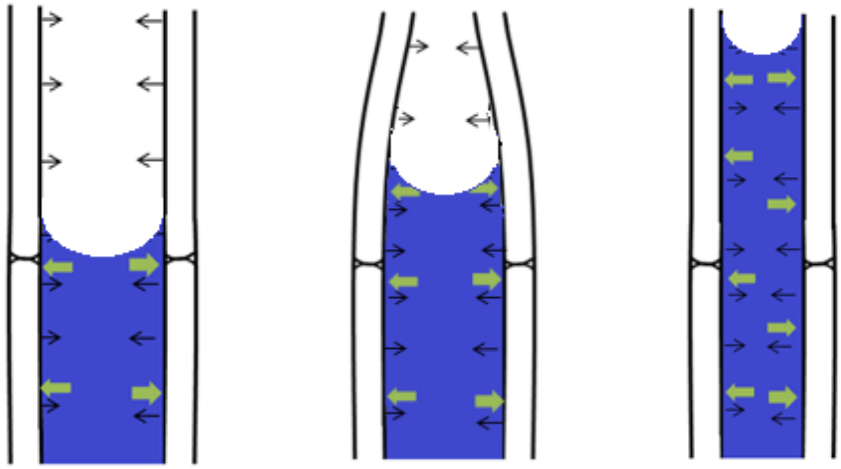


Fig 3.11: The conceptual diagram to describe water loss in mushroom with partially filled capillaries. The small arrow show the direction of collapse of hyphae, the big arrow show the pressure exerted by water against collapse. ***Left****: before thermal treatment.* ***Middle****: Partial collapse of hyphae during the thermal treatment* ***Right****: after the thermal treatment*

Meat is a food material, whose WHC is investigated quite extensively. Water population in meat is grossly divided into the *intramyofibrillar water* which is water present in the muscle fibre by hydrogen bonding and physical entrapment, and *capillary water* or *free water* which is water present in cylindrical extracellular cylindrical capillaries in sarcoplasmic area [1, 27]. In meat, the thermal processing causes a decrease in intra-myofibrillar water population and an increase in water population in the capillary phase [28]. The thermal treatment contracts collagen making this extracellular water to expel. Capillary phase water population in mushroom seems to play even a bigger role in mushroom hydration because of its higher porosity; pore phase holds around 40% of water in a vacuum infiltrated fresh

mushroom. Unlike meat, the capillary in mushroom is not an anisotropic narrow cylindrical passage; but it is a larger space between the hyphae which are randomly intertwined to each other. Upon thermal treatment, hyphae having reduced mechanical strength reach closer to each other that subsequently results in water removal. This phenomenon is in contrast to water loss behaviour shown by other vegetables having lower capillary space such as carrot which can still retain relatively more water than mushroom after its thermal treatment [2]. Next to the contribution of capillary phase, chemical makeup of gel phase might also determine hydration behaviour as every constituent contribute differently on it.

3.4 Conclusion

Description of the hydration behaviour of a gel matrix in many food material is possible with Flory-Rehner theory as is shown by several previous studies. Many food materials such as mushrooms are more complex than just a system consisting of only a gel matrix. Mushroom serves as a good model system to understand the hydration behaviour of a system that consists of a gel phase and a pore phase. The current study shows that porosity and temperature contribute independently to the hydration of heat treated mushroom.

The contribution of capillary water in food material might be different among many foods because of various porosity characteristics and wetting properties of capillary wall. Similarly, WHC of the gel phase of various food material might change differently with temperature because of variation in the constituents that make them. Knowledge of the composition of the cellular constituents and capillary phase contribution, is needed to understand the WHC character of many food materials, including meat and coarse protein gels.

Acknowledgements

We are grateful to C4C holdings B.V., The Netherlands for the financial support. We are also thankful to Erik Esveld, Food and Biobased Research (FBR) group of Wageningen University, for his help with the XRT analysis.

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CHAPTER 4

Effects of Cellular Structure and Cell Wall Components on Water Holding Capacity of Mushrooms

This chapter is submitted as *Paudel E., Boom R.M., van Haaren E., Siccama J., van der Sman R.G.M. Effects of Cellular Structure and Cell Wall Components on Water Holding Capacity of Mushrooms*

Abstract:

Little attention has been paid to the effects of different physical contributions to the water holding capacity of foods. In a sequel of papers we have investigated this by considering the common white button mushroom (*Agaricus bisporus*). The mushroom is especially suited for this research question, because it has two distinct phases: 1) the pore space in between the hyphen, and 2) the cellular phase. In the current paper of our sequel, we consider individual contributions of the cellular phase to water holding. The water holding capacity of hydrated mushroom is studied in relation to cell membrane integrity loss and change of state structural polymers the cell wall. We show that the loss in water holding capacity after heat treatment is closely correlated with cell membrane integrity loss. The intrinsic water holding capacity of the cell wall components, mostly chitin and proteins also play a role. The contribution of protein manifests largely via a significant response of WHC towards change in pH.

Keywords: Cell membrane integrity, cell wall components, water holding capacity, mushroom

4.1 Introduction

In a sequel of papers we have been investigating the physical contributions to water holding capacity of the common white button mushroom (*Agaricus bisporus*). Like in cellular plant foods, water is held in mushrooms in more than one compartment. Distinctive of mushrooms is that they have a clear pore phase, which is a continuous air space in between the hyphae of the fruiting body. During industrial processing, the pore phase in mushroom is filled with tap water via vacuum impregnation [1–3]. We have covered the contribution of capillary water in our previous paper [4]. The other compartments in the mushroom, holding water, are present in the cellular phase. The structure of the cellular phase is similar to plant foods, with 1) the vacuole holding small soluble solutes like salts and sugars, 2) the cytoplasm, and 3) the cell wall. These compartments are separated from each other by a cell membrane that is permeable to water, but impermeable to solutes. The impermeability of the membrane to solutes creates an osmotic pressure inside the vacuole, which will attract water. The cell wall is made up of several crosslinked biopolymers, which will deform elastically due to the influx of water into the vacuole [5]. The elastic deformation of the cell wall will generate the turgor pressure, which will balance the osmotic pressure in the vacuole under homeostasis. The cytoplasm is largely build up by biopolymers such as actin proteins. From the viewpoint of water holding capacity both the cytoplasm and cell wall can be viewed as a crosslinked polymeric gel, whose water retention can be described by the classical Flory-Rehner theory [5–8]. In the following we will consider the cytoplasm and cell wall as a single phase – which we will denote as the gel phase. The vacuole will be denoted as the intracellular phase, which is responsible for the osmotic pressure inside the cell. Note that the cell wall material and pore phase are not separated by a cell membrane, and can freely exchange water until thermodynamic equilibrium is reached.

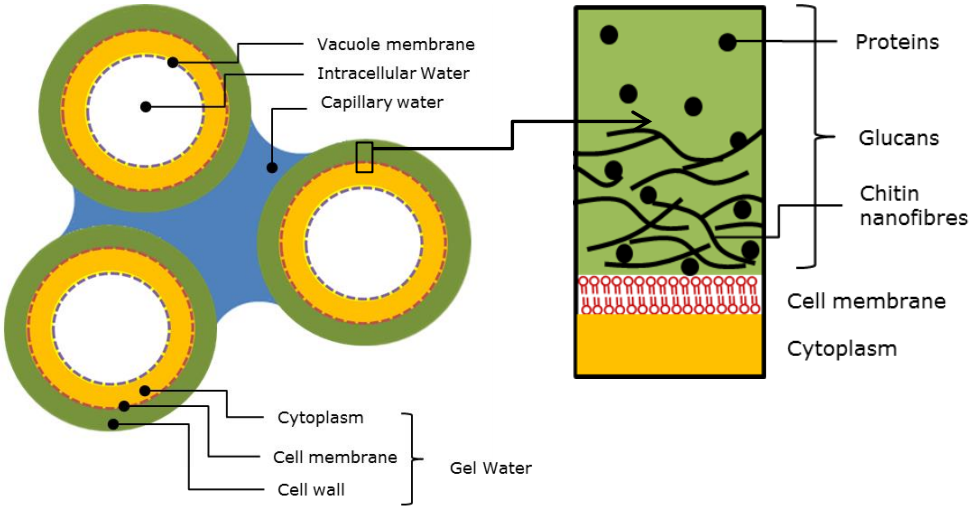

Fig 4.1: ***Left:*** *Schematic diagram of cross section of mushroom cellular (hyphal) structure.* ***Right****: The cell wall of mushroom zoomed in (pictures adopted from Ikafu et al.(2011) and Michalenko GO, Hohl, and Rast (1976) .*

The cell wall of mushroom consists of three major biopolymers, which are crosslinked to each other: 1) chitin which is a polymer of β-(1 → 4) branched N-acetylglucosamine unit [11], 2) beta-glucans and 3) proteins. The biopolymers display a compositional and structural gradient across the cell membrane [9, 10]. The outermost mucilaginous layer is rich in beta-glucans and the lower layers are more rich in chitin and beta-glucans, forming an intertwined network [10]. Proteins are distributed in the cell wall largely as glycoproteins, which are often crosslinked with polysaccharides [9, 12].

In our previous research, we have reported that the amount of *capillary water* in the fresh mushroom correlates linearly with the degree of hydration after thermal treatment [4]. This finding indicates that pores still hold a significant amount of water, even after thermal treatment. In our first paper of the sequel [6], the contribution of the gel phase on the WHC of heat treated mushroom is described with Flory-Rehner theory. There we have assumed that mushroom can be viewed as a gel, i.e. a homogeneous mixture of crosslink biopolymers in an aqueous phase. Similar to previous literature [13–15], we have considered there that protein denaturation is the main cause of the loss of WHC after thermal treatment, and we

have disregarded the structural complexity of the mushroom cells. While the theory has been quite successful in describing the overall WHC of mushroom tissue under a certain mechanical load, it could not fully describe the WHC of mushroom when low external loads are applied [6]. Now, we question whether protein denaturation is the sole cause of the loss of WHC during thermal treatments. We recognize that the loss of cell membrane integrity can also lead to a significant loss of water holding [5, 16]. Hence, in this paper we investigate the contributions of the loss of cell membrane integrity, and physical changes in biopolymers constituting the cell wall material, on water holding capacity of mushroom.

4.2 Methods and materials

In our experiments we will change the cell membrane integrity via heating, freezing and milling of the mushroom. The integrity of the cell membrane is measured via conductivity measurement. The change in electrical conductivity (after cooling or thawing) is used as a measure for the degree of loss of cell membrane integrity.

Mushroom samples are scanned with dynamic scanning calorimetry (DSC) to test whether protein denaturation can be measured and correlated to the WHC loss. The influence of specific structural biopolymers of the gel phase on WHC is investigated by applying enzymes to hydrolyse proteins, chitin and beta-glucans, present in the cell wall of the mushroom. We note here that while protein is known to behave as a polyelectrolyte, chitin does not contain any charged residues [17, 18], and beta-glucans are neutral polysaccharides. Hence, the contribution of proteins as polyelectrolyte to WHC can also be investigated via pH treatment of mushroom.

4.2.1 Cell membrane integrity measurement

The cell membrane integrity can be measured via various techniques. Few examples include examination of the cell after treatment with colorimetric and fluorescent dyes [19], NMR measurement [20], using microfabricated devices [21]. Because of its convenience, we have measured the cell membrane integrity via electrical conductivity, cf. [22]. The conductivity is measured of the fluid that is released from the tissue after thermal or milling treatment. For the conductivity measurement of fresh mushrooms, we have cut them into pieces with kitchen mushroom cutter that

has blades spaced at 4mm. Three grams of mushroom pieces are mixed with 6 ml of sugar solution, and held in tubes. The sugar solution is osmotically balanced with mushroom, which contains 2.25% mannitol and 0.16% trehalose [6]. The mushroom pieces are provided a thermal treatment for 30 min at various temperature from 30 to 90 ºC. After the thermal treatment, the tubes are cooled down to room temperature and the conductivity of the fluid is measured. The relative conductivity change is expressed as :

$$\kappa_R = \frac{\kappa_{90} - \kappa_T}{\kappa_{90} - \kappa_{30}} \quad [4.1]$$

Where κ_{90}, κ_{30}, κ_T represent the electrical conductivity of the fluid that is previously heat treated at 90 °C, 30 °C and the temperature of interest. At 30 °C, the relative conductivity value is at zero and at 90 °C, its value is 1. We correlate the relative conductivity value with relative cell membrane integrity I_R as:

$$I_R = 1 - \kappa_R \quad [4.2]$$

For the conductivity measurement of frozen samples, mushroom pieces are frozen at -18 °C for 24h. After the freezing, they are thawed at room temperature for one hour and then, the samples are well mixed with the sugar solution in the tubes, and the conductivity of fluid is measured. A similar procedure is applied to the milled sample. Here, fresh mushrooms are milled, and the sugar solution is added to the milled mushroom, while mixing, and the conductivity of the fluid is measured.

4.2.2 Water holding capacity measurement

Two types of measurements for the water holding capacity (WHC) are used.

1. The WHC under zero external mechanical load is measured via simple weight measurements using scales. This value is expressed as degree of hydration (Y_H) which is the weight ratio after a certain treatment (vacuum hydration or the thermal treatment) W_T relative to its fresh weight W_F before vacuum impregnation.

$$Y_H = \frac{W_T}{W_F} \quad [4.3]$$

The relative change in degree of hydration of mushroom with temperature is normalized to:

$$Y_R = \frac{Y_{H,30} - Y_{H,T}}{Y_{H,90} - Y_{H,30}} \quad [4.4]$$

Where $Y_{H,30}$, $Y_{H,90}$ and $Y_{H,T}$ represent the WHC of mushroom at 30 °C, 90 °C and temperature of interest. Values of $Y_{H,30}$, $Y_{H,90}$ and $Y_{H,T}$ are adapted from our previous work [6].

2. The water holding capacity of mushroom tissue under mechanical force are measured with the centrifugation technique [6, 23]. Mushroom samples are given vacuum impregnation with mushroom isotonic solution [6]. After vacuum impregnation, mushrooms are stored for 24 h before the thermal treatment to allow relaxation of polymers. Sample of size 7 mm in diameter and 5 mm in height are punched out of mushroom caps for WHC determination. Before the freezing treatment, the samples are punched out of the tissue, and subsequently they are vacuum impregnated. Finally, they are frozen at -18 °C for 24h. The following day, samples are allowed to thaw for one hour at room temperature before their centrifugation.

Mechanical pressure is applied to mushroom samples via centrifugation [6] in a centrifugal filtration tube. The WHC loss of the sample due to centrifugation is measured via weight of the samples before and after the treatment. From these measurements, water fraction of the samples are calculated and expressed as WHC under mechanical weight (Y_W).

$$Y_w = y_{w,1} - (1 - \frac{W_2}{W_1}) y_{w,iso} \quad [4.5]$$

Where, W_1 and W_2 are the weight of sample before and after the centrifugation; $y_{w,iso}$ is the water content in isotonic solution (97%); and $y_{w,1}$ is the water content in the sample before hydration which is calculated from moisture content of mushroom tissue (92.1%) [6, 24] and weight gain by the tissue during its hydration with isotonic solution.

4.2.3 Mushroom milling:

The cut pieces are milled with a laboratory scale rotary mill. The mill first crushed the samples with its rotating blades, and the crushed particles passed 0.5 mm sieve in the form of paste. The resulting paste is collected in plastic tubes. The air in the head-space of the tube is replaced by flushing nitrogen gas to prevent any surface oxidation. The tubes are closed and stored at 4°C. Milled samples are subjected to further analysis always on the same day of milling.

4.2.4 DSC measurement

Ten milligrams of mushroom samples is enclosed in the DSC steel sample pan. The sample is scanned from 25 °C to 100 °C at a rate of 10 °C/min and back to 25 °C. The heating and cooling scanning is continued for 3 cycles. The resulting thermograms are analysed further to determine the peak temperatures and peak areas.

4.2.5 Enzyme treatment:

Commonly, fungal cell wall are degraded with a cocktail of enzymes originating from other fungi [25, 26] which hydolyse several biopolymer at the same time. In our work, biopolymer groups (protein, glucan and chitin) that are present in the cell wall of mushroom are specifically targeted by using their corresponding hydrolyzing enzymes. Instead of using mixture of enzymes, proteases, chitinase and glucanase are used one at a time to hydrolyze one biopolymer type at one time. An aqueous solution of endo-proteases derived from *Bacillus Licheniformis* is obtained from Sigma Aldrich B.V. The protease used in the current work has a broad substrate specificity and is used in applications such as hydrolysis of chicken leg bone protein or egg yolk protein modification [27, 28]. Chitinase from *Streptomyces griseus* is lyophilized powder from the same source. The chitinase used in current work was used previously to inhibit growth of fungi [29] by its action against the chitin and therefore can be expected to work for hydrolysis of chitin in mushroom as well. Endo-1,3 β-D glucanase derived from barley is obtained from Megazyme as a solution containing 50% glycerol, which is removed by dialysing it against milliQ water for 24 hours at 4°C. The enzyme was selected based on its action against the

endo-1,3 β-D linkage which is said to be the most common type of linkage in fungal glucan [30]. The antifugal activity of this enzyme from barley source is reported previously [31]. The selection criteria of the enzymes was based on their optimal temperature. The temperature (range) for optimal activities for the protease [32], chitinase [33], glucanase [34] are reported to be 50 °C, 30-40 °C and 40-50 °C respectively. Enzyme treatment is provided at incubation temperature of 37°C (for 24h) for all the enzymes, to get sufficiently high enzyme activity near their optimal pH but below the temperature where native state of biopolymers in mushroom are altered because of the temperature. All the enzymes are suitably diluted in the isotonic solution to get a final concentration of 200U per litre of isotonic solution.

Enzyme treatment is provided to both *intact* and *milled mushroom* samples. The WHC values of those samples can provide insight on contribution of the biopolymer on question to a) the mechanical strength of the cell wall and b) osmotic binding of water. For the *intact samples,* the centrifugation technique, as described above, is used. Fresh mushroom tissues are vacuum impregnated with the isotonic solution with the enzyme added. After the incubation, the samples are centrifuged using filtration tubes at 4800g for 60 minutes to find their water holding capacity. The filtration tubes have a (0.2 micron) membrane allowing liquid to be expelled from the sample, and collected at the bottom.

For the *milled samples,* the centrifugal filtration tube is not suitable as the milled tissue is shown to block the membrane filter in the tube, Hence, regular centrifugal tubes with closed bottom are used. Now the expelled liquid is at the top of the pellet, and is collected by carefully decanting it. The milled samples are added with the enzyme solution and mixed properly in a vortex mixer. After incubation, the centrifugation is carried out at 4800g for 60 minutes in a 10 ml plastic tube. The weight difference before and after centrifugation is used to express WHC in terms of water fraction.

It is important to note that the centrifugation method applied to intact and milled samples are different, inhibiting a direct comparison of results. However, in both treatments we include a control sample that is *not* treated with enzymes. From comparing relative changes with respect to each control sample, we can have an indirect comparison between intact and milled samples. From this comparison we hope to obtain insights in the contribution of the specific biopolymers in osmotic

binding of water, and the mechanical strength of the cell wall both of which influence the water holding of the cell wall.

4.2.6 pH treatment

The isotonic solution is adjusted to various pH values by addition of 2M HCl. Mushroom are impregnated, then subjected to a thermal treatment and finally cooled in the same pH adjusted isotonic solutions. The degree of hydration of mushroom after such treatment is then measured.

4.3 Results and discussion

4.3.1 Cell membrane integrity loss and WHC of mushroom tissue

Three different techniques are used to compromise the cell membrane integrity: heating, milling and freezing. As an additional check, milled and subsequently heated samples are used as a reference, that are known to have completely destroyed cell membrane. The results are shown in figure 4.2.

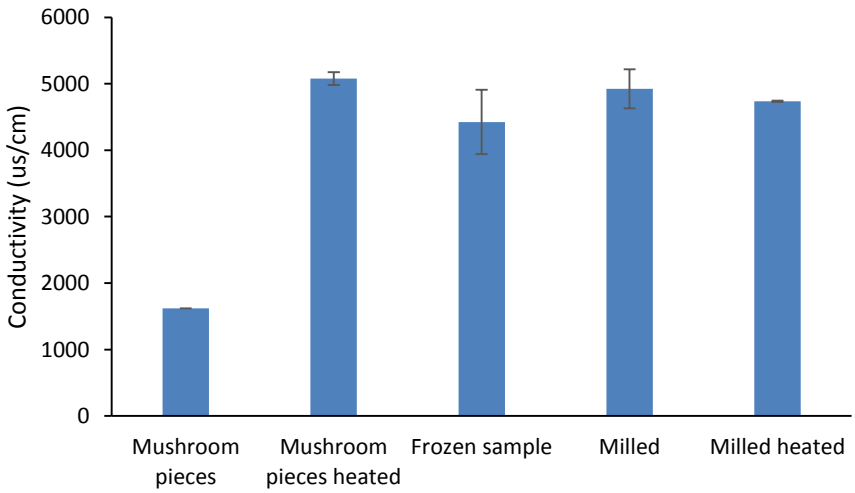

Fig 4.2: Electrical conductivity of fluid released from mushroom samples that are given various types of treatment.

All the treatments have resulted in similar values of the electrical conductivity of the resulting solution as shown in figure 4.2, confirming that in all cases complete cell membrane destruction is achieved. Both the thermal treatment and freezing are known to disrupt the cell membrane integrity of plant cell [20]. The samples that are milled either before or after the thermal treatment show similar values of electrical conductivity. As for all the treatments the conductivity values are similar, we assume that thermal treatment above 90 °C and freezing disrupts the membrane integrity as completely as the reference, which experienced both milling and thermal treatment at 90 degrees.

The effects of all the treatments on water holding capacity are investigated via the centrifugation method by using centrifugal filtration tubes. The WHC of milled mushrooms cannot be compared with the intact sample as the solids in the suspension will block the membrane in centrifugal filtration tube. The results of thermally treated and frozen mushroom samples compared to the hydrated fresh tissue is shown in Fig 4.3.

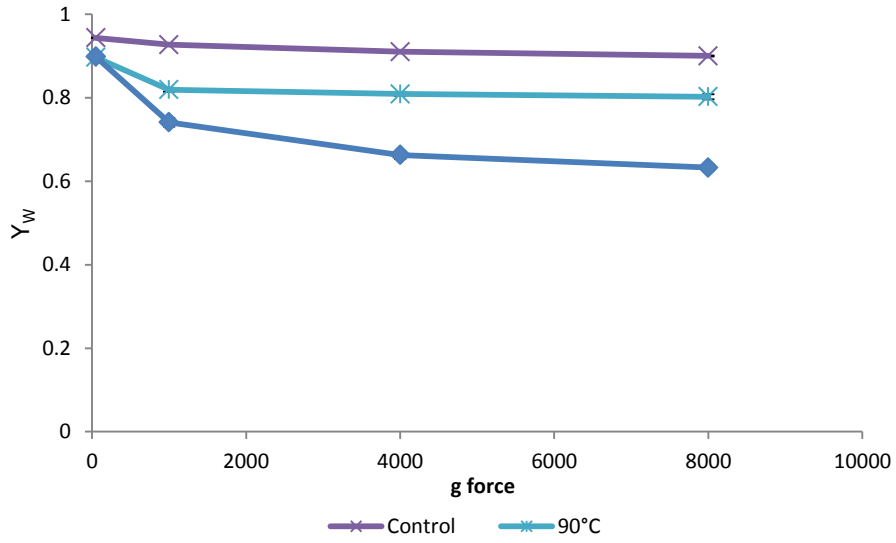

Fig 4.3: Comparison of WHC of heat treated and frozen mushroom sample with the control expressed as fraction of water in sample after centrifugation at various centrifugation speed.

For all the speeds used in our experiment, heat treatment and freezing have shown a lower WHC than that of the fresh samples indicating that the samples can retain less water after such treatments.

Both thermal treatment at 90 °C and freezing destroy the cell membrane integrity as is demonstrated with figure 4.2. However, the loss of the WHC of the frozen sample is larger than that of the heat treated sample which is shown in figure 4.3. A partial denaturation of proteins as a result of freezing [35] and formation of crosslinks in the mushroom polymers in the frozen sample can be the reason for this difference. Many freeze dried vegetables including mushroom [23] and carrot [36] do not rehydrate completely after freeze drying. Berghout et al. [37] also report lower solubility and formation of aggregates in concentrated lupine protein isolate after their freeze drying. This suggests that the formation of crosslinks in the tissue after they are compressed by ice crystals that have grown in the extracellular spaces during freezing. Figure 4.2 and 4.3 show that it is reasonable to consider that loss of membrane integrity contributes significantly to the loss of WHC from mushroom. Both the frozen sample and heat treated sample have lost their cell membrane integrity and exhibit lower water holding capacity, even when the protein in the frozen sample may not have been fully denatured.

The loss of cell membrane integrity is due to a phase transition of the lipid bilayer of the membrane. This occurs in a specific temperature range, which can be different from the temperature range of protein denaturation, as in the case of meat [38]. Hence, we investigate the conductivity as a function of temperature, as an indication of how the cell membrane integrity changes with temperature. The electrical conductivity values of mushroom are plotted against the temperature in figure 4.4 (left). The electrical conductivity value increases from 30 °C to 60 °C and aterwards the value levels off. The electrical conductivity values are calculated in terms of relative membrane integrity value with equation 4.1 and 4.2. Relative hydration and relative membrane integrity values are plotted as a function of temperature in figure 4.4. Both are fitted with the sigmoid function as in our previous paper [4, 6], which is given below:

$$\zeta(\mathrm{T}) = \zeta_{30} - \frac{\zeta_{30} - \zeta_{90}}{1 + \exp(b(T_o - T)} \qquad [4.6]$$

The symbol ζ represents the relative yield ($\mathrm{Y_R}$) or relative conductivity (I_R) values. The symbols ζ_{30} and ζ_{90} represent those values at 30 and 90°C respectively. The symbol T_o Indicates the midpoint of the graph.

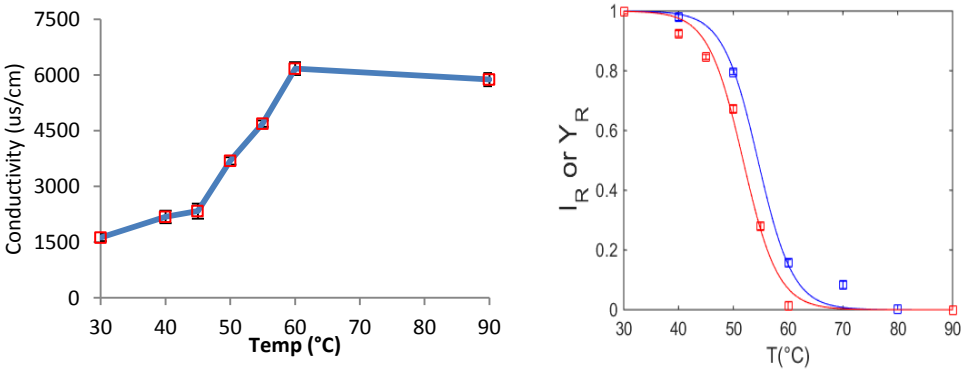

Figure 4.4: **Left**: The electrical conductivity values of mushroom sample treated at various temperatures. **Right**: relative blanching yield (blue) and relative integrity (red) change with temperature. The data point shows the measurements and the line shows the fitted relation.

Non-linear regression using the sigmoid function (equation4.6) shows quite similar values for the slope *b* for the relative hydration and relative cell wall integrity, viz. 0.29 (±0.07), and 0.3139 (±0.10) respectively. That indicates that the losses in Y_R (from water holding measurements) is likely to be explained by the loss in cell membrane integrity I_R (from conductivity measurements). The value of T_o for the relative yield (Y_R) and relative conductivity (I_R) are found to be 54.5 (±1.2)°C and 51.5 (±1.1)°C. In principle the values are statistically significantly different to each other. However, integrity is measured only for one single batch, and there might be significant batch-to-batch variations in mushrooms as has been observed for their porosity [4]. Hence, we conclude that the temperature range of membrane integrity loss is very similar to that of WHC loss with temperature. But, there might still be a

contribution due to protein denaturation as integrity loss occurs at a slightly lower temperature than the hydration loss.

A common method to investigate protein denaturation is differential scanning calorimetry (DSC). In our previous paper [6], and in similar measurements by Min et. al. [39], the peak appearing in the thermogram in DSC scan have been interpreted as protein denaturation. Here, we have done a further DSC study on the mushroom tissue, by comparing fresh mushroom tissue, milled mushroom sample and frozen tissue. The typical thermograms of the analysis are shown in figure 4.5.

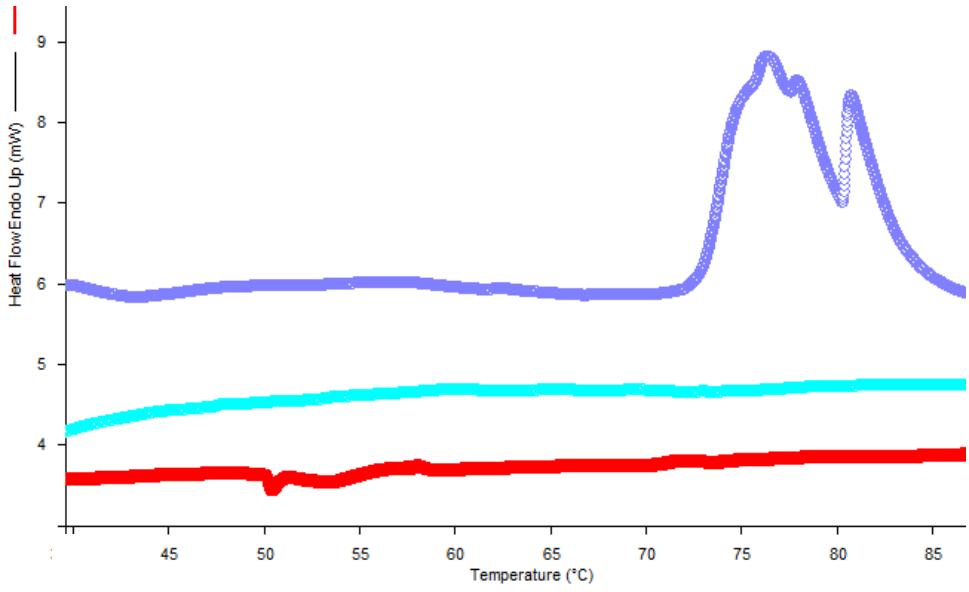

Fig 4.5: DSC curve of fresh (Top), milled (middle) and frozen (bottom) samples.

Among the three types of samples analysed, only the fresh tissue showed a strong peak with a peak temperature of 76°C. Both freezing and milling destroy the cell membrane integrity as discussed earlier. If this peak is indeed due to protein denaturation, all samples should have shown this peak, as we do not expect the milling and freezing to completely denature proteins. Moreover, the value of the enthalpy, obtained after integration the heat flow under the peak, is unusually large

compared to commonly reported values of the heat of denaturation for proteins. The enthalpy of the peak is 1.73 kJ/kg of fresh sample which translates to a denaturation enthalpy of 72 kJ/kg of protein assuming 2.1% protein content on its dry matter [6]. The value is very high for a conformational transition, also compared to other protein sources such as myofibrils from chicken meat protein with 11.7 kJ/kg [40], protein from pork meat with 10.7 kJ/kg [41]. Therefore we think it is more realistic to assign the appearance of the peak to another event that is related to cell membrane integrity destruction, such as stress relaxation of the polymers in the cell walls.

Their small relative amount in the dry matter of mushrooms show that proteins are not a major component contributing of osmotic binding of water in the gel phase. Still, they might be involved as structural components – working effectively as crosslinkers.

4.3.2 Cell wall structural components:

Effects of minor components, such as proteins in the cell wall, on WHC can still be specifically targeted via enzymes. The action of the hydrolysing enzymes on the biopolymer network might be of two types, as is shown in figure 4.6.

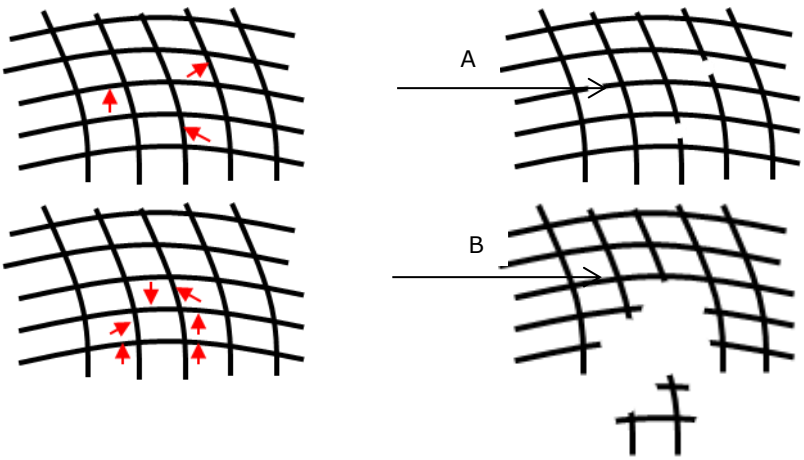

Fig 4.6 Two types of hydrolysis of polymers by enzymes. The red arrow shows the site of enzyme attack.

First, enzymes hydrolyse biopolymers randomly that creates dangling ends. The newly formed sites have newly formed hydroxyl groups, which can bind more water via hydrogen bonding. This will make the WHC to increase WHC after enzymatic hydrolysis. The second type of action can be that enzymes hydrolyse the polymer network more severely that causes a fraction of polymers detach to from polymer network. The hydrolysed fraction of the biopolymer network end up largely in the supernatant. Consequently, the amount of matter in the pellet decreases, leading to a decrease of WHC after enzyme treatment.

For the enzyme treatment, both the intact and the milled samples are used. In a milled sample, all the water fractions are mixed up because the cell membrane integrity is lost. The WHC will represent only the water osmotically bound to the cell wall components and solutes. Enzyme hydrolysis of the biopolymers in the intact sample can influence both osmotic binding and the mechanical strength, as it can also impact the crosslink density. It is quite probable that the effects of enzymatic hydrolysis on the osmotic binding is similar for the intact sample, and for the milled sample. Hence, via comparing the difference responses of samples with respect to the controls, one can have an indication of the effect of the specific biopolymer on the mechanical strength, i.e. crosslinking of the biopolymer network.

Experiments have shown that for both the intact tissue and milled tissue, β-glucanase has not given any difference in WHC compared to the control and hence the results are not presented in a graph. This finding is in contrast to previous reports that the glucan content is related to the hydration of filaments in fungi [42]. β-glucanase used in our study could only target β-(1,3)-glucans which is reported to be the main structural constituent [30]. The structural diversity and complexity of fungal polysaccharides is high and it also contains other polysaccharides having other types of bond system such as α-(1,3), α-(1,4), β-(1,6) as well [26, 30, 43]. Those bonds are not hydrolyzed by the glucanase used in this work. We therefore, do not disregard the contribution of the whole glucan system on WHC, but view our result as possible incomplete hydrolysis of glucans. The WHC of the samples that are treated with enzyme solutions to hydrolyse one specific constituent of the mushroom tissue, is shown in figure 4.7.

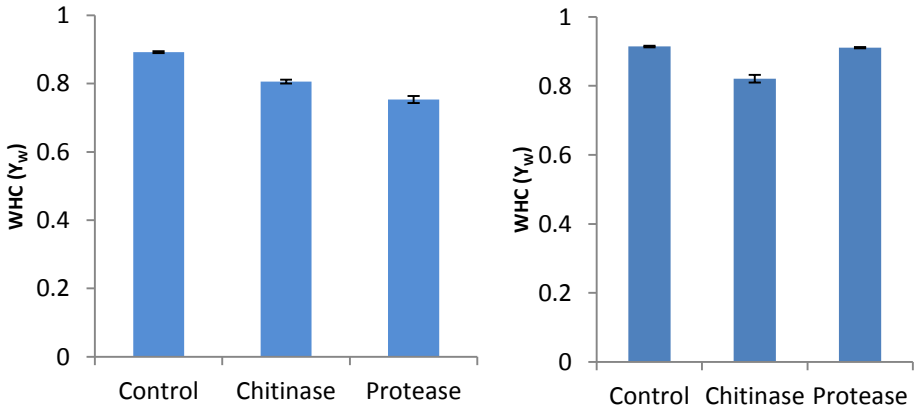

Fig 4.7: WHC expressed as water fraction of enzyme treated intact mushroom samples. The samples were centrifuged at 4800g for 60 minutes **Left**: Intact sample **Right**: Milled sample

For the intact sample which was subsequently given enzyme treatment (**Fig 4.7, Left**), a loss of WHC is evident when the sample is treated with protease or chitinase. The hydrolysis and subsequent solubilisation of cell wall biopolymers in the intact sample may have two effects. First, the mechanical strength of the cell wall may be reduced, which might make the cell membrane more vulnerable to rupture when a centrifugal force is imposed. Second, when the polymers are hydrolyzed to smaller units (figure 4.6 B), the fraction and water bound to it will be expelled in the supernatant. On the other hand, on the milled sample (figure 4.7, Right) the WHC values represent only osmotically bound water with the gel phase. Here, only chitinase treated sample shows lower WHC.

As for the protease only the intact sample has shown a decrease in WHC, while the milled sample has similar WHC as its control sample, we think that the proteases impact is more on the mechanical strength of the cell wall than on the osmotic binding. From these results, together with the above results from DSC, we conclude that proteins in cell walls are a minority compound little involved in osmotic binding of water in the cell wall, but is more involved in the crosslinking of the biopolymer network.

Because the action of the chitinase has led to a decrease in both the intact and milled samples, we conclude that chitin is involved in both osmotic binding of water, as well as in the mechanical strength of the cell wall. Chitin is a semi-crystalline biopolymer. Similar to cellulose in plant cell walls the crystalline part act as a microfibril enhancing the strength of the cell wall, and the amorphous part of chitin can be expected to have good binding of water, as being a polysaccharide likewise beta-glucan and pectin.

A last method for investigating the protein contribution is via change of pH. Being a polyelectrolye, proteins affect the water holding capacity upon change of pH. This effect we have investigated via vacuum impregnation of mushrooms with the isotonic fluid, but with adjusted pH. Figure 4.8 shows the change in the degree of hydration of mushroom at different pH values for hydrated mushroom and the heat treated mushroom. We note that the given pH value holds for the the impregnation liquid. The resulting pH in the mushroom tissue after vaccum hydration might be different because of the mixing with the fluid held in the mushroom (having a pH of 6.3) and buffering effects of the phosphate salts.

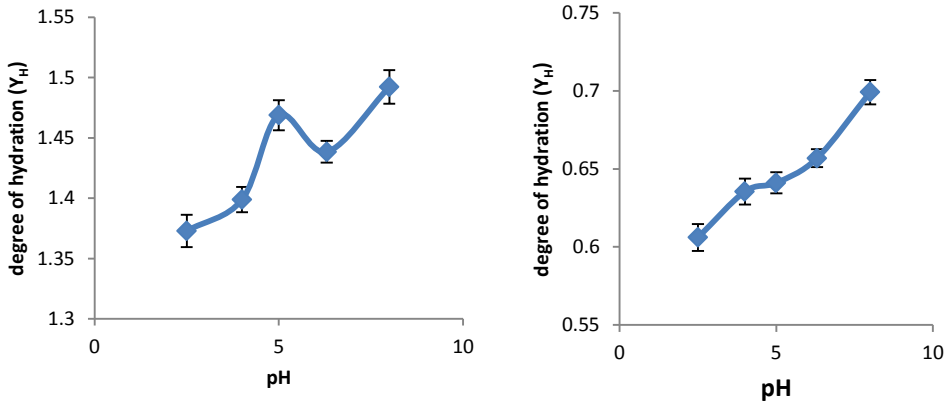

Fig 4.8: The change in degree of hydration of **(Left)** vacuum impregnated mushroom and **(Right)** vacuum impregnated and heat treated mushroom with pH

For both the non-heat treated and heat treated samples, hydration increases with an increase in pH of the bath. A local maximum is apparent for vacuum impregnated samples at pH 5. The overall trend is that WHC increases with pH. This trend

has also been reported by Zivanovic et. al [43]. The increased WHC of mushroom is attributed to increased electrostatic repulsion between proteins. The other biopolymers in mushrooms (chitin and glucans) are neutral, and they can not be charged via pH change. Similar to polyelectrolyte gels (Marcombe) the increase of electrostatic repulsion creates a wider mesh in the polymer network, which can accomodate for more water – thus leading to an increase of WHC. From the perspective of the behaviour of polyelectrolyte gels the local maximum appearing at pH 5 is difficult to explain. Hence, currently we view that as an experimental anomaly, which requires further investigation.

From the change in WHC due to pH treatment, as shown in figure 4.8, we conclude that for at least 15% of the retained water proteins are involved in the WHC. As both DSC and enzyme treatments have shown that proteins are a minority compound, we are inclined to conclude that the major contribution in the irreversible loss of WHC after heating is due to the loss of cell membrane integrity. The loss of cell membrane integrity is complete at temperatures of 70°C, which is the temperature normally used for blanching or pasteurization in mushrooms and vegetables. Thus, via processing one has little influence on the loss of cell membrane integrity, and its associated loss of WHC must be considered as inevitable.

4.4 Conclusion

In the current work, we have investigated the water holding capacity of mushroom in relation to change in cell membrane integrity and the state of biopolymers present in the cell wall. We show that the loss in water holding capacity after heat treatment is largely correlated with cell membrane integrity loss. The remaining WHC after heat treatment is due to osmotic binding of water to solutes and biopolymers present in the cell wall. From the DSC results follow that the effect of protein denaturation on the osmotic binding of water is minor effect. Hence, the osmotically bound water can be assumed to be quite insensitive to temperature. Via enzymatic hydrolysis we have shown that both chitin and protein are involved in WHC, but only chitin is shown to have a significant contribution to the osmotic binding of water. Proteins might be more involved in the mechanical strength of the cell wall. The contribution of protein

also manifests via their behaviour as polyelectrolyte, as shown in the significant change of WHC towards a shift in pH.

Acknowledgement:

We thank C4C holdings B.V for the financial support to carry out this research. Thanks are also due to Caroline van der Horst from C4C holdings BV for providing valuable comments on the manuscript, and to Herman Beukelaer Debeukelaer from Food and Biobased Research (FBR) group of Wageningen University for his support to carry out the DSC measurement.

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CHAPTER 5

More efficient mushroom canning through pinch and exergy analysis

This chapter is submitted as *Paudel E., van der Sman R.G.M., Westerik N., Ashutosh A., Dewi P.C.B., Boom R.M. More efficient mushroom canning through pinch and exergy analysis*

Abstract

Conventional production of canned mushrooms involves multiple processing steps as vacuum hydration, blanching, sterilization, etc. that are intensive in energy and water usage. We analyzed the current mushroom processing technique via pinch and exergy analysis, plus three alternative scenarios. The product yield, utility use, exergy loss, and water use are used as sustainability indicators. Whilst re-arrangement of the production process could maximally save up to 28% of the heat input and up to 25% of the water usage, the most important improvement is obtained by re-using blanch water, which improves the overall yield of the preservation and canning process by 9%, also saving water and exergy use in the production.

Keywords: Pinch analysis, exergy, canned mushroom production, sustainability

5.1 Introduction

The production of canned food products is energy intensive, since these foods need to be blanched, sterilized and cooled. Canning mushroom involves vacuum hydration of the highly porous mushrooms, blanching and sterilization that are energy and water intensive. It is therefore useful to investigate alternative process schemes that may reduce the use of energy and water. In the current study, we use pinch and exergy analysis to assess the efficiency of the existing canned mushroom production process, and that of some possible alternatives.

Pinch technology summarizes the distributed use of a resource such as heat in a complex process in one simple diagram. The insights resulting from this can be used for designing a new process system, or for modification of an existing process [1]. The analysis determines a pinch point based on the minimum driving force (with heat, the minimum temperature difference ΔT_{min}) that is needed to achieve a certain rate of transfer between streams. The minimum amounts of the external utilities in a production system, are estimated with the help of a quality-quantity diagram (in case of heat, temperature–enthalpy) known as the composite curve.

While the simplicity is the greatest strength of pinch technology, it deals with one process resource at a time, such as heat, mass, water etc., and cannot deal with several resources at the same time [2, 3]. For example, heat pinch only uses temperature as quality parameter neglecting other parameters such as changes in pressure and composition of the streams, which often are quite important in food production.

Exergy analysis can do this. The monitoring of the use of resources through heat and mass balances, based on the first law of thermodynamics, has been used for several years to reduce both the use of energy and resulting environmental emissions. Feng & Zhu [1] called this approach "at the best incomplete and at the worst wholly incorrect" as this does not consider that resources have different qualities at different stages.

The quality of a resource can be expressed in terms of its ability to be used, or in terms of its capacity to do work, by exchange with the environment. As an example,

the quality of heat depends on its temperature: the higher the temperature it has, the more can be done with it. If a high-quality heat source is used to heat another stream, the temperature is reduced, and its quality is decreased. Energy sources such as electricity, and chemical energy have a higher ability to do work than the energy contained in other sources such as the exhaust heat that is emitted through a chimney [4]. Likewise, also material streams and other resources can be assigned an exergetic content, which also give a quality to these streams. Unlike pinch technology, exergy analysis does not identify solutions but can be used to compare the exergetic efficiency of alternative scenarios [5].

The evaluation of the process efficiency by using pinch or exergy analysis is relatively novel in the domain of food processing, but well established in other fields, such as the chemical industry, and mechanical and energy engineering. In this work, both techniques are used to evaluate the efficiency of the use of resources. We typically see that the techniques complement each other well.

First, we analyze the current canned mushroom production system, via a detailed analysis of the mass flows, plus the exergy flows during the production of canned mushrooms. Each unit operation is evaluated both in terms of their energy and exergy efficiencies. We then propose three different alternative routes for canned mushroom production. The production yield and quality of each scenario is estimated under pilot scale process simulation experiments. The yield value of each alternatives is used to calculate the amount of raw mushroom that has to be processed to get the same amount of final product. The minimum heating and cooling requirements to process raw mushroom for each of the alternatives are evaluated using pinch technology. Similarly, water and exergy need of the alternatives are also calculated. The production scenarios are compared based on their yield, water consumption and exergy losses.

5.2 Methods and materials

The data of the current production of canned mushroom used in this study was obtained from Lutèce, located in Horst, The Netherlands. For process simulation experiment, white button mushrooms (*Agaricus bisporus*) with a cap diameter of 4-5

cm were used for all the production simulation experiments. The mushrooms were received within 2 hours of their harvest. The blanch water was obtained from Lutèce. The brine solution contained 2% sodium chloride and 0.5% citirc acid (pH 3) [6].

5.2.1 Process simulation experiments

Mushrooms were vacuum impregnated with water at room temperature after their receipt. The mushrooms were first evacuated in a vacuum chamber below 100 mbar. The mushrooms were then hydrated by supplying tap water into the chamber and releasing the pressure. The mushrooms stayed immersed for 5 minutes for sufficient hydration. The vacuum hydrated mushrooms were then blanched at 90°C for 15 minutes by submerging them in a temperature controlled water bath. After blanching, the mushrooms were cooled to room temperature in cold tap water. The mushrooms were sliced and filled in a 300 ml cylindrical glass jars with 2.8 cm diameter and 12 cm height. A brine solution was added to achieve a 1:1 volume ratio of sliced mushroom and brine water. Finally, sterilization was carried out at 126°C for 15 minutes. The canned mushrooms were allowed to stand for one day before their drained weight was measured. The contents of the cans were drained in a sieve for 2 min. After draining, the remaining weight was measured, and expressed in the yield of canned mushrooms as

$$\%yield = Y_b \times Y_c \qquad [5.1]$$

In which Y_b is the weight ratio of blanched mushroom to the fresh mushroom and Y_c is weight ratio of canned to the blanched mushroom.

5.2.2 Pinch analysis

Pinch analysis on heat was carried out following the procedure described in literature [7-9]. All streams were divided into two types: hot streams that should be cooled down, and cold streams that should be heated to a certain temperature, based on the change of enthalpy required in these streams. The minimum temperature difference for heat transfer (ΔT_{min}) was selected to be 10 °C to ensure an adequate driving force for heat transfer. A Grand Composite Curve (GCC) was constructed in a temperature–enthalpy (T-H) diagram, which was used to estimate the minimum

heating and cooling utilities needed to produce the canned mushroom in the current situation and under the various proposed methods.

5.2.3 Exergy analysis

The total exergy of a stream is the sum of its chemical and physical exergy. The chemical exergy is the exergy that is involved in creating the chemical components from a standardized environment, while the physical exergy includes the exergy that is needed to bring those substances to their temperature, pressure and mechanical conditions, starting with those in the standardized environment. In our standardized environment, we adhered to the definitions by Szargut [10], choosing a temperature and pressure of 25°C and 1 bar, respectively.

The chemical exergies were calculated as:

$$B_{Chem} = \Phi \sum \left(b_{o,i} x_i\right) \quad [5.2]$$

in which $b_{o,i}$ is the specific chemical exergy (MJ/kg) of components, x_i is the mass fraction of the components and Φ is the size (flow rate) of the stream. The values of $b_{o,i}$ are adopted following Szargut [10].

The thermal exergy is calculated following [11] with

$$B_T = \Phi\ Cp\ \left[(T - T_0) - T_0 \ln\left(\frac{T}{T_0}\right)\right] \quad [5.3]$$

in which B_T is defined as the exergy due to a different temperature than that of the standardized environment, Cp is the specific heat capacity, T is the temperature of the stream, and T_0 is the temperature of the standardized environment (25 °C). The pressure exergy (of for example steam) was calculated as:

$$B_P = \Phi\ \frac{RT_0}{M_W}\ ln\left(\frac{P}{P_0}\right) \quad [5.4]$$

in which R is the universal gas constant, P is the pressure of a stream, P_0 is the pressure of standardized environment (1 atmospheric pressure) and M_W is the molecular weight of substance.

In principle, the exergy change involved in mixing the chemical compounds needs to be incorporated, however, in systems consisting of mostly biopolymers, its value is very low compared to that of other contributions, and we may safely neglect it in our calculations. Only in the brine solution the exergy of mixing is significant, and there was calculated as:

$$B_M = N_{tot} R T_0 \sum \left(x_i ln(a_i) \right) \quad [5.5]$$

with N_{tot} the total number of moles in the stream calculated from the molecular weight and a_i the activity of component i, which is calculated by finding the activity coefficient using the extended Debye-Hückel relation [12] as described earlier [13].

5.2.4 Sustainability indicators

The unit operations involved in the current production of canned mushroom were evaluated with thermodynamic law efficiencies; the so called first law efficiency (FLE) and the second law efficiency (SLE) as follows.

$$FLE = \frac{\text{theoretical energy need}}{\text{actual energy input}} \quad [5.6]$$

The FLE values of unit operations calculated in the current production system are used to calculate the utility requirement in all the scenario evaluated, assuming a constant FLE for the unit operation.

$$SLE = \frac{\text{Useful physical exergy output}}{\text{Physical exergy input}} \quad [5.7]$$

In our analysis, we only used the physical exergy in calculation of SLE [1] as the chemical exergies did not change in any way during any of the processes

investigated, since no molecular bonds are destroyed. The chemical exergy is not involved in any exchange of heat or mass with other streams and it remained constant in all the scenarios that are investigated in current work. In equation 5.7, the term useful exergy refers to an exergy output in a stream that is used in subsequent operations. The SLE is used to assess the thermodynamic compatibility between the streams, which is discussed further in the results section.

In addition to finding the FLE and SLE of each unit operation in the current production system, the sustainability in different production scenarios was measured in terms of product yield, water use and exergy use. The values are expressed as relative values compared to current mushroom processing. The exergy indicators used are the total exergy loss (B_{Loss}) and the irreversible exergy loss or exergy destruction (B_{Dest}), as used in previous studies [4, 5]. The total exergy loss includes the exergy destruction and the exergy lost with the discarded streams such as cooling water or steam which emitted to the environment without further use.

$$B_{Dest} = B_{In} - B_{Out} \quad [5.8]$$

with B_{In} the total exergy input in a unit operation and B_{Out} the total exergy output. The total exergy loss is expressed as:

$$B_{Loss} = B_{Dest} + B_{Waste} \quad [5.9]$$

in which B_{Waste} represents the exergy loss in the waste stream.

5.2.5 Assumptions

The following assumptions have been made in this study

1. The efficiency of the refrigeration system used to cool mushroom after their vacuum hydration to the storage temperature is assumed to be 30%.

2. For the calculation of energy, exergy and water requirements, the same FLE values of the unit operations in the current production system are used in the other scenarios.

3. The heat capacities of the different streams are constant over the temperature ranges considered.

4. Waste streams (condensate, vapour, hot cooling water, blanch water) are emitted to the environment without additional processing.

5.3 Results and Discussion

5.3.1 The current production system

The canning of mushrooms starts with their vacuum hydration. The mushrooms are hydrated using the technique described elsewhere [14–16]: mushrooms are evacuated below 100 mbar, and immersed in water during the release of the vacuum. The hydrated mushrooms are then stored for 24h at 4 °C, which is known to increase the yield of the final product [17, 18]. As explained elsewhere in literature [15, 19–24], mushrooms are blanched in a bath of nearly boiling water for 15 min. Though the temperature of this bath in practice changes from the entrance towards the completion of the blanching, we assume that the temperature remains at 90°C during the whole blanching process. The blanched mushrooms are then cooled to room temperature in a cooling tank, which is also used as buffer storage for the remaining steps. The cooled mushrooms are sliced and filled into cans or jars. A brine is added to the mushroom in the cans, keeping a small headspace. The cans or jars are closed and sterilized at 135 °C for 15 minutes. Finally, the jars or cans are cooled down to 90°C with pressurized cold water and further cooled to room temperature by environmental air.

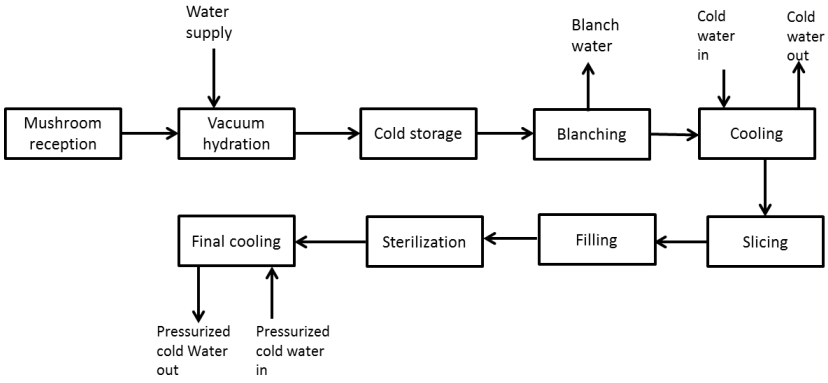

Fig 5.1: Schematic flow diagram of the current production of canned mushrooms

The flows of mass and exergy in the production of the canned mushroom are shown in figure 5.2. The mass flow diagram shows the relative changes in mushroom mass throughout the system, due to the addition and removal of moisture, mostly in the vacuum hydration and during blanching processes. Even though sterilization also removes water from the mushrooms, this water remains contained within the can or bottle, and it stays therefore in the product and is not lost. We choose to show the whole can or bottle of mushrooms as the product and hence, the width of the arrow remains the same after the filling. The exergy losses in processes where steam is used are considerable.

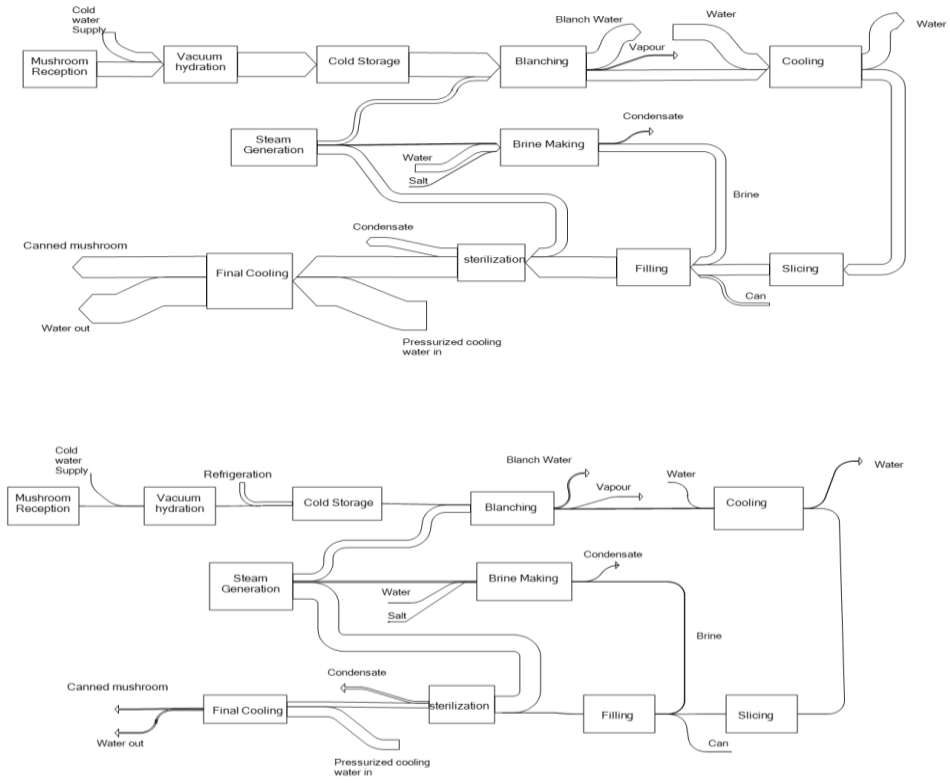

Fig 5.2: The Sankey diagram of mass (top) and physical exergy (bottom) flow of the current canned mushroom production system. The widths of the arrows show the size of the mass or exergy of the streams. In the exergy Sankey, also called Grassmann diagram, the difference between the ingoing and outgoing arrows for any

unit operation shows the loss of exergy during that operation.

Table 5.1: The first law efficiency (FLE), second law efficiency (SLE) and relative exergy loss of unit operations in the current process. The relative exergy loss is the percentage of exergy loss compared to the total exergy loss in the production

Unit operations	FLE (%)	SLE (%)	Relative exergy loss(%)
Vacuum hydration	94.5	15.1	2.3
Storage	30.0	11.1	2.5
Blanching	97.1	7.2	19.6
Cooling	84.1	5.5	5.1
Slicing	93.7	49.0	0.3
Filling	82.8	42.3	2.9
Sterilization	29.2	28.4	28.1
Final cooling	51.1	6.8	38.9

Table 5.1 shows the first and second law efficiencies of the current mushroom production. We describe the differences between FLE and SLE with blanching and sterilization, which both use steam as a source of heat. Both are the unit operations that consume a significant amount of heating utility as shown in figure 5.2. The FLE of blanching is quite high, since it works by steam injection: steam of high temperature (170 °C) and pressure (9 bar) is injected to heat the system to 90 °C at atmospheric pressure. The quality of the steam is however mostly lost in this, as the steam has a specific exergy of 876 kJ/kg steam, while the specific exergy of water at 90 °C is 80 kJ/kg water, or less than 11% of its original content. Using high-pressure steam to directly heat water at this low temperature, therefore wastes most of its potential. Despite a high FLE, the SLE of this unit operation is thus quite low.

During sterilization, again high quality steam is used to heat mushroom in a pressurized chamber, but now to a temperature of 130 °C and pressure of 1.5 bar. This improves the SLE by nearly a factor 4 compared to the blanching step. The heat transfer taking in this case is an indirect transfer via the use of a heat exchanger; this mode of heat transfer reduced the FLE by a factor 3. The table also shows the relative exergy loss of unit operations compared to total exergy loss in the system.

With the biggest exergy loss and lowest SLE value, the final cooling of the jars is the least sustainable processing step, followed by blanching and sterilization. Since cooling step is present in each scenario as long as sterilization by heating is required, we do not consider improving efficiency of this step in this work, even though additional savings could be made with it.

5.3.2 Alternative scenarios

In the following section we assess three alternative scenarios that may lead to more efficient canning of mushrooms.

Scenario 1: Slicing before vacuum hydration

In this scenario (figure 5.3) the sequence of steps is changed, enhancing heat transfer rates, and eliminating intermediate cooling and heating. The mushrooms are now sliced immediately upon their arrival. The sliced mushrooms are then vacuum hydrated in cold water and stored, blanched as before, and hot-filled without intermediate cooling, as is done in the current production system. The hot-filled cans are sterilized and subsequently cooled. An important change is that this scenario assumes no cooling in between the blanching and sterilization.

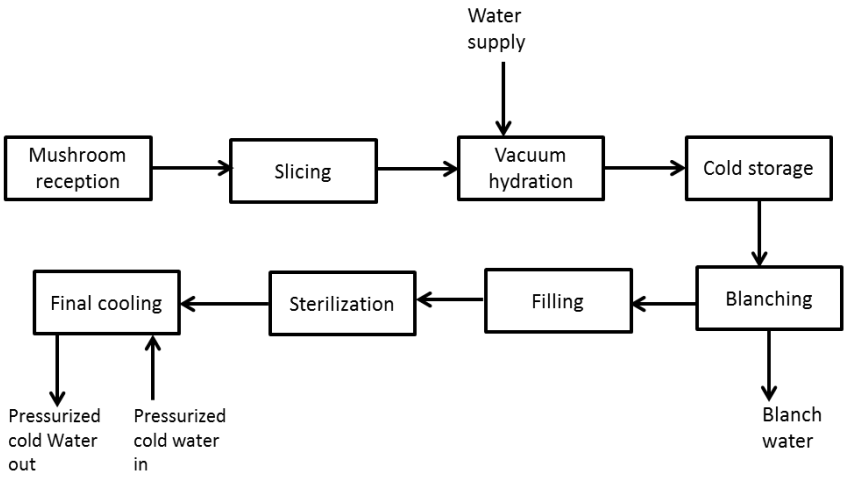

Fig 5.3: Canned mushroom production by scenario 1

Scenario 2: Vacuum hydration with hot water

In this scenario (figure 5.4), vacuum impregnation and blanching are combined in one step. The fresh mushrooms are evacuated at pressure below 100mbar and hot water is supplied to the chamber serving both as hydration and blanching fluid. The water supply to the chamber is first pre-heated by exchanging heat with blanch water, and is further heated to 95°C in a chamber with steam injection. The blanched mushrooms are then cooled in a water bath, sliced, filled in cans, sterilized and subsequently cooled as usual.

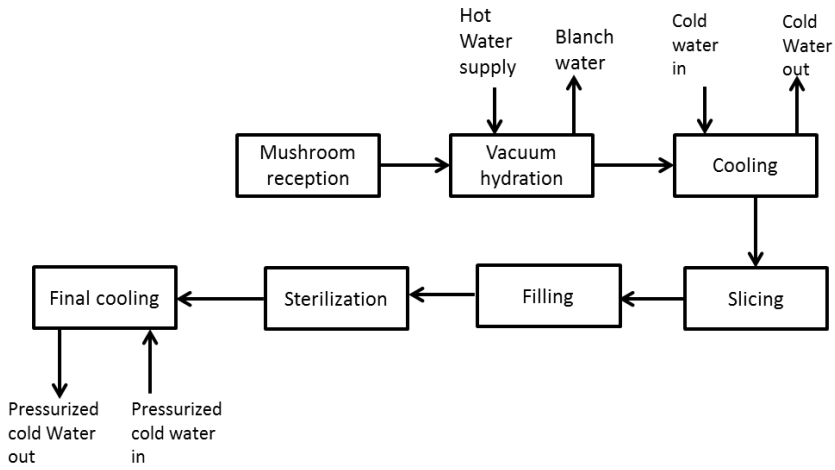

Fig 5.4: Canned mushroom production by scenario 2

This scenario requires hot water at a temperature of 95 °C and 2 times the volume of the mushrooms, serving both as hydration and as blanching fluid. This implies a maximum blanching temperature of 75 °C without external heating. The water used for hydration is first heated to a temperature of 65 °C by exchanging heat with the blanch water; which then was heated further to 95 °C in a chamber with steam injection. In fact, one of the prime aims of the blanching process is to pre-shrink the mushrooms, such that this will not take place after capping of the jars during the sterilisation process, and a lower blanching temperature of 75 °C was found to shrink mushroom sufficiently [15, 22, 23], and therefore one might consider to exclude the additional heating.

Scenario 3: Blanch water as a hydration fluid

In this scenario (figure 5.5), the re-use of the blanching fluid is considered, which could reduce the use of water, but might also increase the yield of the mushrooms, as it would re-introduce the components that were solubilised during blanching, into the mushrooms. The mushrooms are vacuum impregnated with cool blanch water keeping the rest of unit operations the same. The water produced in the blanching step is first cooled in a counter-current heat exchanger, augmented with external cooling by the water that is then used for making the brine (Cooling I). Thus, it also reduces the heat load needed to heat the brine. The blanch water is further cooled by mechanical refrigeration (Cooling II) to 10°C, which is the regular temperature of vacuum hydration fluid. The cooled blanch water is then used for hydration.

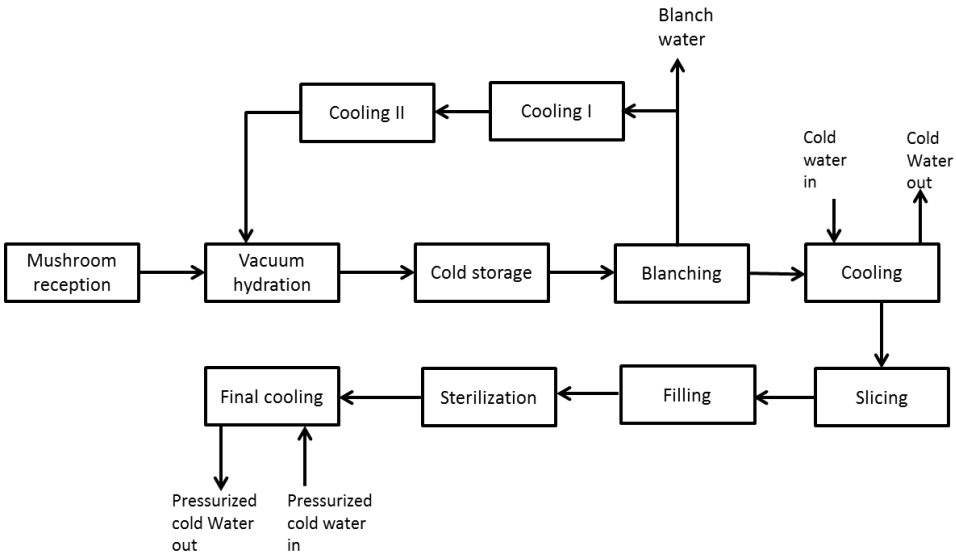

Fig 5.5: Canned mushroom production scheme by scenario 3

The cold water used for making the brine is used to cool the blanch water to a temperature of 22°C ; while at the same time heating the brine to 73°C .

5.3.3 Impact on the yield of product

The impact of the different scenarios was investigated on pilot scale. The yield of the processed mushroom produced under the various scenarios is shown in figure 5.6.

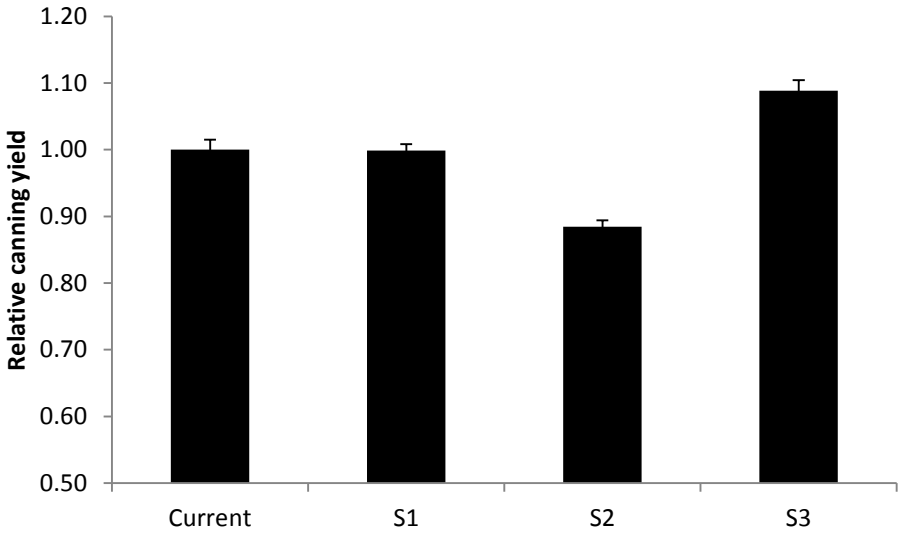

Fig 5.6: The relative canning yield in different scenarios in comparison to the yield in the current process.

An analysis of the yield of the final product with a paired t-test ($p<0.05$) showed a significantly lower yield for scenario 2 and no significant difference in the yield for scenario 1, relative to the current production scheme. In contrast, scenario 3 leads to a significantly higher yield. The reason for this is mostly the re-introduction of the solids into the mushrooms, that are lost during blanching. This may be compared to the increase in canning yield in mushrooms that were infiltrated with solids such as xanthane gum, rice starch, egg protein [25], casein, milk albumin, egg white and carageenan [26]. This therefore represents a true gain in productivity. Therefore scenario 2 requires 14% more mushrooms than the current process, while scenario 3 requires 8% less mushrooms than the current process to obtain the same amount of final product; while there is no difference for scenario 1.

No sensory tests were carried out in this work, so any sensorial differences in canned mushrooms were not noted, except for scenario 3 where the mushrooms became darker in colour because the blanch water had a coffee-brown colour, due to components resulting from enzymatic browning, which leached out of the mushrooms during blanching.

5.3.4 Pinch analysis:

The current heating load of all cold streams together is 8717 kW and the total cooling load of hot streams is 7178 kW. In the current production system, steam is used as a heating utility while cooling water and refrigeration is used for cooling.

Figure 5.7 shows a grand composite curve (GCC), and temperature –enthalpy (T-H) plot of current mushroom production. The pinch analysis of the current mushroom processing shows the potential of saving in the heating and cooling utilities.

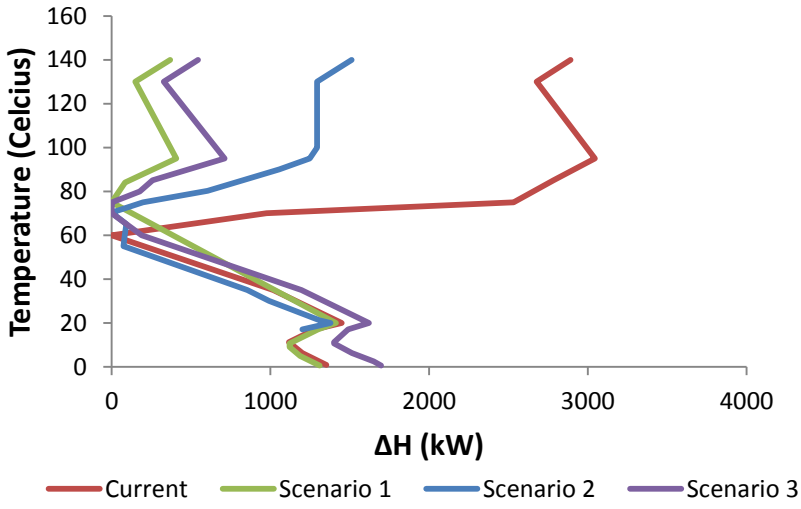

Fig 5.7: The GCC obtained from pinch analysis of current mushroom processing (red), Scenario 1 (Slicing before vacuum hydration, green) Scenario 2 (vacuum hydration with hot water, blue) and Scenario 3 (vacuum hydration with blanch water, purple).

Under the pinch, heating requirement are more than the cooling requirement in the current process. Remarkably, the alternative scenarios, mainly could reduce the use of heating duty under the pinch condition while cooling duty are not much affected. This shows that in terms of heating utility need, the resources are better aligned in the scenarios compared to current process. Total heating and cooling load, and the utility need under pinch as the fraction of total loads, are shown in figure 5.8. Figure 5.8 shows that under the pinch, heating and cooling requirements in the plant decreased up to 52% and 80% respectively in current process. Similarly, the resource need are decreased substantially in the other scenario as well.

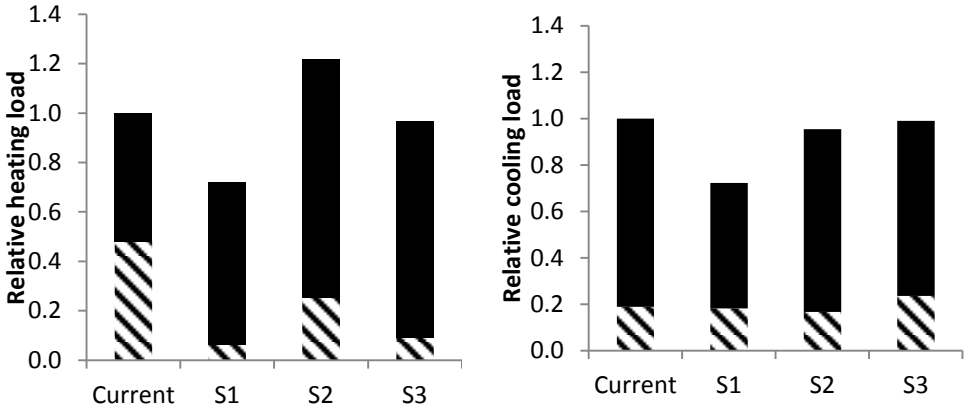

Fig 5.8: The heating and cooling requirement of different scenarios as the fraction of those requirements in the current process. The patterned lower part of the graph shows external utility requirements under the pinch (i.e., the lowest possible heating or cooling load necessary).

As shown in figure 5.8, pre-slicing the mushrooms before their vacuum hydration (scenario 1) decreases the requirement of external cooling by 28%, since the mushrooms do not need to be cooled down after blanching. This also saves 28% on the heating requirements as well. Using blanch water for hydration (scenario 3) decreases the heating load by 3% and decrease the cooling load by 1%. Use of hot water for vacuum hydration (scenario 2) actually increases the requirement of heating load by 23% mainly because large volume of water needs to be supplied for vacuum hydration as supplied water also has to work as heat transfer media. This

approach reduces the need of cooling as there is no refrigeration requirement after vacuum hydration.

Of course, a pinch analysis does not take any constraints into account that may arise from safety or regulatory considerations, nor does it take into account that more complex equipment comes at a price. It does however indicate the potential that is present in re-using heat, and therefore may be used a starting point for a further, more comprehensive analysis.

Water use

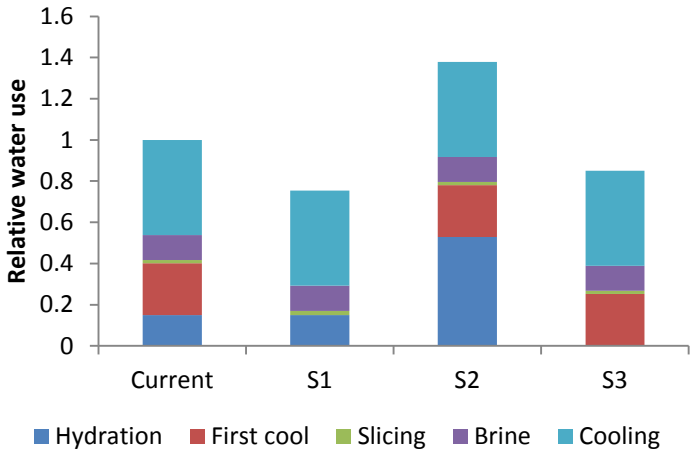

Fig 5.9: Relative water requirement for various unit operations compared to the total water requirement of the current process.

The water use in various scenarios is shown in figure 5.9. It is lowest for scenario 1 followed by scenario 3. In scenario 1, the mushrooms are canned immediately after blanching, which saves cooling water, while in scenario 3, no fresh water is needed for vacuum hydration as the mushrooms were vacuum hydrated with cooled blanch water. In scenario 2, however, more water is used. This is not only because more hot water is needed for vacuum hydration and blanching than in the current process, but also because more mushrooms have to be processed to obtain same amount of final product as in current process. The hot water supplied for the combined vacuum

hydration and blanching is around 3 times more than the normal supply of water used in the impregnation, which increases the overall demand of water for scenario 2. The decrease in water demand in scenario 3 is both because there is no need for external water supply for hydration and also because less mushrooms have to be processed compared to the current process.

5.3.5 Exergy

The relative exergy losses of various mushroom processing scenarios are shown in figure 10. Vacuum hydration, cooling, slicing and brine filling, in total consume less than 5% of the total exergy loss and therefore are shown as 'others' in figure 5.10. As shown in figure 5.10, blanching, sterilization and final cooling consume relatively large amount of exergy. The inefficient use of the exergy in the steam and in the cooling water is evident in the exergy losses in those steps: blanching, sterilization and cooling share 20, 28 and 39% of the total exergy loss in the production. Table 5.1 shows that the SLE of those unit operations are 7, 28 and 7 % respectively, which indicates that significant improvements could be made by using heat sources that are better aligned with the requirements.

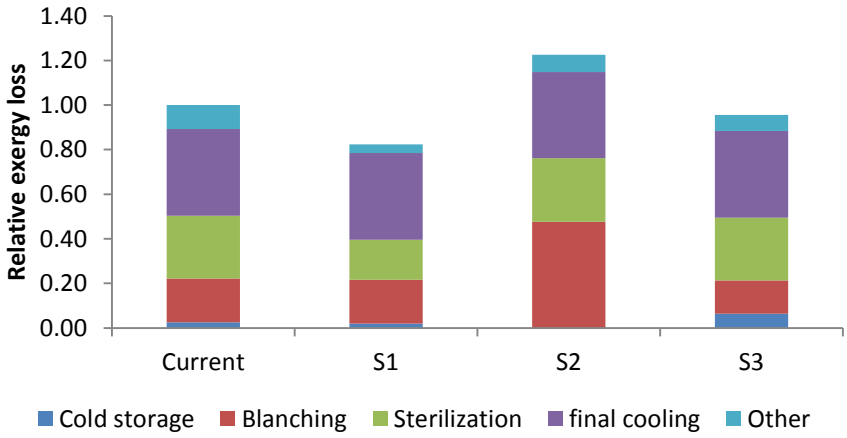

Figure 5.10: Relative exergy losses of various unit operations compared to a total exergy loss in current processing for various scenarios

Scenario 1 (slicing before vacuum hydration) showed the lowest exergy use followed by scenario 3 (using blanch water for vacuum hydration). Scenario 2 showed 22% more exergy loss than the current process. In scenario 1, the mushrooms are not cooled after blanching, and this lowers the total exergy loss by 14% compared to the current process as the heating in the sterilization step is reduced. A comparison of the exergy requirements for scenarios 2 and 3 from figure 5.10 and their product yields from figure 5.6 shows that the difference in the yield of the final product is an important aspect in the sustainability analysis. In scenario 2, though mushrooms do not need to be stored after the blanching treatment, the exergy loss in blanching for this scenario was larger as more raw mushroom has to be blanched because of the low final yield of canned mushrooms. This scenario therefore, shows a total exergy loss of 22% above the current process. On the other hand, despite an additional cooling step needed to cool the blanch water to the vacuum hydration temperature of 10°C, the overall exergy loss in scenario 3 is reduced by 5%, because 9% less mushroom has to be processed.

Fig 5.11 shows the total and the irreversible losses in the scenarios compared to the total losses in current process. In the current process, 73% of total exergy loss is irreversible loss or exergy destruction. The irreversible loss in exergy is the result of incompatible exergy transfer [4] between the streams, which can be reduced if exergy sources that are better aligned to each other are used as this would reduce the driving force for energy transfer. All the scenarios evaluated showed lower irreversible exergy loss compared to current process as is shown in the figure. This indicates that the streams in the scenarios are thermodynamically better aligned than the current process. In contrast, the total loss in scenario 2 increased compared to current process that shows that the exergy loss in the waste stream actually increased. This is because more raw mushrooms are processed in this scenario. In addition, a large volume of hydration water is used in this scenario as the water is also used as heat transfer medium. This made volume of blanch water 2.3 times bigger than the current process, and a large quantity of exergy is lost with this blanch water.

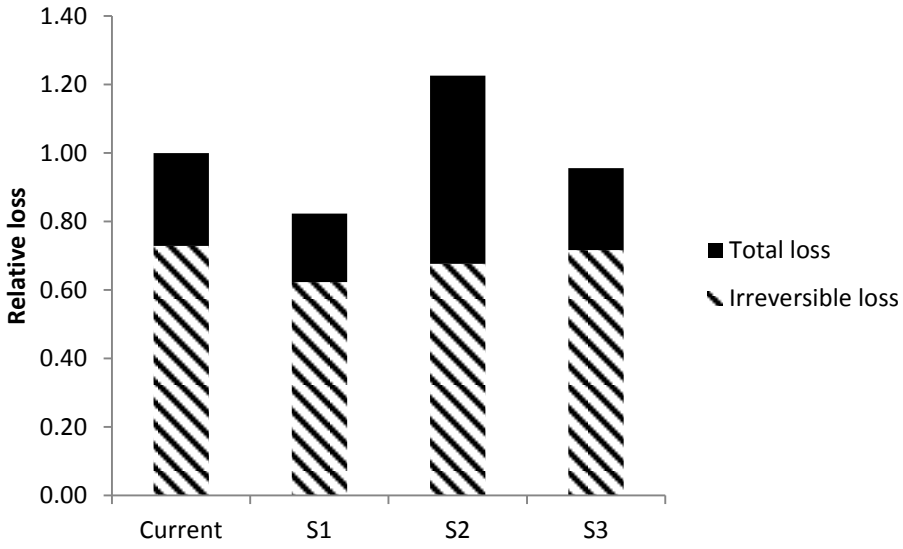

Fig 11: Irreversible exergy loss (stripped lower) shown as a fraction to total exergy loss in current production

5.4 Conclusion

Pinch and exergy analyses were applied on the current process for mushroom canning, and three alternative process schemes that may reduce the use of water and energy. The pinch analysis indicated that via more elaborated heat exchange under pinch, the required heating and cooling utility need can be minimized up to 52% less heating and 81% less cooling compared to current use of those resources. Elimination of cooling after blanching by slicing the mushrooms before impregnation could reduce the water usage by 25%. This also reduces the exergy loss up to 18%. Hydration of the mushrooms with hot water results in the least efficient process among the considered scenarios. Though, this scenario can eliminate the refrigeration need in the plant, it not only reduces the yield of the final product but also increases exergy loss by 22%, and increases the water demand by 37%. In contrast, re-using cooled blanching water for vacuum impregnation of the mushrooms, decreases its overall exergy use by about 4%, but at the same time improves the yield by about 9% and saves water use by 15%.

Acknowledgements

We are grateful to C4C holdings B.V., Gennep, The Netherlands for their financial support. Our sincere thanks to Caroline van der Horst and Geert Weijers (C4C holdings B.V., Gennep, The Netherlands); Jan Broeze (Food & BiobasedResearch, Wageningen, The Netherlands) and Filippos K. Zisopoulos (Food Process Engineering group, Wageningen, The Netherlands) for their valuable comments.

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CHAPTER 6

General Discussion

6.1 Outline

In this chapter the findings of this thesis are summarized and the future scope of the work is discussed. First, the main findings of the current thesis are summarized. Following that, a full thermodynamic description of hydrated mushroom is provided and the effect of thermal treatment on the hydrated mushroom is discussed. After that, the objectives of this thesis, understanding and improving the water retention in processed mushroom while assessing and improving the sustainability of canned mushroom production, are combined in the view of the conceptual process design (CPD) practices. The chapter is concluded with a discussion on the future perspectives of the findings in this thesis.

6.2 Main findings

The phenomenon of water holding capacity (WHC) loss in heat treated mushroom is interpreted with the Flory-Rehner theory in **chapter 2**. Here, we have assumed that the WHC loss in mushroom can be attributed to the protein denaturation as is done with meat. This assumption is based on the experimental observation that, like meat, the WHC of mushroom follows a typical sigmoid curve if plotted against temperature. In this chapter, mushroom is regarded as a lightly crosslinked, homogeneous biopolymer gel in an aqueous solvent that has salts and sugars dissolved in it. The thermodynamic interaction between the biopolymeric network and the solvent is characterized by the so-called swelling pressure, which is decomposed into three independent contributions; the osmotic pressure, the ionic pressure and the elastic pressure. Heating causes denaturation of the mushroom protein which weakens the interaction between water and protein. This is considered by assuming that the change of the Flory-Huggins interaction parameter for protein with temperature is similar to the change with temperature of the water holding capacity of mushroom at zero mechanical load. The temperature dependency of this parameter is tested with independent sorption measurements, which support this assumption. Comparison of the Flory-Rehner model with the WHC under various mechanical loads on the mushroom tissue shows that the other model parameters (the crosslink density N_c, and the polymer fraction in the relaxed state, ϕ_o) are temperature dependent as well: while ϕ_o increased, N_C decreased with the temperature, up to 70 °C. This implies that heating increases the polymer chain length between two crosslinks because the

original conformation of polymers is lost with the denaturation. At the same time, more cross links are formed because of the aggregation of polymers due to the denaturation. In the comparison of experiments with theory we have excluded data points at low external pressure values, where the capillaries are not yet collapsed, and are still filled with liquid. The cellular structure of a mushroom is more complex than just a homogeneous mixture of biopolymer in an aqueous solution as is considered in **chapter 2.** The fruiting body of the mushroom is composed of intertwined fibrous cells (hyphae), with a distinct pore phase in between the hyphae. The cells consist of cell wall, cytoplasm and vacuole, with each compartment separated by a membrane, impermeable to solute but permeable to water. The contribution of the cellular structure is considered in **chapter 3** and **chapter 4**. The contributions of the capillary water in the pore phase on the WHC of mushroom tissue is studied in **chapter 3**. There, we have shown that the porosity of the mushroom also contributes significantly to the WHC: the hydration of heated mushroom increases linearly with the initial porosity of the mushroom tissue. If mushroom is stored under cold condition prior to heating, the hydration of heat treated mushroom increases with the storage time. This effect can largely be explained by the increase in porosity during storage. Therefore, we conclude that the initial porosity of mushroom (prior to heating) is an important parameter for the final hydration of the heated mushroom. A complete thermodynamic approach to describe WHC should not only include a theory for the water holding in the gel phase of cell wall and cytoplasm, but should also take into account the capillary liquid present in the pore phase.

The influence of the cellular structural complexity of mushroom on the WHC is considered in **chapter 4**, and specifically the effects of cell membrane integrity, and the composite character of the cell wall, which is composed of several biopolymers. The loss of cell membrane integrity can largely explain the loss of water from the heated mushroom. The separate enzymatic hydrolysis of specific cell wall components shows that chitin and protein contribute to the water retention by the osmotic binding and/or by their role to provide mechanical strength to the cell. In addition, proteins, being polyelectrolytes, contribute to the water retention by the mushroom tissue also via electrostatic interaction – as evident by the response of WHC on the pH.

Chapter 5 examines the efficiency of the use of raw materials, energy and water (one aspect of sustainability) of the mushroom processing system with pinch and exergy analysis. In the current mushroom canning process, blanching, cooling and sterilization are identified as the unit operations that are the least sustainable in terms of their relative exergy efficiency. Based on concepts from Process Intensification, three alternative production system layouts are proposed and compared with the current system regarding the product yield, water use and exergy use: 1. slicing before vacuum hydration; 2. using hot water for vacuum hydration; and 3. using blanch water for hydration. Slicing mushroom before vacuum hydration lowers the water and exergy demand for the production because the cooling step after the blanching is eliminated. In this scenario, the final product yield remains unaffected. Using hot water for vacuum hydration of the mushrooms eliminates the need for refrigeration after hydration by combining the hydration and blanching operation at one, but it consumes more water and exergy for the production of certain amount of product. It also gives the lowest product yield. Using blanching water for the hydration of mushroom lowers the water and exergy demand for production of certain amount of final product, and it increases the product yield. However, it requires decolouring of the blanch water, or may have consequences for the quality (colour) of the product.

6.3 Describing water loss from mushroom

6.3.1 Mechanisms involved in water holding capacity loss in mushroom processing

In a structured food, the molecular interaction of the constituents with water and the structural make-up of the material are essential in the overall water retention of the material. In the current section the findings from previous chapters are combined. We will formulate the framework of a complete thermodynamic description for the WHC in mushroom, accounting for all compartments in mushroom containing water.

The cell membrane integrity loss is the largest factor associated with the reduction of WHC as a result of thermal processing. Upon the loss of cell membrane integrity the stretched cell wall will relax and squeeze out the water and solutes present in the vacuole. The water and solute will distribute over cell wall, cytoplasm and pore phase.

Equally important, other contributions to the WHC are 1) the stiffness of the hyphae and 2) the porosity of the overall tissue. The loss of the stiffness of the cell wall upon heating causes a collapse of the pore phase under mechanical load [1]. As vacuum impregnated mushroom hold liquid in the pore phase, the collapse will result in more water loss from the mushroom. This assumption is supported by the behaviour of other vegetable foods upon heating: carrot is less porous and does not lose as much water upon heating [2], while more porous onions [3] and apples [4] lose up to 10% and 20% of their original weight on thermal processing.

Cell wall components have a double contribution to the water retention by mushroom tissue. First, they bind water (mainly by hydrogen bonding) and second, they provide stiffness to the hyphae which acts against structural collapse, and thus allows water retention in the intercellular pores. Heating may affect the stiffness of the cell walls because of various reasons: a change in the configuration of the biopolymers by denaturation (proteins), melting of crystalline structure (in proteins and polysaccharides), and thermal hydrolysis of beta-glucans [5]. However, apart from changing the stiffness of cell wall, the heating also changes the osmotic binding of the *gel* phase due to protein denaturation.

Though the effect of thermal treatment on the *gel* phase is discussed in **chapter 2**, the *capillary* water and the intracellular water could not be completely removed from the samples. In Figure 6.1, the effect of the thermal treatment on a milled sample is shown. Milling destroys the cell wall, and also destroys the capillary (intercellular) pore space, so the water retention in this sample is primarily due to the retention by the cell wall constituents due to osmotic binding. The WHC of milled and heated samples is lower than that of the samples which were milled but not heated, for all external stresses (centrifugal forces) employed in this experiment. The results show that thermal treatment, indeed, lowers the water retention capacity of the *gel* phase as was assumed in **chapter 2**.

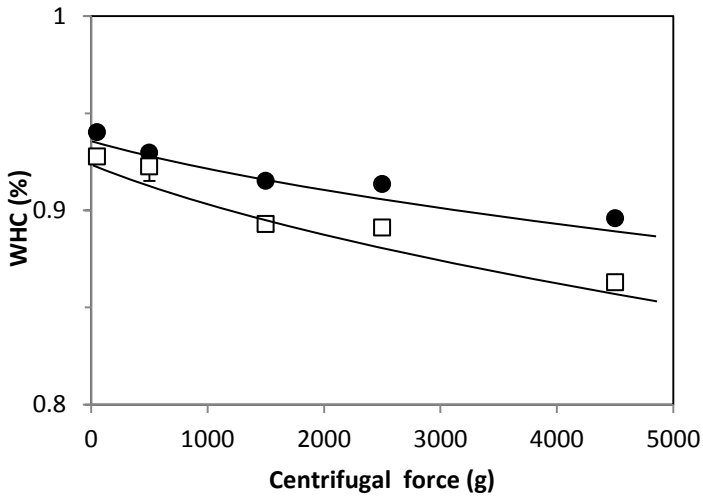

Fig 6.1: Water holding capacity of the milled sample before (circles) and after the thermal treatment (square). The lines are shown to guide the eyes.

6.3.2 Thermodynamic description of the hydration of mushroom tissue

In this section, we explore how a complete thermodynamic description of the water holding in mushroom can be synthesized, using the given fact that water is distributed in three different compartments: the vacuole, the *gel* phase, and the capillary phase as shown in figure 6.2. For the thermodynamic description we take three simplifying assumptions: 1) all biopolymers from cell wall and cytoplasm are thought to be part of the *gel* phase, 2) all solutes like sugars, salts and amino acids are thought to be dissolved in the vacuole, and 3) in fresh mushrooms the *gel* phase and vacuole are seperated by a single cell membrane, which is impermeable to solutes, but allows transport of water. The *gel* phase is assumed to be homogeneously crosslinked biopolymer network. If the cell membrane is intact, the solutes in the vacuole create an osmotic pressure, which will attract water, and make the vacuole and the cell wall swell until the osmotic pressure is balanced by the turgor pressure, excerted by the stretched biopolymer network of the *gel* phase. The capillaries are assumed to be filled with water, as happens during industrial mushroom processing via vacuum impregnation.

Below, we will give the conditions for thermodynamic equilibrium for both fresh mushrooms and heat treated mushrooms. From these thermodynamic conditions follows how water is distributed over the different compartments. We assume that in the heat treated mushroom the cell wall has lost its integrity, and proteins are fully denatured. Now the cell membrane is permeable, the solutes can diffuse and distribute over all compartments, which will also be determined by a separate thermodynamic condition. Furthermore, we will not distinguish between water in the remnants of the vacuole, and the capillary water. From a thermodynamic point of view, it is a single phase, being a mixture of water and solutes. The *gel* phase is now a ternary mixture of biopolymer, solutes and water.

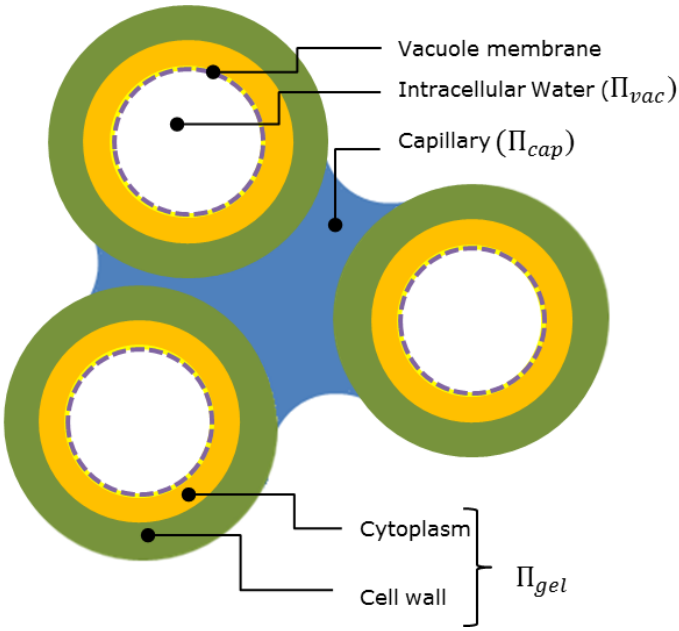

Fig 6.2: A simplified diagram of mushroom tissue.

The condition for thermodynamic equilibrium in the fresh mushroom is that the liquid pressure is equal in all compartments, meaning that:

$$\Pi_{cap} = \Pi_{gel} = \Pi_{vac} \qquad [6.1]$$

Where, Π_{cap} , Π_{gel} ,Π_{vac} represent capillary pressure, gel pressure and vacuole pressures, respectively.

The water distribution in three compartments at equilibrium determines:

1) The radius of capillary, which determines the capillary pressure
2) The volume fractions of components in the gel phase, that determine the gel pressure
3) The volume fractions of salt and sugars in the vacuole, that determine the vacuole pressure

Below, we briefly describe how the above set of equations for the fresh mushroom can be solved in principle. As a given we have a certain amount of water V_w, and mushrooms characterized by the amount of solutes and biopolymers (having a given crosslink density). The calculation starts with an assumed distribution of water over the three phases. From the given amounts of solutes and biopolymers we can compute the volume fractions of solutes and biopolymers, which are required to compute the liquid pressures of the gel phase and the vacuole. If the liquid pressures are unequal, the water distribution has to be refined, until the condition of thermodynamic equilibrium is satisfied. Via this iterative procedure water distribution can be computed.

$$V_w = V_{vac} + V_{gel} + V_{cap} \qquad [6.2]$$

Vacuole pressure calculation

Sugars (mannitol, trehalose) and salt (potassium phostphate) are contained in the vacoule. The total osmotic pressure generated in the vacuole is given by:

$$\Pi_{\mathrm{vac}} = \Pi_{\mathrm{Mix}} + \Pi_{\mathrm{ion}} \qquad [6.3]$$

Mixing pressure is calculated using the Flory Huggins equation as was described in chapter 2. For the calculation of the ionic pressure Debye Hückel relation is used which was also described in chapter 2.

Gel pressure calculation

We assume that the gel phase is a homogeneous mixture of polymers and water. We use the Flory Rehner theory for swelling of crosslinked polymer gels to calculate the gel pressure as:

$$\Pi_{\text{gel}} = \Pi_{\text{mix}} - \Pi_{\text{elas}} \qquad [6.4]$$

The mixing pressure is described by the Flory Huggins theory. The Flory Huggins interaction parameter for polymers is composition dependent and they are specified in chapter 2. The elastic pressure can be described by the Flory Rehner theory, as was also described in chapter 2. The values of the model parameters ϕ_o (the polymer fraction at the relaxed state) and $\mathrm{N_c}$ (the crosslink density) vary from material to material. In the case of mushrooms, their values are temperature dependent and are provided in chapter 2. Values of ϕ_o and N_c can be determined from comparing experimental observations of the polymer fractions against the external pressure values as was described in chapter 2. A recent paper has shown that both model parameters can be related to each other via the c*-theorem of deGennes [6].More advanced approaches can also be used for the elastic pressure, which can be derived from advanced strain energy functions that incorporate effects of micrifibrils in the cell wall [7]. The theory also reduces the model parameters in elastic pressure from two to one. However, for our current purposes, we consider Flory Rehner theory for the calculation of the elastic pressure because of its simplicity.

The capillary pressure calculation*:*

The capillary pressure can be calculated with the Young-Laplace [1] relation as:

$$\Pi_{Capil} = \frac{2\sigma \cos\theta}{r} \qquad [6.5]$$

in which σ is the surface tension of water (7.2×10^{-2} N/m), θ is the contact angle. We simplify the relation by assuming that the contact angle θ is known. The presence of hydrophobins in mushroom [8] makes mushroom hyphae at least partially hydrophobic; the exact value of θ may be obtaind from the experiments. The

estimation of the pore radius r is not straightforward as mushrooms do not have uniform, cylindrical capillaries; the capillary system is a continuos, highly irregular phase. When hyphae are assumed solid cylinders, the actual value of r can be calculated from the distance between the mushroom hyphae and the diameter of the hyphae [9]. We analyzed scanning elecron microscopy pitcures of fresh mushrooms, which yielded an average distance between two hyphae of around 16 μm. Even though this value is only tentative and should receive further investigation, we may use it here as a first estimate of the capillary pressure.

We can now describe the situation in the mushroom tissue during and after heating:

a) *During thermal treatment:*
The cell membrane integrity is lost, which is temperature dependent and follows a typical sigmoid curve (chapter 5). The integrity loss is complete at 60 °C and above. The water and solutes are then free to move across the cell membrane and cell wall to be mixed with the water present in the capillaries. The protein in the *gel* phase denatures, which increases the Flory-Huggins interaction parameter for protein-water. This increase also follows a typical, though somewhat different sigmoid curve. Its value stabilizes at 60 °C. The change in enthalpic interaction induces a change in the gel pressure. The values of model parameters $\emptyset_0$ and N_c also change with temperature (described in chapter 2) which also affect the elastic pressure.

b) *After the heat treatment:*
The combined aquous phase is a solution of salts and sugars, and their concentrations depend on the exchange of water. After permeabilisation of the vacuole and cell membranes, the solutes are mixed with the water present in the capillaries, while this solution is expelled out of the tissue. We assume that the concentrations in the tissue solution and the expelled solution are the same, and that the vacuole phase is completely lost (or merged with the capillary phase). The gel pressure at this condition is equal to the total pressure in the capillaries:

$$\Pi_{\mathrm{cap,H}} = \Pi_{\mathrm{gel,H}} \qquad [6.6]$$

The aquous phase in the capillaries is now a solution of salts and sugars. The capillary phase therefore imparts the capillary pressure as a function of radius of capillary for pure water ($\Pi_{\mathrm{capil,o}}$) which is descibed with equation 6.5, and the osmotic pressure because of solutes (salts and sugars, $\Pi_{\mathrm{cap,S}}$) which are calculated as done for Π_{vac}. We note here that effects of solutes on in interfactial tension is neglected.

$$\Pi_{\mathrm{cap,H}} = \Pi_{\mathrm{capil,o}} + \Pi_{\mathrm{cap,S}} \qquad [6.7]$$

The gel phase after the heat treatment is a mixture of biopolymers in a solution that now also contains salts and sugars. The mixing pressure of this ternary solution of biopolymer, sugars and water can also be described by Flory-Huggins theory [10].

The distribution of solutes over the capillary and gel phase is governed by the equality of the activities of the solutes (salts and sugars) in both phases: :

$$a_{s,gel} = a_{s,cap} \qquad [6.8]$$

The Flory-Huggins interaction parameter for denatured protein and all other compounds are known (chapter 2). The constants in elastic pressure $\emptyset_0$and N_c for heat treted mushroom are known as well (Chapter 2). The water volume fraction ($\emptyset_w$) that is necessary in the relation for the mixing pressure and polymer volume fraction (ϕ) appearing in the elastic pressrue are composition dependant. The values can be found by equating the gel pressure to the capillary pressure. This again requires knowledge of radius of the pores – of course a first approximation could be to assume that the effective pore radius does not change because of heat treatment, however we do know that the overall structure collapses, so one would expect the effective pore radius to be reduced.

6.4 Towards conceptual process design (CPD) of mushroom processing

The main objective of this thesis has been to understand and improve the processing of fresh mushrooms into preserved, canned or jarred mushrooms, while balancing

product quality and optimal use of resources (raw materials, water, energy). The previous section focussed on understanding the quality (simplified into the retention of water) as a result of processing; here we will consider the efficiency in the use of resources, as function of the choices made in the process configuration.

This analysis is on the unit operations level, while the understanding of the product quality (water retention) focusses more on the molecular level, as they are governed by the interaction of biopolymer interactions with water, and mechanical properties of cell wall. Such a decomposed analysis is part of the conceptual process design methodology, and in particular the microscale/*mesoscale* analysis of Li and Kraslawski (2004).

6.4.1 Mesoscale analysis

We consider product yield, water saving and exergy use for production as is discussed in **chapter 5**. Three scenarios are tested: scenario 1 (slicing before vacuum hydration), scenario 2 (hydration with the hot water), and scenario 3 (vacuum hydration with blanch water). Scenario 2, which uses hot water for hydration, can combine the hydration and blanching at one unit operation eliminating the intermediate refrigeration need; however it requires more water for production and yielded less product after processing and costs more exergy to produce a certain mass of final product. This scenario is therefore excluded from further analysis. The variant scenario 1, in which the mushrooms are sliced immediately upon the arrival followed by the vacuum impregnation, does not increase the product yield, but it required the lowest volume of water and consumed the least exergy among all the process scenarios considered. Scenario 3, which uses blanch water for vacuum hydration purpose after cooling, significantly increases the product yield, lowers the water demand and decreases the exergy loss in the production. However the product quality is affected: if the blanching water is used without decolourization step, the product becomes dark in colour due to the browning reactions during and before blanching, which dissolve in the blanch water.

The lower exergy loss and water use in scenario 1 (slicing before vacuum hydration) is achieved by avoiding the cooling of the mushrooms after blanching which saves cooling water and the exergy associated with cooling. This also decreases the heat

load in the subsequent sterilization step as the sterilizer would receive filled bottles at a higher temperature of 79°C instead of the current temperature of 55°C. In scenario 3, some of the solids that are suspended or dissolved in the blanching water during blanching, are re-introduced into the mushrooms during vacuum impregnation, and therefore, the product actually retains more solids. Water is saved as well, since the blanch water replaces the hydration water, and no additional water is required for this.

From the insights learned, we can now say that a combination of scenarios 1 and 3, as proposed in figure 5.3, would be even more sustainable. In this scenario, the mushrooms are sliced before vacuum hydration, with decolorized blanch water after cooling it. Decolourization of the blanch water, either by reaction (e.g., further oxidation) or separation (adsorption or membrane separation) solves the problem of mushroom browning. Our current data shows that only about 70% of blanch water that is produced would be required for the hydration; the other 30% of blanch water can still be used as brine solution as a filler in the jars or cans, which can further reduce the demand of water by 37%, which is needed to make the brine solution. Thus, we combine the redundancy of cooling water, with a significant increase of the final product yield.

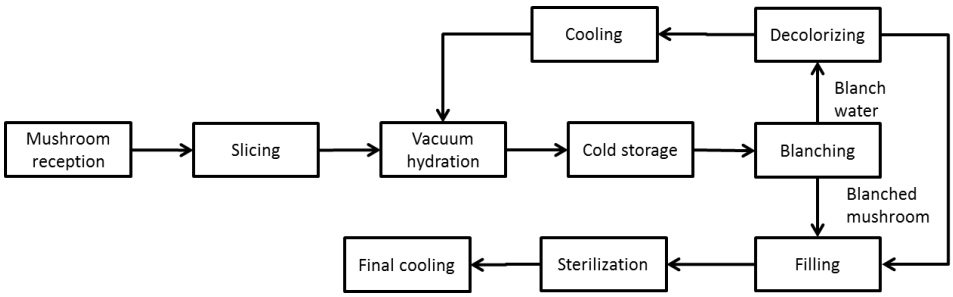

Fig 5.3: A conceptual sustainable mushroom production scheme

In preliminary experiments, we have not seen any difference in the yield of the product whether mushroom were sliced before the hydration step, or after the blanching step. Slicing before vacuum hydration may have the additional advantage that the mechanical stresses on the mushrooms, e.g., during conveying, are

minimised. Mushrooms loose most of their elasticity after their thermal treatment, and are thus susceptible to plastic deformation if subjected to any external force for slicing. Cooling and slicing after blanching may deform and damage the mushrooms due to the mechanical stresses needed. This may result in a lower final product yield. When slicing is done before hydration, the mushroom tissue is still elastic, and less damage due to mechanical deformation is expected.

The use of this new production scenario does impose a few challenges. A proper measure for removing the colour from the blanch water is needed. In the current work we did not investigate on the colour removal techniques of the blanch water. A detailed investigation on the compounds responsible for the colour is needed. Techniques such as filtration [11] or colour absorbent activated carbon [12, 13] column may be suitable. The shifting of slicing before vacuum hydration will need a different type of mushroom cutter than what is used for the blanched mushroom because of the differences in the mechanical properties between the fresh mushrooms and the blanched mushrooms. Though fresh mushrooms are more elastic than the heat treated mushroom, the puncture force for fresh mushrooms is lower than the blanched mushrooms [14]. This indicates that fresh mushroom are more fragile than the heat treated mushroom and more care will be needed to avoid breakage.

6.4.2 Microscale analysis

The temperature of the thermal treatment (**chapter 2, chapter 3**), the pH of the brine (**chapter 4**) and the porosity (**chapter 4**) of the fresh mushrooms influence the water holding capacity of the heated mushroom. The minimum requirements for thermal treatment on the canned mushroom are set by food safety requirements, legal constraints and market demands. We therefore only discuss the use of the porosity of fresh mushroom and the pH of the brine as design parameters in the conceptual design of mushroom processing.

In conceptual process design, the properties of the product have to be defined as concisely as possible. Typical of fresh produce is that the product properties change over different individual products, and over time. This includes variations in average size, moisture level, and composition, and there is a substantial natural variation in

the porosity of mushrooms within the same batch and between batches. On production scale, those variations in porosity result in many overfilled cans as the filling is configured to ensure volumetric filling of the can based on the lowest expected yield of the mushroom.

The pH of the brine can be used as a parameter as well. **Chapter 4** shows that a higher pH of the brine solution is associated with a higher water retention of the mushrooms. Similar results were shown previously [15]. Canned products with a higher pH, however, have a higher risk of survival of spores of bacillus or clostridium species which can later grow into vegetative cells in such products [16, 17], while they cannot grow below a pH value of 4.6 [16]. The pH of brine solution in most of the canned mushroom is reported to be much lower than 4.6. In general, components such as citric acid and ascorbic acid are added. Vivar-Quintana et al [18] report that the addition of 0.5% citric acid and 1000 ppm of ascorbic acid yielded a pH value of 3.7. **Chapter 4** shows that the increase in the degree of hydration for heat treated mushroom in the pH range of 3.7 to 4.6 is substantial, which is also reported by Zivanovic et al. [5]. Thus, carefully controlling the pH of the brine solution may provide opportunities to produce mushrooms with better water retention.

6.5. Future perspectives

In this section, future perspectives of the work based on current findings are discussed briefly. Porosity is important as it is one of the key parameters in our model for water retention. Having accurate estimations of its value, and of the pore size and shape distribution will enhance our understanding of the pore (capillary) water.

In addition, the blanch water from mushroom processing contains compounds that are important for water retention, for its sensory properties and for the nutritional quality. Reuse of blanch water can make processing more sustainable as well. However, the direct use of blanch water in mushroom hydration produces a visually unappealing brown product. Therefore, the options for decolourization of the blanching water must be investigated.

The damage to mushroom tissue by thermal treatment cannot be avoided as cell membrane integrity and protein denaturation already take place at lower temperatures. Non-thermal preservation techniques may offer the benefit of retaining better quality of the mushrooms while still giving a sufficient shelf life; however, these techniques are generally much more costly than heat based preservation processes.

We may also make use of the loss of water holding, in using mushroom tissue for other products. Meat like sensorial attributes of mushroom have been acknowledged. Mushroom has a high water holding fibrous structure similar to meat fibre and has a chewy-meat like texture and other sensorial attributes [19]. This property can be explored more to look at the possibility of using mushroom to partly replace meat from meat products. The loss of water holding capacity is good, since a typical meat analogue would have around 25 weight% protein. One may combine the use of mushroom with other vegetable protein sources for example with lupine protein concentrate which cannot form a solid fibrous structure [20] to produce meat analogue.

Finally an accurate description of WHC of mushroom is important to know effects of processing variables in the water retention behaviour. Though both the cell wall material and cytoplasm are mainly made of polymeric materials, the physical characters are different: the cell wall is an elastic solid with a high crosslink density, whereas cytoplasm is a fluid and the crosslink density is probably low. The further development of model can have a look at this aspect.

Overall, this thesis demonstrated that there is substantial scope in improving the efficiency in the use of resources in producing preserved mushroom, and in retention of water in the mushroom tissue, thus showing that both aspects, product and process efficiency, can be improved at the same time.

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Summary

Summary

This thesis deals with the understanding of the water holding capacity of mushroom, in the context of a redesign of their industrial processing. For designing food process the retention of food quality is of the utmost importance. Water holding capacity is an important quality aspect of mushrooms. A convenient process design methodology which accounts also for product quality is Conceptual Process Design (CPD). An approach to follow CPD methodology is first to explore, the material properties of the products to find optimal processing conditions. In this stage the constraints of (existing) processing equipment are not considered. Later in the second stage, suboptimal processing conditions are determined considering the constrains of equipment.

In mushroom canning, temperature induced loss of water holding capacity (WHC) of tissue manifests as a lower product yield. This loss of water is accompanied with the loss of nutrients, dissolved in the water. In addition to the loss of product quality like water holding capacity, mushroom canning (with alternating heating and cooling steps) also induces losses of useful resources as (potable) water. In terms of water use, water is added at several steps, and is discarded at other places. This shows that there is opportunity to improve the sustainability of the production system, but with the constraint that product quality is not impaired, or evenly improved.

The WHC is an important property that determines several aspects of foods. For example, it determines the juiciness of fruits, vegetables and meat products; the freshness (firmness, or crispiness) of green-leafy vegetables; and the calorie intake per serving for high calorie containing foods such as cheese. Despite being a widely used term in food science, there is no clear definition of water holding capacity and its thermodynamic nature is not fully acknowledged. The understanding of the WHC is even poorer in structured cellular foods such as mushroom, where different water fractions are present in various compartments. In a cellular system water is present as 1) a solution in the vacuoles, 2) water osmotically bound to the cytoplasmic and the cell wall materials and 3) capillary water in pores, which might be filled during processing. Because they have a distinct capillary phase, the mushroom is a good system to study the contributions of various water fraction on the total water retention.

The main aim of the current work is to provide insight for the development of canned mushroom processing where: 1) the resources of energy and water are efficiently used, and 2) the quality of mushroom is maintained. These two aims are related to the efficient use of raw materials and maintenance of full weight of mushroom during processing. The WHC is an important quality indicator of mushrooms. The analysis related to water retention of mushroom has been carried out at *microscale* where molecular and structural interactions in relation to water retention are studied. The micro scale analysis is discussed in **chapter 2**, **chapter 3** and **chapter 4**. The sustainability analysis is carried out at *mesoscale* where analysis is carried out at the unit operation level which is described in **chapter 5**.

In **chapter 2**, the heat-induced change in water holding capacity of particular the gel phase of the mushroom is interpreted with the Flory-Rehner theory, commonly applied to polymer gels. As done earlier for meat, we have first assumed that WHC loss in mushroom can also be attributed to the protein denaturation. This assumption is based on the experimental observation that, like meat, mushroom also follows a typical sigmoid relation with change in temperature. In the theory, we have regarded mushroom as a homogeneous biopolymer hydrogel, in which salt and sugar are dissolved. The water holding capacity is then understood as the swelling capacity of the biopolymer gel. The thermodynamic state of this simplified system is characterized by the so-called swelling pressure, which is decomposed into three independent contributions: 1) the mixing pressure induced by sugars and polymers, 2) the ionic pressure induced by the salt, and 3) the elastic pressure induced by the polymers. An assumption was made that the heat treatment denatures mushroom protein, which is reflected in the change of the Flory Huggins interaction for protein. It follows the same temperature dependency as the WHC loss by mushroom under zero mechanical load. The assumption of the temperature dependency of the interaction parameter is tested with an independent sorption measurement. With the assumption, the sorption curve for mushroom sample which were preheated previously at 30, 60 and 90 °C could accurately be predicted. Curve fitting of WHC under various mechanical loads has shown that model parameters that are associated with the elastic pressure, the crosslink density N_c and fraction of the polymer in the relaxed state, $\emptyset_o$ are temperature dependant. The values of $\emptyset_o$ increased in contrast to the decrease of N_C upon heating of mushroom tissue up to

temperature of 70°C. The result indicates that heat treatment increases the polymer chain length between the cross links as original conformation of mushroom is lost. At the same time, more crosslinks are formed by a polymer because of aggregation of polymers. However, in our fitting procedure, we have excluded WHC data at low external pressure values, as water is present in both gel phase as in in the capillaries. This is done as the pores in this range are not fully collapsed and the Flory Rehner theory is valid only for the gel phase

In **chapter 2** mushroom is simplified in the sense that only compositional contribution is considered in WHC but not the structural contributions. In subsequent chapters we have acknowledged that mushroom has a cellular structure with a distinct pore phase. The pores are intentionally filled during processing via vacuum impregnation. The contribution of water present in the capillaries due to vacuum impregnation of mushroom has been discussed in **chapter 3**. Both the temperature of heat treatment and the initial porosity of mushroom contribute independently to water holding capacity of heat treated mushroom. The hydration of heat treated mushroom increases linearly with the initial porosity of mushroom for all the temperatures from 30 to 90 °C. The porosity of mushroom can also largely explain the increase in hydration of heat treated mushroom with storage as both porosity and the hydration increase simultaneously with the storage days. The fluid that filled in the capillaries acts against collapse of the hyphae which have inherent elastic force that works in the other direction. The initial porosity of mushroom is an important aspect that determines the hydration of the heat treated mushroom and therefore, cannot be ignored. In addition, the Flory-Rehner theory alone cannot capture the contribution of the capillary water. Hence an addition is needed in the theory to capture this effect.

The cellular phase in mushroom tissue is even more complicated because water is present in this phase in two other forms, as *gel water* and the *intracellular water*. **Chapter 4** takes into account the role of structure in the WHC. The role of cell membrane integrity and the cell-wall structural components is investigated for retention of the water fraction. The cell membrane integrity is calculated from the conductivity measurement of the fluid that leaches out from the vacuole that has salts in it. The loss of the cell membrane integrity largely explains the water loss from heat treated mushroom sample. The loss of cell membrane integrity is also

related with the water loss from frozen mushroom, but additional losses occur during freezing due to novel crosslinks formed during the growth of ice crystals compressing the unfrozen cell wall material. The enzymatic hydrolysis of mushroom cellular components shows that chitin and mushroom protein both contribute to the water holding capacity either via osmotic binding or by their role to provide the mechanical strength to the mushroom hyphae. In addition, proteins have additional contributions to water retention by mushroom because of their electrostatic interaction as polyelectrolyte. This is evident as the hydration of the mushroom increases with pH of mushroom.

In **chapter 5**, the efficiency of the use of the resources (raw materials, energy water) is investigated. The mass and exergy flow in the current production system is visualized with the Sankey diagrams. The sustainability of unit operations involved in the current production system of canned are analysed with the second law efficiency using exergy. Using ideas from Process Intensification three alternative routes are proposed for the production of canned mushrooms namely: 1). Slicing before vacuum hydration, and 2) Using hot water for vacuum hydration and 3) Using blanch water for vacuum hydration. Using hot water for vacuum hydration is not seen as a feasible option, since it consumed more resources. Slicing mushroom before their vacuum hydration and using blanch water for hydration of mushroom lowers the resources requirement for production. In addition, using blanch water for hydration also increases the final product yield.

Finally, the main findings of this thesis are summarized in the *general discussion* in **chapter 6.** The findings from previous chapters are combined to an overall description of water loss from heat treated mushroom. The overall description of water holding capacity in mushrooms is given in terms of the thermodynamic conditions for equilibrium between the different compartments holding water. The two dimensions of the thesis, the higher water retention of processed mushroom and more sustainable operation are discussed in the light of conceptual process design, using a micro/mesoscale approach. At the microscale material properties of mushroom are discussed. The biggest effect comes from cell membrane integrity loss. The porosity of fresh mushroom and the ionic interactions of polymers are the other effects that influence the WHC. Mesoscale analysis shows that shifting the sequence of unit operations and reusing the blanch water that is discarded in the current

production process can improve the sustainability. Finally based on outcome of current work, future perspective of current work is discussed briefly.

Overall, this thesis demonstrated that there is substantial scope in improving the efficiency in the use of resources in producing preserved mushroom. Also scope in retention of water in the mushroom tissue is demonstrated. Thus this thesis shows that both aspects, product and process efficiency, can be improved at the same time.

Acknowledgements

It's done, finally. What a great sense relief (and happiness)!. The booklet in your hand, where I appear as an author, is in no way crafted by me alone. There are several supporters and contributors to whom I want to thank here. Firstly, I would like to thank entire Food Process Engineering (FPE) group of Wageningen University and C4C holdings, B.V., The Netherlands for giving me the opportunity to work on the current project. I think, this opportunity gave me a chance to grow personally and professionally in the last four years of my project.

My special thanks goes to Remko as the promotor of my project. After every single meeting with you, I could feel my interior filled with a great positive energy which made me think about various options of doing things. You were always so supportive to me. Thank you. You are certainly a great leader and will remain as a great source of inspiration for me in future as well. Thanks are also due to Ruud for your great support for this work. Open to discussions, generous in providing time and support, you taught me how to re-think more scientifically and come over pre-occupied ideas. And, how to work and re-work to make a work better and better every time. Many thanks.

An interesting part of this project was its close collaboration with C4C holdings B.V. (and Lute'ce). I want to extend my special thanks to Geert Weijers for all of your time and effort in the project. The frequent update meetings were tonic to move ahead and to design a new phase of experiment. My special thanks are also due to Caroline van der Horst, director R&D at C4C holdings for your encouragement and valuable scientific insight to the work. Thanks are also due to Eddy Teernstra (former production director Lutece B.V) and Jan Broeze (FBR) as the other steering committee members for their valuable input.

I also want to thank our staffs and technicians: Jos, Maurice, Martin, Jarno and Marjan for various types of lab related and administrative support. An extra thanks to Maurice for game nights and movie trips :). Thanks are also due to Martijntje (FBR), Lena and Jimmy for your time to tell me about separate aspects that were related to my thesis.

I also want to thank Henk van As, Norbert de Ruijter, Ton Marcelis, Erik Esveld, Tiny Franssen-Verheijen, and Herman de Beukelaer from various groups in WUR for their help to carry out various measurements related to this thesis. I want to thank my students, Nieke, Belinda, Ashutosh, Du Vu Linh, Konstantina, Joanne and Els for their contributions. Thank you for your work!. Your contribution is certainly one of the biggest strength of this thesis.

I want to thank (Dokhtaa) Arun, Puja, Sami, Nirajan, Udit, Huma didi and Suraj for finding time to proof-read my thesis. Arun and Puja, thank you for helping me by providing resources and tips from time to time.

I am indebted to all the wonderful people of wonderful FPE group for wonderful time (I have three wonderful here, that means I am really thankful :)). Coffee break, birthday cakes, FPE borrel, game events, Christmas dinner, PhD trip, FPE day, labuitje...There are so many things to remember. Thank you Atze Jan as a head of theme group food structuring, and to all the members of the group, for organizing several meetings and dinners. Thank you Anja Janssen for motivational words, every now and then. Thank you everyone specially to Angellica, Fillippos, Laura, Jorien, Jaqueline, Francisco, Anja, Victor, Marta, Nicolas, Ali, Mauricio, Dimitri, Kostas. Special thanks to my current office mates Kelly and Meinou for their support (and a gift food pack) especially during the last stage of my PhD. My special thanks to Sami and Jue for accepting to become my paranymphs and for many more reasons.

The list will really be incomplete if I do not thank the people who made stay at the Netherlands more homely with dinner every now and then. Thank you guys, Rabindra, Sampurna (for your words to help me!!!), Ram(s), Deepak(s), Susma, Shailendra, Sajendra, Rakesh, Rajita, Tilak dai, Indra, Suresh, Sanjeeb dai, Bihani, Anita, Binita and Sudip and many other friends for wonderful time we had together, here in Holland. Exclusive thanks to Suman and Trishna for being a very good friend and always supportive.

At the last, I thank all of the family members. You are in my heart. Always.

Ekaraj

About the author

Ekaraj Paudel was born on December 4th, 1981, in Gauradaha, Jhapa, Nepal. He completed his secondary school from Gyanjyoti High school Gauradaha, Nepal in 1997. After finishing his intermediate education in science from Amrit Campus Kathmandu in 1999, Ekaraj started his career in food science from Central campus of Technology Dharan Nepal, from where he completed his bachelor degree in food technology in 2003 and master degree in 2005. After the university education, he started his teaching career from College of Applied Food and Dairy Technology (CAFODAT), Kathmandu, Nepal and, later at National college of Food Sciences (NCFS), Kathmandu Nepal as a program coordinator for Food science program. In 2009, Ekaraj was selected to study in Netherlands under NFP fellowship program and started his master degree in Food science from Wageningen University. During his master in Wageningen University he carried out his thesis on "*Kinetics of glucosidase enzymes using isothermal titration calorimetry*" in FPE group and did his internship in NIZO food research, Netherlands on *"Isolation of alkaline phosphatase in milk"*. In 2011, he joined Food process Engineering group (FPE) as a PhD student. Under the supervision of promotor Prof. Dr Remko M. Boom and co-promotor Dr Ruud van der Sman, he worked on "Water retention in mushroom during sustainable processing", which is presented in this thesis. At the moment Ekaraj is working as a Postdoc in FPE group.

Publications

Van der Sman RGM, Paudel E. Voda A. Khalloufi S (2013) Hydration properties of vegetable foods explained by Flory Rehner theory. *Food Research International* 54, 804-811

Paudel E., Boom R.M., Van der Sman RGM (2014) Change in water holding capacity in mushroom with temperature analyzed by Flory Rehner theory. *Food and Bioprocess Tech. 8(5),960-970*

Paudel E., Boom R.M., Van der Sman RGM (2015) Effects of porosity and thermal treatment oh hydration of mushroom, *Accepted* by Food and Bioprocess Tech.

Paudel E., Boom R.M., Haaren E., Siccama J., Van der Sman RGM Effects of cellular structure and cell wall components on water holding capacity of mushrooms, *Submitted* manuscript

Paudel E., Van der Sman RGM, Westerik N., Ashutosh A., Dewi B., Boom R.M. More efficient mushroom canning through pinch and exergy analysis, *Submitted* manuscript

Warmerdam A., Paudel E., Jia W., Boom R.M. Janssen AEM (2013) Characterization of β galactosidase isoforms from *Bacillus circulans* and their contribution to GOS production. *Appl Biochem Biotechnol* 170 (2) 340-358

OVERVIEW OF COMPLETED TRAINING ACTIVITIES

Discipline Specific Courses	
Netherlands Process Technology Symposium (NPS11), Arnhem, The Netherlands	2011
Food Structure and Rheology, VLAG, Wageningen, The Netherlands	2012
Faraday Discussion: Soft Matter Approaches to Structured Foods (FD158), Wageningen, The Netherlands	2012
Macromolecular Physics and Chemistry, Forschungszentrum Jülich, Germany	2012
7th Baltic Conf. on Food Sci and Tech (FoodBalt2012), Kaunas, Lithuania.	2012
Advanced Food Analysis, VLAG, Wageningen, The Netherlands	2013
European Congress of Chemical Engineering(ECCE9), The Hague, The Netherlands	2013
13th ASEAN Food Conference (AFC2013), Singapore	2013
Reaction Kinetics in Food Science, VLAG, Wageningen, The Netherlands	2013
8th International Conference on Mushroom Biology and Mushroom Products (ICMBMP), New Delhi, India	2014
12th International Congress on Engineering and Food (ICEF12), Quebec, Canada	2015
General Courses	
PhD week, VLAG, Wageningen, The Netherlands	2012
Scientific Publishing, WGS, Wageningen, The Netherlands	2012
Data Management, WGS, Wageningen, The Netherlands	2013
Scientific Writing, WGS, Wageningen, The Netherlands	2013
Project and Time Management, WGS, Wageningen, The Netherlands	2014
Workshop Carousel, WGS, Wageningen, The Netherlands	2014
Optional Activities	
Preparation of Research Proposal, Food Process Engineering Group	2011
PhD Trip, Finland and Baltics, Food Process Engineering Group	2011
PhD trip, Chile, Food Process Engineering Group	2013
Weekly Group Meetings, Food Process Engineering Group	2011-2015

This research was conducted under the auspices of the Graduate School VLAG (Advanced studies in Food Technology, Agrobiotechnology, Nutrition and Health Sciences).