Journal of Food Engineering 290 (2021) 110283

Contents lists available at ScienceDirect

Journal of Food Engineering

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

Enhancing the water holding capacity of model meat analogues through marinade composition

Steven H.V. Cornet [a,b,*], Silvia J.E. Snel [b], Judith Lesschen [b], Atze Jan van der Goot [b], Ruud G.M. van der Sman [a,b]

[a] Food and Biobased Research, Wageningen University & Research, The Netherlands
[b] Food Process Engineering, Agrotechnology and Food Sciences Group, Wageningen University & Research, The Netherlands

ARTICLE INFO

Keywords:
Meat analogue
Swelling
Juiciness
Flory–Rehner theory
Marinade
Plant protein

ABSTRACT

Meat analogues can offer consumers a more sustainable alternative to meat. A successful meat analogue is characterized by a meat-like texture and high juiciness. Juiciness is related to the water holding capacity (WHC). To gain an understanding of how to control the WHC via external conditions, we investigate the effect of ionic strength and pH on water uptake. Model meat analogues were prepared in a Shear Cell and swollen in baths of known pH and ionic strength. The effect of bath composition on water uptake was determined experimentally, and simulated using Flory–Rehner theory. Experiments and simulations were in qualitative agreement. The results show that water uptake increases with an increasing difference between bath pH and the protein's iso-electric point (*pI*). At low ionic strengths, the internal pH is near the *pI*, resulting in reduced swelling. At high ionic strengths, the charge imbalance between gel and bath is limited, also resulting in reduced swelling. At intermediate ionic strengths, swelling increases with decreasing bath ionic strength. Cross-link density negatively relates to WHC and can be controlled via the addition of cross-linking and reducing agents. This work shows that by carefully choosing marinade pH and ionic strength, the WHC of meat analogues can be controlled. These advancements can help improve the sensory characteristics and yield of meat analogues and could enable the production of reduced-salt products.

1. Introduction

Meat products can offer consumers an excellent sensory experience. However, their production puts excessive strain on our food production system and the environment (Pimentel and Pimentel, 2003; Aiking, 2011). Since consumers are not expected to simply stop eating meat (OECD-FAO, 2018; Tilman et al., 2002), an alternative is needed. Plant-based meat alternatives may offer a more sustainable alternative to meat (Smetana et al., 2015). This need has intensified the research focused on developing meat-like products from plant-based ingredients.

Consumer studies indicate that consumers are most attracted to meat alternatives that accurately mimic the beloved sensory attributes of meat (Hoek, 2010). Several attributes of meat, including fibrousness, can already be found in structures produced with the novel shear cell technology. The shear cell can structure different combinations of bio-polymers into fibrous gels that visually and structurally resemble meat (Grabowska et al., 2014, 2016; Dekkers et al., 2016; Schreuders et al., 2019).

Together with fibrousness, juiciness is one of meat's most desirable attributes (Warner, 2017; Bertram and Aaslyng, 2007; Hoek, 2010). Taste and textural properties of meat and meat analogue products can be enhanced by marination and impregnation (Burke and Monahan, 2003; Alvarado and Mckee, 2007; Lee et al., 1986; Sheard and Tali, 2004; Gómez et al., 2019). A marinade can be a carrier of salt, as well as water-soluble flavours, or fat-soluble flavours when using an emulsion. In addition to flavour, marinade composition could affect the water holding capacity (WHC) of meat and meat analogue products (Lebert and Daudin, 2014; English, 1996).

The WHC and moisture content of meat and meat products are related to their juiciness (Aaslyng et al., 2003; Warner, 2017; Pearce et al., 2011; Puolanne and Halonen, 2010). The WHC of meat can be described with the Flory–Rehner (FR) theory of cross-linked polymer networks (van der Sman, 2007, 2012). FR theory relates the WHC of the polymer network to material properties such as polymer–water affinity and cross-link density (Flory and Rehner, 1943; Quesada-Pére et al., 2011). We have recently shown that the FR theory for neutral gels can also adequately describe the WHC of simplified meat analogues made from soy protein and gluten (Cornet et al., 2020).

* Correspondence to: Food and Biobased Research, Wageningen University & Research, Bornse Weilanden 9, 6708 WG, Wageningen, the Netherlands.
E-mail address: steven.cornet@wur.nl (S.H.V. Cornet).

https://doi.org/10.1016/j.jfoodeng.2020.110283
Received 21 January 2020; Received in revised form 6 August 2020; Accepted 8 August 2020
Available online 12 August 2020

Since proteins are poly-ampholytes they can carry both positive and negative charges depending on the pH. English (1996) provided an extension for the FR theory that takes the additional swelling due to the local charge density into account (English, 1996; Rička and Tanaka, 1984). Variations in pH and ionic strength can, therefore, affect the WHC. It was shown that sensory juiciness is strongly affected by marinade pH, although no relationship with WHC was found for short marination times (Yusop et al., 2010). How the WHC of meat analogues is affected by marinade properties such as ionic strength and pH is currently not well understood.

We use a combination of experiments and simulations to improve our understanding of the effect of pH and ionic strength on the WHC of meat analogues. Model meat analogues prepared with a conical shear cell were swollen in marinades with different pH and ionic strength. Ionic strength was controlled using either NaCl or KCl. KCl was selected alongside NaCl as it is a commonly used sodium-free alternative for NaCl (Inguglia. et al., 2017). The effect of pH and ionic strength on swelling was also simulated using a model based on the extended FR theory. The simulations were aimed at qualitatively explaining the outcomes of the swelling experiments. They should provide insight into how WHC and juiciness can be controlled with pH and ionic strength, alongside cross-link density and structure.

2. Theory

2.1. Flory–Rehner theory

The water holding capacity of polymer networks can be described with Flory–Rehner theory by relating it to the swelling pressure, Π_{swell}. In the original theory for neutral gels, Π_{swell} has two contributions. The first contribution, Π_{mix}, accounts for the interaction between polymer and solvent. The second contribution, Π_{elas}, accounts for the elastic pressure generated as a result of network deformation upon swelling or de-swelling. Π_{elas} counteracts the moisture sorption due to Π_{mix} at sufficiently high moisture contents. At equilibrium, Π_{swell} is equal to the pressure applied to the gel:

$$\Pi_{ext} = \Pi_{swell} = \Pi_{mix} - \Pi_{elas} \tag{1}$$

Π_{ext} is thus the pressure externally applied on the gel (e.g. via centrifugation). Under free swelling conditions, such as when submerged in a bath, Π_{ext} can also be zero. In that case Π_{swell} is also zero and thus Π_{mix} equals Π_{elas}. Flory–Rehner theory has been extended to describe the swelling as a result of different ion concentrations inside and outside the gel, as captured by the ionic pressure Π_{ion} (English et al., 1996). Π_{ion} contributes to Π_{swell} as:

$$\Pi_{ext} = \Pi_{swell} = \Pi_{mix} + \Pi_{ion} - \Pi_{elas} \tag{2}$$

According to the Rehner hypothesis all three contributions are independent. The three contributions to the swelling pressure are described below.

2.2. Mixing pressure

We describe Π_{mix} with Flory–Huggins (FH) theory, which depends on the Flory–Huggins interaction parameter χ. The mixing pressure is taken as:

$$\Pi_{mix} = -\frac{RT}{v_w}\left[\ln(1-\varphi) + \varphi(1-\frac{1}{N}) + \chi\varphi^2\right] \tag{3}$$

v_w is the molar volume of water, R is the universal gas constant, T is the absolute temperature, and φ is the volume fraction of polymer. N is the ratio between molar volumes of polymer and water (Paudel et al., 2015). Because of the relatively large volume of proteins, the term $\frac{1}{N}$ is effectively zero. We have previously determined the values of χ for soy protein isolate and gluten by fitting the sorption data with the extended Free Volume Flory–Huggins (FVFH) theory (Vrentas and Vrentas, 1991). χ is 0.91 ± 0.02 for soy protein; for gluten χ is 1.16 ± 0.04 (Cornet et al., 2020).

The applicability of FVFH theory has already been demonstrated for numerous foods (Paudel et al., 2015; van der Sman et al., 2013; Jin et al., 2014; van der Sman et al., 2013; van der Sman, 2012; Cornet et al., 2020) and bio-polymer gels (van der Sman, 2015a). Note that FVFH theory includes an additional term to account for the increased sorption in the glassy regime. Since food polymers in wet systems, such as meat analogues, are in the rubbery regime, we have omitted the FV term our expression for Π_{mix} (Eq. (3)).

The interaction parameter χ is composition dependent (van der Sman, 2011):

$$\chi = \chi_0 + (\chi_1 - \chi_0)(1-\varphi)^2 \tag{4}$$

with χ as the effective Flory–Huggins interaction parameter and χ_0 and χ_1 as the interaction parameters under dilute and concentrated conditions respectively. Water is a theta solvent for proteins at very low polymer concentrations; χ_0 is therefore 0.5.

2.3. Elastic pressure

We describe the network deformation and resulting elastic pressure Π_{elas} with the affine network model.

$$\Pi_{elas} = G_{ref}[\tilde{\varphi}^{1/3} - \frac{\tilde{\varphi}}{2}] \tag{5}$$

$\tilde{\varphi}$ is defined as:

$$\tilde{\varphi} = \frac{\varphi}{\varphi_{ref}} \tag{6}$$

G_{ref} and φ_{ref} are the shear modulus and polymer volume fraction in the reference state. The reference state refers to the value of φ at which the polymer chains are neither extended nor compressed (van der Sman, 2015b). It must be mentioned that the physical meaning of φ_{ref} and the conditions at which φ_{ref} should be determined are still under debate (Khokhlov, 1980; Quesada-Pére et al., 2011). φ_0 is related to G_{ref} via (Horkay and Zrínyi, 1982; De Gennes, 1979):

$$G_{ref} \propto \varphi_0^{9/4} \tag{7}$$

φ_0 and φ_{ref} are related via $\varphi_0 = 2/3\varphi_{ref}$ (van der Sman, 2015a). We use the relation between G_{ref} and φ_{ref} to select reasonable values for φ_{ref} and G_{ref} in our simulations (Cornet et al., 2020).

2.4. Ionic pressure

The approach developed by English (1996) was used to describe the swelling as a result of an unequal distribution of charges between bath and gel using an approach based on the work by (English, 1996). The bath is assumed to be in excess and is not affected by the gel. The bath is a NaCl solution of known ionic strength and pH, comparable to the experimental set-up. The ionic strength is regulated by varying the salt concentration c_{NaCl}. The counter ions for the protein side-groups in the gel phase are assumed to be Na^+ and Cl^-.

When ion concentrations become sufficiently high, their activity (γ) can deviate from 1. The conditions used in our model simulations are in the regime where $\gamma \neq 1$ and activity coefficients should be taken into account. We initially accounted for the non-ideal behaviour using the Pitzer equations for the bath, and Debye–Hueckel for the gel phase (van der Sman et al., 2020). However, accounting for the non-ideal behaviour had only a minor effect on the absolute outcome of the simulations and did not change the qualitative conclusions that could be drawn. van der Sman et al. (2020) similarly showed that the qualitative outcomes of simulated swelling experiments are similar when non-ideal effects are ignored (van der Sman et al., 2020). The lengthy equations were therefore omitted and ideality was assumed throughout.

We first describe the equilibria in the bath, followed by the equilibria in the gel. The subscripts α and β were used to differentiate between the gel and bath respectively.

Table 1
Concentrations and pKa values of the different ionizable protein side-groups on soy protein as determined by van der Sman et al. (2020) via acid titration (van der Sman et al., 2020).

AA (three letter code)	Concentration ($mol\,kg^{-1}$)	pKa
Glutamic acid (Glu)	10	4.25
Aspartic acid (Asp)	66	3.67
Histidine (His)	43	6.54
Lysine (Lys)	30.6	10.67

2.4.1. Equilibria in the bath

The bath is a NaCl solution of known concentration (c_{NaCl}) and pH. NaCl is assumed to be fully dissociated. The pH of the bath is regulated by the addition of HCl or NaOH. The bath pH depends on the concentration of protons in the bath $c_{H,\beta}$. The dissociation of water is governed by its dissociation constant K_w and the bath water activity a_w:

$$K_w = \frac{c_{H,\beta}c_{OH,\beta}}{a_w} \tag{8}$$

K_w is taken to be a constant with value 10^{-14}. a_w follows from Raoult's law.

2.4.2. Equilibria in the gel

We consider a soy protein network with both acidic and basic side-groups, capable of carrying either negative or positive charges respectively. The concentrations and pKa values of the ionizable side groups on soy protein were determined by van der Sman et al. (2020) via acid titration (Table 1) (van der Sman et al., 2020). van der Sman et al. (2020) determined the concentration of the basic amino acids based on the premise that the effective charge of the protein is zero at the iso-electric point (pI). We use the subscripted three letter codes to refer to the different amino acids (Table 1). These are Glu, Asp, His and Lys for Glutamic acid, Aspartic acid, Histidine and Lysine respectively. The ionizable groups were reported as the number of residues per gram protein n_i. By multiplying n_i with the density of protein ρ_p and the polymer volume fraction φ, the number of ionizable groups per unit volume N_i is obtained:

$$N_i = n_i\rho_p\varphi \tag{9}$$

The concentration of the different side-groups is given by $c_{A,i}$. (De-)protonation of the acidic (Glu, Asp) and basic side-groups (His, Lys) is indicated with the subscripts ${}_{AH} \leftrightarrow_{A^-}$ and ${}_{A} \leftrightarrow_{AH^+}$ respectively. The dissociation reactions of the acidic side-groups depend on $K_{A,i}$ via:

$$K_{A,i} = \frac{c_{H,\alpha}c_{A^-,i}}{c_{AH,i}} \qquad i = Glu, Asp \tag{10}$$

The dissociation of basic side groups His and Lys depends on $K_{A,i}$ via:

$$K_{A,i} = \frac{c_{H,\alpha}c_{A,i}}{c_{AH^+,}} \qquad i = His, Lys \tag{11}$$

The degree of side-group dissociation θ_i depends on the ratio of dissociated side-groups over all side-groups of species i. For Glu and Asp, θ_i is given by:

$$\theta_i = \frac{c_{A^-,i}}{c_{A,i}} = \frac{K_{A,i}}{K_{A,i} + c_{H,\alpha}} \qquad i = Glu, Asp \tag{12}$$

Since His and Lys are charged at low pH, θ_{His} and θ_{Lys} are given by:

$$\theta_i = \frac{c_{AH^+,i}}{c_{A,i}} = 1 - \frac{K_{A,i}}{K_{A,i} + c_{H,\alpha}} \qquad i = His, Lys \tag{13}$$

The conservation of ionizable side-groups is ensured by:

$$c_{A,i} = c_{AH,i} + c_{A^-,i} \qquad i = Glu, Asp \tag{14}$$

$$c_{A,i} = c_{AH^+,i} + c_{A,i} \qquad i = His, Lys \tag{15}$$

The concentration of positive and negative counter ions are c_+ and c_-. $c_{H,i}$ and $c_{OH,i}$ are assumed negligible compared to c_{NaCl}. Therefore, c_+ and c_- are taken as $c_+ \approx c_{Na^+}$ and $c_- \approx c_{Cl^-}$. The ionic strength is thus related to c_+ and c_- via:

$$c_+c_- = I^2 \tag{16}$$

The Donnan equilibria are:

$$\frac{c_{Na,\alpha}}{c_{Na,\beta}} = \frac{c_{OH,\beta}}{c_{OH,\alpha}}$$
$$\frac{c_{Cl,\alpha}}{c_{Cl,\beta}} = \frac{c_{H,\beta}}{c_{H,\alpha}} \tag{17}$$

Since $\frac{c_{H,\alpha}}{c_{H,\beta}} = \frac{c_{OH,\beta}}{c_{OH,\alpha}}$, we can express c_- and c_+ as the ratio between $c_{H,\alpha}$ and $c_{H,\beta}$:

$$c_+ = \frac{Ic_{H,\alpha}}{c_{H,\beta}} \tag{18}$$

$$c_- = \frac{Ic_{H,\beta}}{c_{H,\alpha}} \tag{19}$$

The electro-neutrality condition of the gel is therefore given by:

$$c_{A,His}\left(1 - \frac{K_{His}}{K_{His} + c_{H,\alpha}}\right) + c_{A,Lys}\left(1 - \frac{K_{Lys}}{K_{Lys} + c_{H,\alpha}}\right) + \frac{Ic_{H,\alpha}}{c_{H,\beta}} + c_{H,\alpha}$$
$$= \frac{c_{A,Glu}K_{Glu}}{K_{Glu} + c_{H,\alpha}} + \frac{c_{A,Asp}K_{Asp}}{K_{Asp} + c_{H,\alpha}} + \frac{K_w}{c_{H,\alpha}} + \frac{Ic_{H,\beta}}{c_{H,\alpha}} \tag{20}$$

By filling out the above equations into the electro-neutrality condition and simplifying, we arrive at a lengthy sixth-order polynomial similar to English (1996), (English, 1996). The polynomial was omitted because of its size and poor readability.

The proton concentration inside the gel was obtained by solving the electro-neutrality condition with respect to $c_{H,\alpha}$, using a numerical solver in Python. The degrees of dissociation for the different side-groups, θ_i, are then obtained from their dissociation constants. The molar concentration of ionized groups on the protein follows from:

$$n_p = \sum_i z_i\theta_i c_{A,i} \tag{21}$$

With n_p as the molar concentration of ionized side-groups and z as side-group valency. We use the expression by Horkay et al. (2001) for the ionic pressure (Horkay et al., 2001):

$$\Pi_{ion} = 2RT\left[\sqrt{I^2 + (\frac{1}{2}n_p)^2} - I\right] \tag{22}$$

3. Materials and methods

3.1. Materials

Soy protein isolate (SPI, Supro 500E, Solae, St Louis MO, USA; 81.7±1.1% protein Nx5.7; 95.2 ± 0.4wt% DMC) and vital wheat gluten (Roquette, Lestrem, France; protein 77.9±0.1% Nx5.7; 92.5 ± 0.7wt% DMC) were obtained from commercial sources. The protein isolates contained 1.33 ± 0.01 and 0.39 ± 0.01wt% NaCl equivalents respectively (Cornet et al., 2020); this was accounted for when setting the ionic strength of the solutions. Glutaraldehyde (25% aqueous solution), dithiothreitol (DTT; 99%), sodium chloride (NaCl), potassium chloride (KCl), disodium phosphate (Na_2HPO_4), monosodium phosphate (NaH_2PO_4), and all other reagents used were of analytical grade and were obtained from Sigma-Aldrich (Steinheim, Germany). Mili-Q water was used for all experiments (IQ7000, Merck KGaA, Darmstadt, Germany).

3.2. Sample preparation

Fibrous model meat analogue samples were prepared from soy protein isolate (SPI), gluten, NaCl, and water in the weight ratio of 23:7:1:69 using an in-house built high-temperature shear cell (HTSC; Wageningen University and Research; (Grabowska et al., 2016)), resulting in a DMC of 29.4wt%. The sample preparation procedure and the used formulation have previously been described in detail by Grabowska et al. (2014). Samples were prepared as follows. NaCl was dissolved in water and the SPI added, followed by vigorous mixing with a spatula until a homogeneous paste was obtained. The paste was covered with Parafilm (Pechiney Plastic Packaging, Inc., IL, USA) to prevent water evaporation and left to hydrate for 30 min. After hydration, gluten was added, followed by further mixing. The obtained mixture was transferred to the shear cell, pre-heated at 95 °C. The shear cell was sealed hermetically by lowering the top cone and applying a sealing pressure of 2 bar. The shearing process was started immediately thereafter by applying a shear rate of 39 s^{-1} at the edge sample's edge (30 rpm) for 15 min. After shearing, the system was cooled down in 5 min to a temperature of 50 °C by connecting it to a cooling oil bath (Julabo PrestoPlus LH85, Seelbach, Germany). After opening the shear cell, the sample was immediately transferred to a hermetically sealed bag to prevent evaporative moisture loss. After the bagged samples reached room temperature (22 °C) they were horizontally placed in a freezer and frozen at −18 °C until use to prevent sample spoilage.

3.3. Vacuum impregnation and swelling

Samples were thawed at room temperature before use. Samples with a diameter of 7 mm and a height of 5 mm were taken with a sharp biopsy punch. Vacuum impregnation was carried out by placing samples in the bath solution and exposing them to a vacuum of 30 mbar for 60 min using a vacuum pump (SC 950, KNF Neuberger GmbH, Freiburg, Germany). After the vacuum was released sample containers were sealed and left to equilibrate overnight at 4 °C. Various bath compositions were used as described in the next paragraph. Some samples underwent a washing treatment by replacing the water frequently until the washing water reached a constant conductivity.

3.3.1. Bath composition

Bath composition was varied with respect to pH and ionic strength. The pH was varied between pH 6 and pH 8 with a step size of 0.5, and stabilized using phosphate buffers. The buffer solutions were prepared by mixing stock solutions of Na_2HPO_4 and NaH_2PO_4 in appropriate ratios. The ionic strength was adjusted to 0.002, 0.01, 0.05, 0.09, 0.15, 0.25, 0.43, or 0.8 m by adding either water or a NaCl solution. Actual ionic strength were calculated afterwards for each sample separately. In some experiments, NaCl was replaced with KCl. Where applicable, glutaraldehyde or DTT were diluted with buffer to achieve the desired concentrations. The buffer's ability to stabilize the pH was confirmed in preliminary tests.

3.4. Determination of the water holding capacity

The water holding capacity (WHC) is expressed as the swelling ratio Q and was determined using a similar method as Paudel et al. (2015). Q was determined based on the polymer volume fraction at maximum swelling, φ_0. φ_0 is based on the dry weight fraction of polymer, y_p, and the assumption that no polymer is lost during swelling. Dry weight was determined after drying at 105 °C for 24 h. φ_0 was calculated as:

$$\varphi_0 = \frac{y_p/\rho_p}{(y_p/\rho_p) + (1 - y_p)/\rho_w} \tag{23}$$

Swelling ratio Q relates to φ_0 via:

$$Q = \varphi_0^{-1} \tag{24}$$

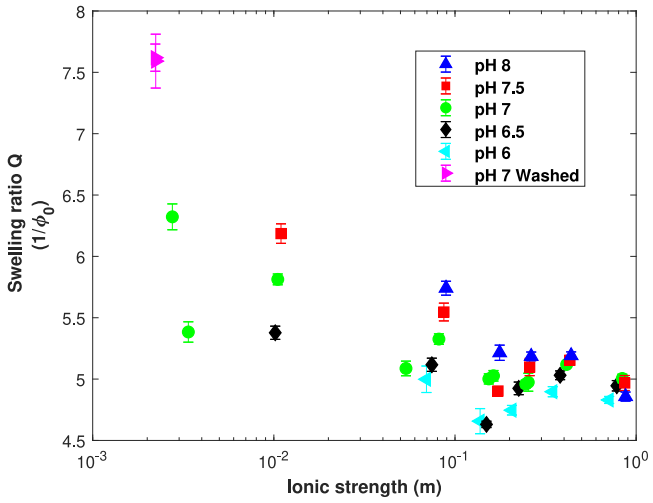

Fig. 1. Maximum level of swelling expressed as a function of bath ionic strength and pH. The bath was a phosphate buffered saline solution, or water in the case of the lowest ionic strength. Error bars are the 95% confidence intervals with $n = 3$.

ρ_p and ρ_w are the polymer and water densities with values 1330 kg m^{-3} and 1000 kg m^{-3} respectively. y_p was determined gravimetrically after drying at 105 °C for 24h. Simulation results were normalized to the level of swelling at the *pI* (pH 5) to obtain a measure for Q.

3.5. Statistics

The number of repetitions differed between experiments and is indicated with n. Error bars indicate the 95% confidence interval of the observation. Where applicable, significant differences were tested using a one-way ANOVA and Tukey test.

4. Results and discussion

We have studied the swelling of a soy protein and wheat gluten-based model meat analogue. The pH and ionic strength of the bath were varied to investigate their effect on swelling. Cross-link density was varied by adding cross-linking or reducing agents to the marinades. Model simulations based on the extended Flory–Rehner theory were run alongside the experimental work and are part of the discussion.

4.1. Experimental results

4.1.1. Effect of pH and I on maximum swelling

Model meat analogues were swollen in bath solutions with different pH and ionic strength. The level of swelling was determined and is expressed as the swelling ratio Q (Fig. 1). Bath ionic strength, I, affects Q, with low I resulting in more swelling than high I. Washing samples with water until constant conductivity, and low I, resulted in the highest level of swelling. There is an apparent plateau at $Q \approx 5$ when $I > 0.5$. At moderate I ($I \approx 0.01$–0.2), pH has a pronounced effect on Q, with an increase in pH resulting in more swelling. The effect of pH on swelling is weakened as I increases, as indicated by the similarity in Q between samples around $I \approx 0.8$.

The effect of potassium chloride (KCl) on the WHC was also determined, as KCl is a commonly used alternative to NaCl (Fig. 2). To exclude any effect of the phosphate buffer, bath solutions were prepared without a buffer. The solutions thus had a pH equal to the initial sample pH of 7. Both NaCl and KCl showed a decrease in WHC as the ionic strength increased, and an apparent plateau at high ionic strength. Samples swollen in KCl solutions show similar levels of swelling compared to those swollen in NaCl solutions at high

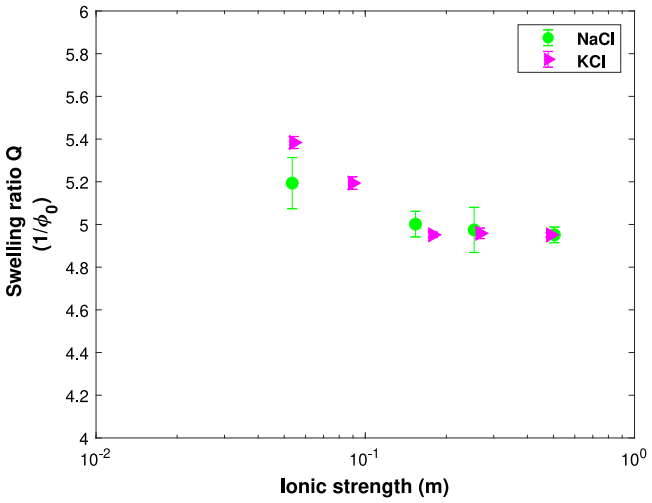

Fig. 2. Maximum level of swelling as a function of bath ionic strength. The ionic strength was adjusted with either NaCl or KCl. The pH was 7 and was not adjusted. Error bars are the 95% confidence intervals with $n = 3$.

Table 2
Effect of salt addition to the soy protein–gluten mixture prior to shear-induced structuring on WHC. Standard formulation contained 1wt% NaCl. All baths had pH 7. Values are presented as swelling ratio Q ± confidence interval. Letters indicate different significant groups ($p = 0.05$).

Formulation	Bath ionic strength (m)	Swelling ratio Q
Standard	0.25	4.9 ± 0.2[a]
No NaCl added	0.25	5.1 ± 0.1[a]
Standard	0.002	7.6 ± 0.1[b]
No NaCl added	0.002	7.6 ± 0.1[b]

ionic strengths ($I > 0.1$). A minor but significant increase in swelling is noticeable at low ionic strength when using KCl instead of NaCl, as indicated by the lack of overlap between confidence intervals. No significant effect was observed at higher ionic strengths (as confirmed with ANOVA; not shown). The origin of the minor increase at low ionic strength cannot be identified based on our results. We do note that commercially available meat analogue products tend to have ionic strengths over 0.1 m (Bohrer, 2019). At such ionic strengths the effect of using KCl on WHC does not differ from that of NaCl.

4.1.2. Salt addition before shear-induced structuring

Salt (NaCl) is commonly added to the hydrated soy–gluten mixture prior to shear-induced structuring (Grabowska et al., 2014; Dekkers et al., 2016). We have omitted the addition of NaCl before shear-induced structuring and tested its effect on WHC. The samples with and without added NaCl had a visually similar structure. Swelling in bath solutions of high ionic strength revealed no significant effect on the WHC (Table 2). Also when both samples were washed, no significant effect on the WHC was found. This suggests salt addition before structuring has no irreversible effects on WHC.

4.1.3. Effect of cross-link density on swelling

The cross-link density was altered through the addition of cross-linking agent glutaraldehyde and reducing agent DTT. Glutaraldehyde can form cross-links between various functional groups of proteins (Migneault et al., 2004), while DTT disrupts disulphide bonds (Peters et al., 2015). Cross-linking with glutaraldehyde had a negative effect on WHC, as indicated by the decrease in swelling ratio Q (Fig. 3a). WHC reduced proportionally upon increasing the glutaraldehyde concentration. DTT had the opposite effect and increased the WHC (Fig. 3b). For DTT, no concentration dependence was observed. Conductivity measurements revealed no effect of glutaraldehyde or DTT addition on ionic strength (data not shown).

4.2. Model simulations and discussion

4.2.1. Model considerations

The effects of pH and I on the swelling of a simplified meat analogue were simulated using the extended Flory–Rehner theory. The bath is a NaCl containing solution with a pH between 2 to 12. The cross-link density was varied by adjusting G_{ref}. The experimental results were explained based on the model simulations and their implications discussed. Gluten swelling accounts for only a minor part of mixed soy protein–gluten gel swelling (Cornet et al., 2020). However, gluten can exert an additional (mechanical) pressure on soy in mixed soy–gluten gels when present at sufficiently high concentrations (Cornet et al., 2020). Since the magnitude of this effect cannot be predicted accurately at this time, a more simplified meat analogue containing solely soy protein was assumed for the simulations. Given the minor contribution of gluten to the overall swelling, our simulations can still provide meaningful qualitative information. The degree of swelling was determined by solving $\Pi_{ext} = \Pi_{swell}(\varphi) = 0$. The degree of swelling is expressed with the normalized swelling ratio Q. Q is obtained by normalizing with the degree of swelling at the pI ($Q = \phi/\phi_{pI}$).

4.2.2. The effect of ionic strength and pH on Q near the pI

The simulated swelling data is presented as a function of pH instead of ionic strength (like in Fig. 1) to improve visibility of the observable effects (Fig. 4). The insert in Fig. 4 serves to ease comparison with Fig. 1. The simulation results show a minimum in swelling ratio Q around the pI of soy protein (Fig. 4; pH 5). The swelling minimum is in good agreement with the reported pI of soy protein ($pI \approx$ 4.5–5; e.g. (Virkar et al., 1982)), as van der Sman et al. (2020) determined the number of lysine residues based on the pI (van der Sman et al., 2020). Swelling increases on either side of the swelling minimum as the bath pH moves away from the pI. This is a result of increased protein net charge due to additional side-group dissociation. Lowering the ionic strength results in a progressive widening of the swelling minimum (Fig. 4 left). When the ionic strength becomes sufficiently high ($0.01 > I > 1$), ionic strength no longer affects swelling around the pI (Fig. 4 right). As the ionic strength takes extreme values ($I > 1$), the swelling minimum widens once more. At high I, non-ideal ion behaviour could be of importance.

4.2.3. pH buffering by proteins near the pI

The observed widening and narrowing of the swelling minimum around the pI upon changing the ionic strength in the range $I < 1$ is a result of the ability of proteins to act as pH buffers. At the pI, net protein charge is zero. As the pH changes, side-groups dissociate while satisfying the electro-neutrality condition. When sufficient ions are present in the bath solution (moderate to high ionic strength), the electro-neutrality condition can be satisfied by taking up counter-ions from the bath. However, at low bath ionic strengths, counter-ion availability is limited. To achieve gel electro-neutrality, side-group dissociation must be limited to reduce the number of counter-ions required. Since this occurs near the pI, the gel's internal pH will have deviated from the bath pH and be close to the pI (Fig. 5). The internal gel pH close to the pI thus explains the widened swelling minimum near the pI at low ionic strength.

The reduced swelling at low ionic strengths was not observed experimentally, even after simulating an infinitely large bath of low I through repeated washing with de-ionized water (Fig. 1). A possible explanation lies in the composition of the (commercial) protein isolates used in this study. Commercial isolates can contain a considerable amount of ions. Possibly, these ions were only partially washed out during the swelling experiments, either due to slow diffusion or protein–ion binding. As shown by Borukhov et al. (2000), polymer charge might also contribute to the ionic strength, and elevate the ionic strength (Borukhov et al., 2000). Both situations could result in an increase of the gel's internal ionic strength.

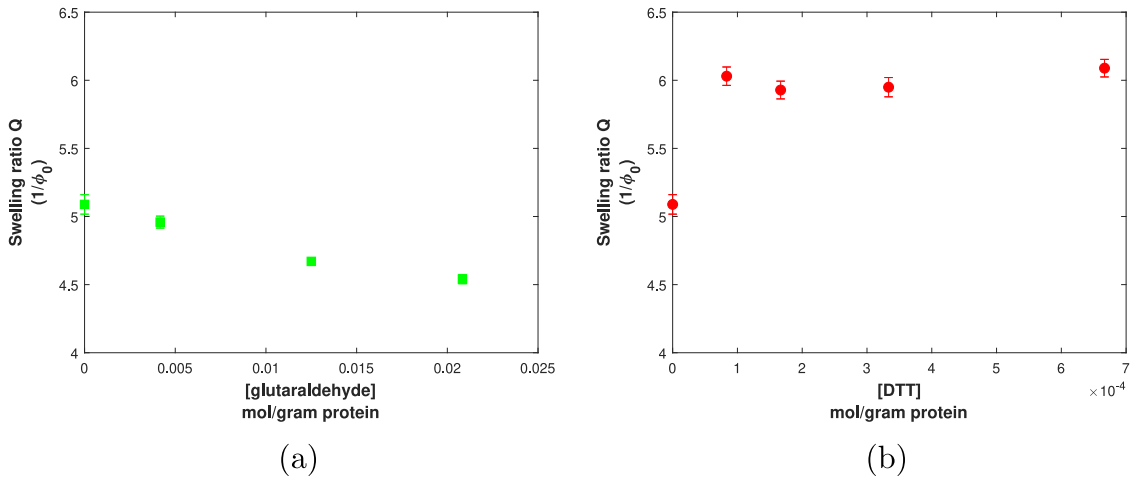

Fig. 3. Swelling as affected by the addition of glutaraldehyde (a) and DTT (b). The ionic strength was 0.03 m for all samples. The pH was 7 and was not adjusted. Error bars are the 95% confidence intervals with $n = 3$.

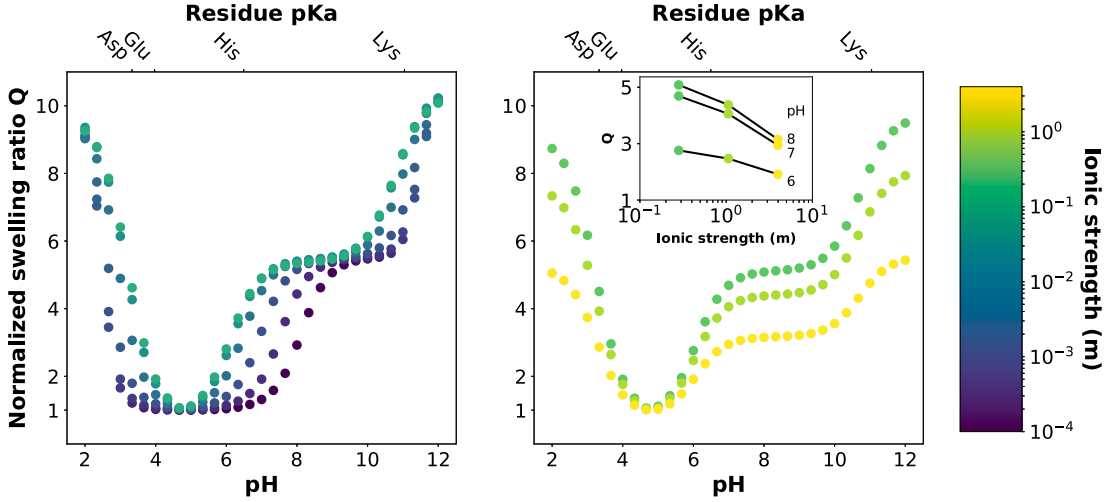

Fig. 4. Model simulations of the level of maximum swelling, based on the extended Flory–Rehner theory with $\Pi_{ext} = 0$ for different ionic strength and pH. Low (left) and high (right) ionic strengths are presented separately for clarity. The insert serves to ease qualitative comparison with Fig. 1. The gel's elastic properties were taken as $G_{ref} = 50$ kPa and $\varphi_{ref} = 0.091$. The used colour map should scale well with (printed) greyscale. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4.2.4. The effect of pH on Q

When the pH moves from the pI, swelling ratio Q increases as a result of increased protein charge (Fig. 4). Changes in Q occur near the pKa of the different amino acid residues. In the pH range, 7.5–9, an apparent plateau forms, indicating swelling is not affected by pH. At these pH values, the two acidic side-groups are deprotonated and charged, while histidine is neutral and lysine is protonated. As the pH approaches the pKa of Lysine (pH 10.7), Lysine is deprotonated. The protein net charge will have increased, which explains the additional swelling. At the limit of high and low pH, all available groups will have dissociated. Further increasing or decreasing the pH does not result in increased polymer charge and, therefore, does not affect swelling.

In reality, the pKa of an individual amino acid residue is affected by its local environment (Alexov et al., 2011). The effect of pH on swelling is, therefore, more gradual as the pH passes through the range of pKa values of a given amino acid. This explains why there was still a pronounced effect of pH on swelling in the pH range 7.5–9 (Fig. 1).

Increasing the ionic strength results in reduced swelling at all pHs except the pI. The greater ionic strength results in a lower ionic pressure and therefore reduced swelling. This also explains the widening of the swelling minimum around the pI at high ionic strength. Since the proteins have no net charge, swelling at the pI is not affected by ionic strength.

Although the experimental pH range is narrower, the same trend is observed; moving the bath pH from the pI results in additional swelling (Fig. 1). The detrimental effect of high ionic strength on swelling as shown by the simulations is in line with our experimental observations.

Plotting Q as a function of the internal gel pH, all but the highest ionic strength data points collapse onto a single curve (Fig. 6). This shows that the main function of ionic strength is to modulate the effect of pH. Thus, the ionic strength indirectly controls swelling by affecting the internal gel pH.

4.2.5. Effect of cross-link density on swelling

The shear modulus, G_{ref}, is proportional to the cross-link density (Gosline, 1978). By varying G_{ref} we can further study the effect of the cross-link density on WHC (Fig. 7). In our simulations, increasing the modulus resulted in reduced swelling. Similarly, reducing the modulus increased WHC. This is what would be expected based on Eq. (1) and (5), which show that a greater modulus results in a greater resistance to swelling. Reducing G_{ref} thus results in a larger relative contribution of the mixing and ionic pressure to the swelling pressure, and results in more swelling.

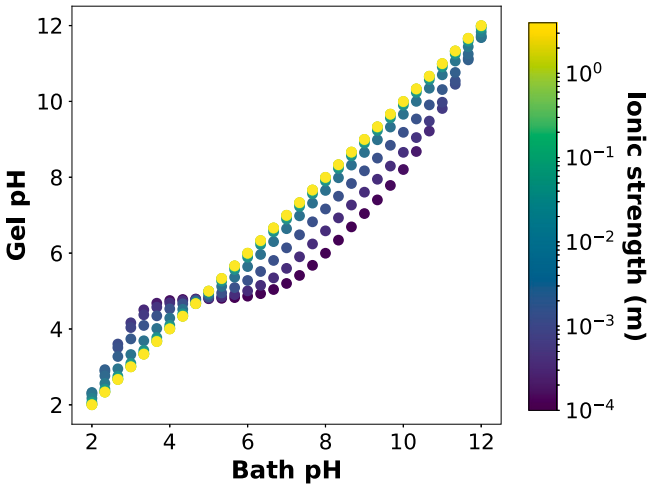

Fig. 5. Internal gel pH as a function of the bath pH and buffer concentration, as determined by solving the electro-neutrality condition (Eq. (20)). The gel's elastic properties were taken as $G_{ref} = 50$ kPa and $\varphi_{ref} = 0.091$. The used colourmap should scale well with (printed) greyscale. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

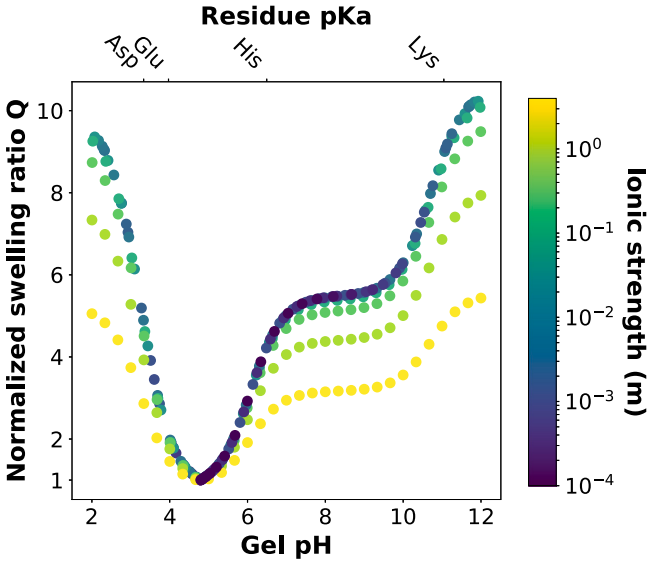

Fig. 6. Normalized swelling ratio Q as function of the internal gel pH and bath ionic strength. The gel's elastic properties were taken as $G_{ref} = 50$ kPa and $\varphi_{ref} = 0.091$. The used colourmap should scale well with (printed) greyscale. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The experimental results show a similar relation between WHC and cross-link density. Upon increasing the cross-link density with glutaraldehyde, the WHC progressively declined. Reducing the disulphide bonds with DTT did have a positive effect on WHC, but no effect of concentration was visible. Glycinin, the main protein in soy, has 2 mole disulphide bonds per mole protein (approximately $6.7 \mu mol\, g^{-1}$) (Renkema, 2001), which is lower than the concentrations of DTT used in the experiments. Glycinin also contains free sulfhydryl groups, which decrease upon heating (Berghout et al., 2014). This suggests additional disulphide bonds may have been present. Furthermore, gluten also contains disulphide bonds (Delcour et al., 2012). However, the lack of a concentration-dependence in the WHC suggests all disulphide bonds were already reduced at the used lowest concentration of DTT, and that the actual number of disulphide bonds is below the lowest concentration of DTT used. Peters et al. (2015) also reported a positive effect of

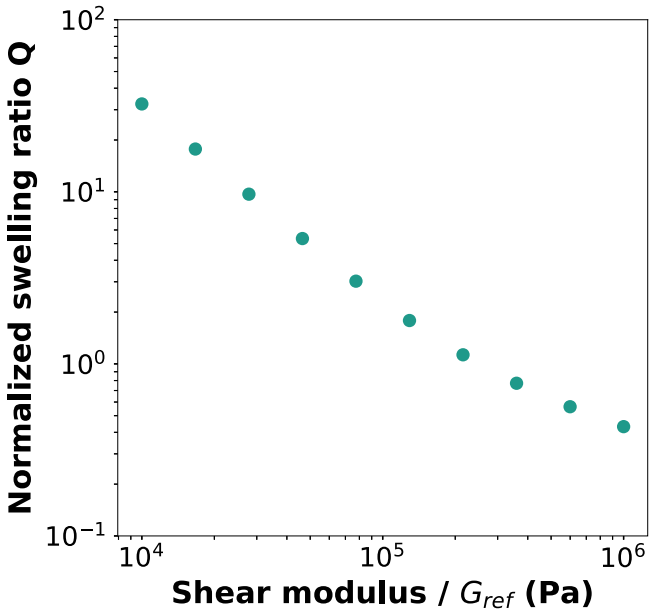

Fig. 7. Simulations of the effect of cross-link density on swelling ratio Q. Cross-link density was altered by varying G_{ref}. The corresponding values of ϕ_{ref} range from 0.048 to 0.30. The ionic strength was 0.03 m and the pH was 7.

DTT on the WHC of whey protein particles and did find a concentration dependent response (Peters et al., 2015). This suggests that such an effect could have been visible at lower DTT concentrations. Glutaraldehyde acts on several functional groups on proteins (Migneault et al., 2004), which could explain why it did show a concentration effect. Soy protein gels and extrudates are stabilized not only by disulphide bonds but also by physical interactions such as hydrogen bonds, hydrophobic interactions and ionic interactions (Shimada and Cheftel, 1988; Pietsch et al., 2019). The physical bonds remaining after DTT treatment formed a sufficiently strong network to prevent further swelling. Lowering salt content prior to shear-induced structuring showed that the addition of NaCl has no irreversible effects on WHC (Table 2). Furthermore, the fibrous structure was not affected. This suggests the importance of ionic cross-links is limited.

4.3. General discussion

We have shown that simulations based on Flory–Rehner theory are in good qualitative agreement with experimental observations of the swelling of model meat analogues at a range of pH values and ionic strengths. Our simulations show that low ionic strength results in reduced swelling over a wide range of pH values due to an internal gel pH close to the pI. The gel minimizes side-group dissociation to achieve electro-neutrality when counter ions are limited. The presence of more ions can, therefore, be beneficial to the water uptake by meat analogues. Ions present in soybean and its derived ingredients, or added during processing, increase the gel's internal ionic strength and thereby limit the gel's buffering capacity. This could explain the experimentally observed swelling maximum at low bath (/marinade) ionic strengths. While some ions should be present to act as counter-ions, the addition of copious amounts of salt is detrimental to the WHC. The development of salt-reduced meat analogue products with improved juiciness might, therefore, be possible.

Small pH alterations can greatly improve water uptake by model meat analogues and improve product yield. Increased moisture content might be beneficial for the product's sensory properties by enhancing the juiciness. Tailoring product formulation and post-structuring process conditions can, therefore, have a substantial effect on the final

product properties. We do note that pH adjustment of concentrated protein products is challenging due to the high buffering capacity of proteins. The use of concentrated acid or base might, therefore, be unavoidable.

Cross-link density can be used to modify the WHC. Increasing the cross-link density reduces WHC while reducing cross-link density results in improved WHC. Since glutaraldehyde and DTT are by no means fit for human consumption, cross-linking and proteolytic enzymes such as trans-glutaminase (Peters et al., 2015) and papain could be considered to achieve the desired level of WHC.

Product reformulation could be an alternative path towards salt reduction. Omitting the addition of NaCl to the pre-mix prior to structuring could be beneficial as it resulted in improved WHC while maintaining a fibrous appearance. Replacing NaCl by another salt such as KCl also has potential for sodium reduction. KCl addition had a similar negative effect on WHC as NaCl in the regime most relevant for meat analogue products. Given its similar effect on WHC, the use of KCl could help reduce the sodium content of meat analogue products (Inguglia. et al., 2017).

5. Conclusions

The effect of pH and ionic strength on the WHC of soy protein and gluten-based model meat analogues was studied using both experimental data and model simulations. The experimental results and simulations are in qualitative agreement on the effect of pH. They show that a marinade pH far from the iso-electric point of the protein results in additional swelling. Minimizing the ionic strength experimentally resulted in the highest level of swelling, while our simulations showed limited swelling at low ionic strength. The simulations showed that the buffering capacity of the protein limits protein side-group dissociation at low ionic strengths. This effectively widens the iso-electric point of the protein, which in turn reduces swelling. However, the internal gel pH is decisive in setting the degree of swelling. We have shown that marinade pH and ionic strength are tools to control water uptake by simplified meat analogues. Furthermore, altering the cross-link density could be a route to alter WHC without the addition of ions. With these tools, food manufacturers can develop meat analogue products that more closely resemble real meat, and improve product yields. Low-salt marinades showed the highest water uptake, which might enable the development of salt-reduced meat analogues.

CRediT authorship contribution statement

Steven H.V. Cornet: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **Silvia J.E. Snel:** Investigation, Formal analysis. **Judith Lesschen:** Investigation. **Atze Jan van der Goot:** Writing - review & editing, Supervision. **Ruud G.M. van der Sman:** Methodology, Writing - review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research is part of the project Plant Meat Matters, which is co-financed by Top Consortium for Knowledge, The Netherlands and Innovation Agri & Food by the Dutch Ministry of Economic Affairs, The Netherlands; the project is registered under contract number TKI-AF-16011.

References

Aaslyng, M.D., Bejerholm, C., Ertbjerg, P., Bertram, H.C., Andersen, H.J., 2003. Cooking loss and juiciness of pork in relation to raw meat quality and cooking procedure. Food Qual. Pref. (ISSN: 09503293) 14 (4), 277–288. http://dx.doi.org/10.1016/S0950-3293(02)00086-1.

Aiking, H., 2011. Future protein supply. Trends Food Sci. Technol. (ISSN: 09242244) 22 (2), 112–120. http://dx.doi.org/10.1016/j.tifs.2010.04.005,

Alexov, E., Mehler, E.L., Baker, N., Baptista, A.M., Huang, Y., Milletti, F., Erik Nielsen, Jens, Farrell, Damien, Carstensen, Tommy, Olsson, Mats H.M., Shen, Jana K., Warwicker, Jim, Williams, Sarah, Michael Word, J., 2011. Progress in the prediction of pK a values in proteins. Proteins Struct. Funct. Bioinformatics (ISSN: 08873585) 79 (12), 3260–3275. http://dx.doi.org/10.1002/prot.23189, URL: https://www.ncbi.nlm.nih.gov/pubmed/22002859.

Alvarado, C., Mckee, S., 2007. Marination to improve functional properties. J. Appl. Poultry Res. 16 (1), 113–120, URL: https://www.sciencedirect.com/science/article/pii/S1056617119316022?via{%}3Dihub.

Berghout, J.A.M., Boom, R.M., van der Goot, A.J., 2014. Understanding the differences in gelling properties between lupin protein isolate and soy protein isolate. Food Hydrocolloids (ISSN: 0268005X) 43, 465–472. http://dx.doi.org/10.1016/j.foodhyd.2014.07.003.

Bertram, H.C., Aaslyng, M.D., 2007. Pelvic suspension and fast post-mortem chilling: Effects on technological and sensory quality of pork - A combined NMR and sensory study. Meat Sci. (ISSN: 03091740) 76 (3), 524–535. http://dx.doi.org/10.1016/j.meatsci.2007.01.010, URL: https://www.ncbi.nlm.nih.gov/pubmed/22060996,

Bohrer, B.M., 2019. An investigation of the formulation and nutritional composition of modern meat analogue products. Food Sci. Hum. Wellness (ISSN: 22134530) 8 (4), 320–329. http://dx.doi.org/10.1016/j.fshw.2019.11.006.

Borukhov, I., Andelman, D., Borrega, R., Cloitre, M., Leibler, L., Orland, H., 2000. Polyelectrolyte titration: Theory and experiment. J. Phys. Chem. B (ISSN: 15206106) 104 (47), 11027–11034. http://dx.doi.org/10.1021/jp001892s, cond-mat.

Burke, R.M., Monahan, F.J., 2003. The tenderisation of shin beef using a citrus juice marinade. Meat Sci. (ISSN: 03091740) 63 (2), 161–168. http://dx.doi.org/10.1016/S0309-1740(02)00062-1, URL: https://www.ncbi.nlm.nih.gov/pubmed/22062175.

Cornet, Steven H.V., van der Goot, Atze Jan, van der Sman, Ruud G.M., 2020. Effect of mechanical interaction on the hydration of mixed soy protein and gluten gels. Curr. Res. Food Sci. (ISSN: 26659271) 3, 134–145. http://dx.doi.org/10.1016/j.crfs.2020.03.007.

De Gennes, P.G., 1979. Scaling concepts in Polymer Physics. Cornell University Press, Ithaca and London, ISBN: 080141203X, URL: https://link.springer.com/chapter/10.1007/978-1-4757-1402-9{_}42.

Dekkers, B.L., Nikiforidis, C.V., van der Goot, A.J., 2016. Shear-induced fibrous structure formation from a pectin/SPI blend. Innovative Food Sci. Emerg. Technol. (ISSN: 14668564) 36, 193–200. http://dx.doi.org/10.1016/j.ifset.2016.07.003.

Delcour, J.A., Joye, I.J., Pareyt, B., Wilderjans, E., Brijs, K., Lagrain, B., 2012. Wheat gluten functionality as a quality determinant in cereal-based food products. Annu. Rev. Food Sci. Technol. (ISSN: 1941-1413) 3 (1), 469–492. http://dx.doi.org/10.1146/annurev-food-022811-101303.

English, A.E., 1996. Phase Transitions in Polyampholytic Polymers and Hydrogels (Doctor of Philosophy in Medical Engineering and Medical Physics). Massachusetts Institute of Technology, URL: https://dspace.mit.edu/handle/1721.1/38378.

English, A.E., Mafé, S., Manzanares, J.A., Yu, X., Grosberg, A.Y., Tanaka, T., 1996. Equilibrium swelling properties of polyamapholytic hydrogels. J. Chem. Phys. (ISSN: 00219606) 104 (21), 8713–8720. http://dx.doi.org/10.1063/1.471560,

Flory, P.J., Rehner, J., 1943. Statistical mechanics of cross-linked polymer networks II. Swelling. J. Chem. Phys. (ISSN: 00219606) 11 (11), 521–526. http://dx.doi.org/10.1063/1.1723792, arXiv:gr-qc.

Gómez, I, Ibañez, F.C., Beriain, M.J., 2019. Physicochemical and sensory properties of sous vide meat and meat analog products marinated and cooked at different temperature-time combinations. Int. J. Food Prop. (ISSN: 15322386) 22 (1), 1693–1708. http://dx.doi.org/10.1080/10942912.2019.1666869.

Gosline, J.M., 1978. The temperature-dependent swelling of elastin. Biopolymers (ISSN: 10970282) 17 (3), 697–707. http://dx.doi.org/10.1002/bip.1978.360170312,

Grabowska, K.J., Tekidou, S., Boom, R.M., van der Goot, A.J., 2014. Shear structuring as a new method to make anisotropic structures from soy-gluten blends. Food Res. Int. (ISSN: 09639969) 64, 743–751. http://dx.doi.org/10.1016/j.foodres.2014.08.010,

Grabowska, K.J., Zhu, S., Dekkers, B.L., De Ruijter, N.C.A., Gieteling, J., van der Goot, A.J., 2016. Shear-induced structuring as a tool to make anisotropic materials using soy protein concentrate. J. Food Eng. (ISSN: 02608774) 188, 77–86. http://dx.doi.org/10.1016/j.jfoodeng.2016.05.010.

Hoek, A.C., 2010. Will Novel Protein Foods beat meat? Consumer Acceptance of Meat Substitutes - A Multidisciplinary Research Approach (Ph.D. thesis). Wageningen UR, ISBN: 9789085855361, p. 220, URL: http://library.wur.nl/WebQuery/clc/1926634.

Horkay, F., Tasaki, I., Basser, P.J., 2001. Effect of monovalent-divalent cation exchange on the swelling of polyacrylate hydrogels in physiological salt solutions. Biomacromolecules (ISSN: 15257797) 2 (1), 195–199. http://dx.doi.org/10.1021/bm0056153,

Horkay, F., Zrínyi, M., 1982. Studies on the mechanical and swelling behavior of polymer networks based on the scaling concept. 4. Extension of the scaling approach to gels swollen to equilibrium in a diluent of arbitrary activity. Macromolecules (ISSN: 15205835) 15 (5), 1306–1310. http://dx.doi.org/10.1021/ma00233a018.

Inguglia., E.S., Zhang, Z., Tiwari, B.K., Kerry, J.P., Burgess, C.M., 2017. Salt reduction strategies in processed meat products – A review. Trends Food Sci. Technol. (ISSN: 09242244) 59, 70–78. http://dx.doi.org/10.1016/j.tifs.2016.10.016.

Jin, X., van der Sman, R.G.M., van Maanen, J.F.C., van Deventer, H.C., van Straten, G., Boom, R.M., van Boxtel, A.J.B., 2014. Moisture sorption isotherms of broccoli interpreted with the Flory–Huggins free volume theory. Food Biophys. (ISSN: 15571858) 9 (1), 1–9,

Khokhlov, A.R., 1980. Swelling and collapse of polymer networks. Polymer (ISSN: 00323861) 21 (4), 376–380. http://dx.doi.org/10.1016/0032-3861(80)90005-1.

Lebert, A., Daudin, J.D., 2014. Modelling the distribution of aw, pH and ions in marinated beef meat. Meat Sci. (ISSN: 03091740) 97 (3), 347–357. http://dx.doi.org/10.1016/j.meatsci.2013.10.017.

Lee, Y.B., Sehnert, D.J., Ashmore, C.R., 1986. Tenderization of meat with ginger rhizome protease. J. Food Sci. (ISSN: 17503841) 51 (6), 1558–1559. http://dx.doi.org/10.1111/j.1365-2621.1986.tb13860.x.

Migneault, I., Dartiguenave, C., Bertrand, M.J., Walrdon, K.C., 2004. Glutaraldehyde: behavior in aqueous solution, reaction with proteins, and application to enzyme crosslinking. BioTechiques (ISSN: 20507496) 5 (37), 790–802. http://dx.doi.org/10.1039/C6TA07211A, URL: https://www.future-science.com/doi/pdf/10.2144/04375RV01.

OECD-FAO, 2018. Agricultural Outlook 2018–2027. Tech. Rep., ISBN: 8889337419, pp. 17–66, (Chapter 1) URL: https://www.oecd-ilibrary.org/agriculture-and-food/oecd-fao-agricultural-outlook-2018-2027{_}agr{_}outlook-2018-en.

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 Bioprocess Technol. (ISSN: 19355149) 8 (5), 960–970. http://dx.doi.org/10.1007/s11947-014-1459-7.

Pearce, K.L., Rosenvold, K., Andersen, H.J., Hopkins, D.L., 2011. Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts on fresh meat quality attributes - A review. Meat Sci. (ISSN: 03091740) 89 (2), 111–124. http://dx.doi.org/10.1016/j.meatsci.2011.04.007.

Peters, J.P.C.M., Luyten, H., Alting, A.C., Boom, R.M., van der Goot, A.J., 2015. Effect of crosslink density on the water-binding capacity of whey protein microparticles. Food Hydrocolloids (ISSN: 0268005X) 44, 277–284. http://dx.doi.org/10.1016/j.foodhyd.2014.09.030.

Pietsch, V.L., Bühler, J.M., Karbstein, H.P., Emin, M.A., 2019. High moisture extrusion of soy protein concentrate: Influence of thermomechanical treatment on protein-protein interactions and rheological properties. J. Food Eng. (ISSN: 02608774) 251 (August 2018), 11–18. http://dx.doi.org/10.1016/j.jfoodeng.2019.01.001.

Pimentel, D., Pimentel, M., 2003. Sustainability of meat-based and plant-based diets and the environment. Am. J. Clin. Nutr. (ISSN: 00029165) 78 (Suppl. 3), http://dx.doi.org/10.1093/ajcn/78.3.660s, URL: https://www.ncbi.nlm.nih.gov/pubmed/12936963.

Puolanne, E., Halonen, M., 2010. Theoretical aspects of water-holding in meat. Meat Sci. (ISSN: 03091740) 86 (1), 151–165. http://dx.doi.org/10.1016/j.meatsci.2010.04.038.

Quesada-Pére, M., Maroto-Centeno, J.A., Forcada, J., Hidalgo-Alvarez, R., 2011. Gel swelling theories: the classical formalism and recent approaches. Soft Matter (ISSN: 1744-683X) 7 (22), 10536. http://dx.doi.org/10.1039/c1sm06031g, URL: https://pubs.rsc.org/en/content/articlelanding/2011/sm/c1sm06031g/unauth{#}!divAbstract.

Renkema, J.M.S., 2001. Formation, Structure and Rheological Properties of Soy Protein Gels (Ph.D.). Wageningen UR, ISBN: 9058085015, p. 137.

Rička, J., Tanaka, T., 1984. Swelling of ionic gels: Quantitative performance of the donnan theory. Macromolecules (ISSN: 15205835) 17 (12), 2916–2921. http://dx.doi.org/10.1021/ma00142a081,

Schreuders, F.K.G., Dekkers, B.L., Bodnár, I., Erni, P., Boom, R.M., van der Goot, A.J., 2019. Comparing structuring potential of pea and soy protein with gluten for meat analogue preparation. J. Food Eng. (ISSN: 02608774) 261 (May), 32–39. http://dx.doi.org/10.1016/j.jfoodeng.2019.04.022, URL: https://linkinghub.elsevier.com/retrieve/pii/S0260877419301803.

Sheard, P.R., Tali, A., 2004. Injection of salt, tripolyphosphate and bicarbonate marinade solutions to improve the yield and tenderness of cooked pork loin. Meat Sci. (ISSN: 03091740) 68 (2), 305–311. http://dx.doi.org/10.1016/j.meatsci.2004.03.012, URL: https://www.ncbi.nlm.nih.gov/pubmed/22062241.

Shimada, K., Cheftel, J.C., 1988. Determination of sulfhydryl groups and disulfide bonds in heat-induced gels of soy protein isolate. J. Agricult. Food Chem. (ISSN: 15205118) 36 (1), 147–153. http://dx.doi.org/10.1021/jf00079a038,

Smetana, S., Mathys, A., Knoch, A., Heinz, V., 2015. Meat alternatives – life cycle assessment of most known meat substitutes. Int. J. Life Cycle Assess. (ISSN: 0948-3349) 2050, 1254–1267. http://dx.doi.org/10.1007/s11367-015-0931-6.

Tilman, D., Cassman, K., Matson, P., Naylor, R., Polasky, S., 2002. Agricultural sustainability and intensive production practices. Nature 418, 671–677, URL: https://www.nature.com/articles/nature01014.

van der Sman, R.G.M., 2007. Moisture transport during cooking of meat: An analysis based on Flory-Rehner theory. Meat Sci. (ISSN: 03091740) 76 (4), 730–738. http://dx.doi.org/10.1016/j.meatsci.2007.02.014, URL: https://www.ncbi.nlm.nih.gov/pubmed/22061251,

van der Sman, R.G.M., 2011. Prediction of the state diagram of starch water mixtures using the Flory–Huggins free volume theory. Soft Matter (ISSN: 1744-683X) 7, 429. http://dx.doi.org/10.1039/c0sm00280a.

van der Sman, R.G.M., 2012. Thermodynamics of meat proteins. Food Hydrocolloids (ISSN: 0268005X) 27 (2), 529–535. http://dx.doi.org/10.1016/j.foodhyd.2011.08.016,

van der Sman, R.G.M., 2015a. Biopolymer gel swelling analysed with scaling laws and Flory-Rehner theory. Food Hydrocolloids (ISSN: 0268005X) 48, 94–101. http://dx.doi.org/10.1016/j.foodhyd.2015.01.025.

van der Sman, R.G.M., 2015b. Hyperelastic models for hydration of cellular tissue. Soft Matter (ISSN: 1744-683X) 11, 7579–7591. http://dx.doi.org/10.1039/C5SM01032B, URl: http://pubs.rsc.org/en/Content/ArticleLanding/2015/SM/C5SM01032B.

van der Sman, R.G.M., Houlder, S., Cornet, S.H.V., Janssen, A., 2020. Physical chemistry of gastric digestion of proteins gels. Curr. Res. Food Sci. (ISSN: 26659271) 2, 45–60. http://dx.doi.org/10.1016/j.crfs.2019.11.003.

van der Sman, R.G.M., Paudel, A., Voda, A., Khalloufi, S., 2013. Hydration properties of vegetable foods explained by Flory-Rehner theory. Food Res. Int. (ISSN: 09639969) 54 (1), 804–811. http://dx.doi.org/10.1016/j.foodres.2013.08.032, URL: https://www.sciencedirect.com/science/article/pii/S0963996913004766.

Virkar, P.D., Hoare, M., Chan, M.Y.Y., Dunnill, P., 1982. Kinetics of the acid precipitation of soya protein in a continuous-flow tubular reactor. Biotechnol. Bioeng. (ISSN: 10970290) 24 (4), 871–887. http://dx.doi.org/10.1002/bit.260240410.

Vrentas, J.S., Vrentas, C.M., 1991. Sorption in glassy polymers. Macromolecules (ISSN: 15205835) 24 (9), 2404–2412. http://dx.doi.org/10.1021/ma00009a043.

Warner, R.D., 2017. Chapter 14 – The Eating Quality of Meat—IV Water-Holding Capacity and Juiciness, eighth ed. Woodhead Publishing Limited, ISBN: 9780081006948, pp. 419–459. http://dx.doi.org/10.1016/B978-0-08-100694-8.00014-5, URL: http://www.sciencedirect.com.ezp.lib.unimelb.edu.au/science/article/pii/B9780081006948000145.

Yusop, S.M., O'Sullivan, M.G., Kerry, J.F., Kerry, J.P., 2010. Effect of marinating time and low pH on marinade performance and sensory acceptability of poultry meat. Meat Sci. (ISSN: 03091740) 85 (4), 657–663. http://dx.doi.org/10.1016/j.meatsci.2010.03.020,