The mechanism of salt effects on starch gelatinization from a statistical thermodynamic perspective

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**Abstract**

Understanding the gelatinization of starch requires a theoretical framework that can extract microscopic information from the literature’s abundant experimental data in a quantitative manner. Recent developments in statistical thermodynamics have been adapted here to extract the starch-water and starch-salt affinity changes that occur during gelatinization, in a manner beyond the capabilities of traditional polymer theory. We have clarified the mechanism that leads to the increases of salt binding upon gelatinization at high concentrations. Contrary to previous assumptions, salt-starch affinity has been shown to be predominant in the salt effect on gelatinization over water structure contributions. How the temperature of gelatinization depends on salt concentration can be rationalized by the exclusion of salts at low salt concentrations from the gelatinized state and salt anion accessibility to the inside of the starch granule at increased concentration. Finally, the salt anion accessibility informs whether gelatinization progresses from the hilum or the periphery of the starch granule.

**1. Introduction**

Starch exists in the form of semi-crystalline granules that consist of two types of polysaccharides: non-branched amylose and branched amylopectin. Upon heating in the presence of excess water, the granule goes through gelatinization, which is the process comprised of swelling of granules and partial leaching of amylose (Day, Fayet, & Homer, 2013; Evans & Haisman, 1982). What is particularly important is the control of gelatinization process by the addition of small molecule components (co-solutes), such as salts and sugars (Ahmad & Williams, 1999; Day et al., 2013; Li, Li, & Gao, 2015), which has been exploited routinely in cooking and food processing. The aim of this paper is to clarify the mechanism on a molecular scale by which salts and sugars influence the gelatinization process.

Despite decades of investigations by the employment of a variety of experimental techniques ranging from microscopy and calorimetry (Evans & Haisman, 1982; Jane, 1993) to rheological and NMR measurements (Ahmad & Williams, 1999; Chinachoti, White, Lo, & Stengle, 1991; Zhu, 2017), the understanding on a molecular scale of how salts affects gelatinization remains incomplete. Indeed, the influence of salts on starch gelatinization can be measured through how gelatinization temperature and the enthalpy of gelatinization change with cosolute concentration, which is probed using differential scanning calorimetry (DSC) (Biliaderis, 2009). The advantage of this approach is that, despite the irreversibility of the transition (Lelievre, 1976), sufficiently slow heating rates can eliminate kinetic contributions (Kohyama & Nishinari, 1991; Shiotsubo & Takahashi, 1984), thereby enabling the application of equilibrium statistical thermodynamics, such as the Flory theory (Habeych, Guo, van Soest, der Goot, & Boom, 2009). Still, the universal difficulty which has yet been overcome is the lack of a direct link between the experimental observation and gelatinization temperature shift in a quantifiable manner, namely putting numbers to each of the contributions and comparing the relative importance between them.

However, experimental observation generally points out the importance of the following two contributions to salt induced changes in gelatinization (Ahmad & Williams, 1999; Chiotelli, Pilosio, & Le Meste, 2002; Jane, 1993; Li, Zhang, Ye, & Gao, 2015; Wang et al., 2017):

1. Starch-cation interaction (leading to the decrease of )
2. “Water structure” (leading to additional changes in )

Beyond the consensus on the existence of above two driving forces, there is little agreement on their causes and relative importance (Ahmad & Williams, 1999; Jane, 1993), which reflects the lack of a reliable link between the change of and the microscopic interactions that take place in solution.

The paradigm for explaining how salts affect hydration of solutes and polymers has long been the concept of the “water structure” (Frank & Franks, 1968), which is considered to be the driving force for the hydrophobic effect (Dill, 1990), and the ion-induced hydration change is rationalized via the change of water structure. The difficulty of this paradigm arises from the fact that not only has the “water structure” been ambiguously defined but also the how it influences the thermodynamics of hydration has never been quantified (Collins & Washabaugh, 1985; Shimizu, Booth, & Abbott, 2013).

Hence the aim of this paper is threefold:

1. to quantify starch-water and starch-salt interactions and to identify the dominant contributions;
2. to clarify if there is any relationship between starch-water and starch-salt interactions;
3. to identify the molecular scenario behind 1 and 2.

To achieve these aims, it is indispensable to base our analysis on statistical thermodynamics, because it is the only branch of physics that can provide an explanation for macroscopic phenomena based on microscopic behaviour of the molecules (Ben-Naim, 1992). Previously, the Fluctuation Adsorption-Solvation Theory (FAST), our statistical thermodynamic theory of solvation in solution mixtures with a long tradition from Kirkwood and Buff (Ben-Naim, 1977; Kirkwood & Buff, 1951), has clarified how additives and co-solutes can influence solubility, macromolecular stability and gelation based solely on experimental data (Booth, Omar, Abbott, & Shimizu, 2015; Shimizu & Boon, 2004; Shimizu & Matubayasi, 2014a). Based on this track record, here we extend our approach to starch gelatinization, by utilising the extensive experimental data on the role of salts and sugars on gelatinization of sago starch (Ahmad & Williams, 1999, 2000; Jane, 1993). The systems studied in this study are starch/ salt/ water and starch/ sugar/ water, with the main focus being on salts. This focus had been chosen because salts have a larger range of different ways gelatinization temperature and enthalpy depend on concentration. Varying the anion/cation can extract information about these dependencies.

**2. Statistical thermodynamics of starch gelatinization in the presence of salts**

**2.1 Thermodynamics of starch gelatinization**

To elucidate from calorimetric data how the addition of co-solutes, such as salts, affect the gelatinization on a molecular scale, here we construct a statistical thermodynamic theory. To this end, let us consider a three-component solution consisting of starch (), water (), and co-solute (). For co-solute concentration, we use the molar scale, , or its chemical, .

We refer to the endotherm transition as gelatinization for simplicity (Cooke & Gidley, 1992). Following Ahmad and Williams, the data used in our study is the peak temperature. Since initial and final temperatures are affected by increasing co-solute concentration, the peak temperature is more likely to be the most consistent measure of attainable (Kohyama & Nishinari, 1991). Under this setup, how salt concentration changes the free energy (and therefore the chemical potential) difference between the gelatinized and granular states of the starch, , can directly be obtained from and . To do so, , which is the function of temperature , pressure and salt chemical potential can be expanded around a point on the gelatinization equilibrium as:

 (1)

where signifies a small change of the variables in question.

Along the equilibrium line, , Eq. (1) can be rewritten as:

 (2)

where the equilibrium condition was used to yield and is the volume change that accompany gelatinization. Under a constant pressure, , we obtain (Shimizu & Matubayasi, 2014a):

 (3)

Eq. (3) will lead to the determination of starch-water and starch-salt affinities, as will be shown in the next section. The present theoretical framework relies only on the existence of the gelatinization as a phase transition, which is observed in pure water and in the presence of salts alike.

**2.2. Statistical thermodynamics of starch gelatinization**

Calorimetric data on gelatinization, analysed via Eq. (3) yields the free energy change that accompanies gelatinization transition. How depends on salt concentration reveals quantitative microscopic information by the help of statistical thermodynamics, as will be shown below.

To this end, let us summarise first what gelatinization process consist of according to the consensus of the field (Ahmad & Williams, 2002; Ai & Jane, 2015; Evans & Haisman, 1982; Kohyama & Nishinari, 1991):

1. the leaching of amylose into solution,
2. loss of crystallinity and swelling of the granules.

In the scope of statistical thermodynamics, these processes correspond to:

1. solvation of amylose from the solid state to a molecularly dispersed solution with water and salts
2. expansion of a granule and penetration of solution into the granule.

Hence both processes accompany solvation changes.

Such solvation changes can be interpreted microscopically, based on the difference in solvent and co-solute densities between the vicinity of starch and the bulk solution. This vicinity-bulk density difference can be captured quantitatively using the change of Kirkwood-Buff integrals (KBIs),(Hall, 1971; Shimizu, 2004) defined as (Kirkwood & Buff, 1951):

 (4)

where refers to the distribution function between a state of the starch (either gelatinized or semi-crystalline state) and species (either water or cosolute ). signifies the net increase or depletion of the species around each state of starch compared to the bulk solution (Shimizu & Matubayasi, 2014a, 2018). This, at times, has been interpreted as the averaged “affinity” between species and (Ben-Naim, 1977; Nicol, Isobe, Clark, & Shimizu, 2017) as terminology which can assist our understanding of . The KBI changes in Eq. (4) accompany the semi-crystalline (granular) state gelatinized state transition, such that . Note that we deal with “starch” (which consists of amylose and amylopectin) as an averaged single entity. We emphasize that the part of amylose that leaches upon gelatinization is still being an integral part, statistical thermodynamically, of starch, or the species .

 The KBI changes are linked to the free energy change upon gelatinization through the following relationship (Shimizu, Stenner, & Matubayasi, 2017):

 (5)

Eq. (5) plays a central role in the analysis of this paper.

**2.3. Determining starch-water and starch-salt affinity changes**

The KBI change for the starch-water and starch-salt affinities and how they vary with co-solute concentration can tell us about the changes in interactions upon gelatinization and how they are affected by co-solutes, which will allow us to determine whether the co-solute or the solvent have the biggest effect on variations in gelatinization temperature. These KBIs can be determined by supplementing Eq. (3) with the following independent relationship (Shimizu et al., 2017):

 (6)

where represent the partial molar volume of the species . Eqs. (3), (5) and (6) yields the following useful formulae for the calculation of KBIs at limit:

 (7)

 (8)

In bulk water, , whereas in aqueous starch solution, may deviate from 1. However, this does not affect the result of the interpretation of due to its negligibly small value as discussed in Section 3. Eqs. (7) and (8) can yield starch-water and starch-salt affinity changes whenever calorimetric and volumetric data on gelatinization are both available.

**2.4. The effect of bulk solution structure**

Salts, according to the classical paradigm, change water structure, which modulates the strength of the hydrophobic effect and consequently the gelatinization free energy (Shimizu et al., 2017). Here we aim to quantify how the bulk solution structure affects gelatinization. To do so, note that we have considered or (salt ion activity) as the measure of co-solute concentration.

However, co-solute concentration is considered usually in molarity. The gap between the chemical potential () and molarity gives rise to (Booth, Omar, Abbott, & Shimizu, 2015):

 (9)

This means that the self-association of salts () in preference to salt hydration () effectively reduces the number of salts, thereby leading to the per-mole inefficiency for starch solvation (Booth et al., 2015). However, note that the l.h.s. of Eq. (9) tends to 1 as , meaning that this inefficiency term disappears at low salt concentration.

**2.5. Source and analysis of experimental data – non-linear regressions**

At dilute salt concentration, it is possible to obtain both calorimetric and volumetric data for starch gelatinization based on data available in the literature (Evans & Haisman, 1982; Shiotsubo & Takahashi, 1984; Thevelein, Van Assche, Heremans, & Gerlsma, 1981). The most systematic set of data that the authors found uses sago starch (Evans & Haisman, 1982; Shiotsubo & Takahashi, 1984; Thevelein et al., 1981). It should be noted that sago starch retains typical compositional, structural, and physical characteristics similar to the other starches such as potato and corn (Ahmad, Williams, Doublier, Durand, & Buleon, 1999). However, whether the salt concentration dependence of other starches proceeds in a qualitatively similar way to sago (Brumovsky, Brumovsky, & Thompson, 2006; Jane, 1993; Rahman, 1995; Wootton & Bamunuarachchi, 1979) or differently (Day et al., 2013; Hirashima, Takahashi, & Nishinari, 2004; Li, Zhang, et al., 2015; Wang et al., 2017) still awaits an extensive experimental clarification. We emphasize nevertheless that the theoretical method developed in this paper can readily be applied to any starch, in so far as the calorimetric and volumetric data on gelatinization has been measured along the concentration of salts.

 can be calculated for dilute solutions from the pressure dependence of using the following relationship (Shimizu et al., 2017):

 (10)

Indeed, the enthalpy change that accompanies gelatinization justifies the treatment of gelatinization just like the first-order transition.

 This means that both and can be determined from the experimental data at this limit. However, the volume change on gelatinization has not been measured at higher salt concentrations, and is qualitatively inconsistent in the literature (Day et al., 2013; Hirashima et al., 2004; Zhu, Gayin, Chatel, Dewettinck, & Van der Meeren, 2009), which means that only the KBI difference is available. And consequently, due to the lack of experimental data, and cannot be determined separately at higher salt concentrations.

For processing the experimental data, the following expression, equivalent to Eq. (4) has been used for convenience (Shimizu et al., 2017; Stenner, Matubayasi, & Shimizu, 2016):

 (11)

To study molecular level interactions using KBIs calculated using Eqs. (5) and (11), several fitting functions were required. Namely, enthalpy (, temperature of gelatinization ( and salt activity ( = ) dependence on salt concentration ().

The enthalpy fitting equations, shown in Table 1, produced continuous data points for each salt studied. Its fitting is shown in Figure 1 below.

 The fitting was required to obtain . Its fitting is shown in Figure 2 and Table 2 shows the equations and coefficients used. and data for NaCl, KCl, NaI and Na2SO4 were obtained from Ahmad & Williams and for KI was obtained from Jane (Ahmad & Williams, 1999; Jane, 1993).

The method for fitting activity data for was established in a previous paper and based on work by Robinson and Stokes (Nicol et al., 2017; Robinson & Stokes, 1959). The activity data used was from various sources (El Guendouzi & Aboufaris, 2015; Jakli & Van Hook, 1972; Moore, Humphries, & Patterson, 1972; Patil, Tripathi, Pathak, & Katti, 1991). Note that activity data for certain salts (KSCN and NaNO3) were not available at the temperature required; their dilute concentration behavior has nevertheless been calculated. The fitting and methodology is summarized in the supporting information. It should be noted here that these same fitting values were used to calculate the salt self-association provided by Eq. (9) through the straightforward relationship between and .

From the values for, and obtained in this section, we calculate preferential interaction KBIs using Eqs. (3), (5) and (11). These are presented in Figure 3.It should be noted that, at times the equations used to fit the data were a general trend and some points lay far from the curve due to the small number of data points. In these cases, to avoid overfitting, the outlying data points were used in place of the smoothed data when calculating KBIs. This has led to a small number of individual points that lie slightly away from the curve in Figures 1 – 3. These points do not appear to break observed trends in the data.

**3. Starch gelatinization under dilute salts**

Based on the theory presented in Section 2, here we clarify how salts affect gelatinization through the starch-water and starch-salt average affinity changes that have been quantified via the KBI changes summarised in Figure 3. It should be noted, again, that we deal with “starch” (which consists of amylose and amylopectin) as an averaged single entity.

At low salt concentration, all the salts studied here (KCl, NaCl, Na2SO4, NaI and KI) raise the gelatinization temperature. This salt-induced destabilization of gelatinization can be attributed to the dominant and large negative compared to the negligible contribution from (100-300 times smaller) seen in Table 3, where and denote KBIs under infinite salt dilution. This negligibility is contradictory to the prevalent hypothesis (Jane, 1993; Li, Li, et al., 2015; Li, Zhang, et al., 2015) that salt-induced gelatinization change is driven by the starch hydration changes caused by the ion’s effect on the “water structure”. Thus, the dominance of the salt-starch affinity and negligibility of starch hydration was established quantitatively for the first time, which was made possible by the independent determination of and enabled by statistical thermodynamics.

What, then, does the dominant contribution, i.e., the negative signify? The spectroscopic evidence demonstrates binding of cations to the OH groups of starch (Ahmad & Williams, 1999; Chinachoti et al., 1991; Jane, 1993), and the ability of anions to form complexes with amylose (Manca, Woortman, Loos, & Loi, 2015; Saenger, 1984). Considering that the direction for KBI changes is defined as granular gelatinized, this means that cations and/or anions are bound more strongly to the granular state than to the gelatinized state at low concentration. This interpretation may seem at odds with the changes that take place upon gelatinization, in particular the leaching of amylose into aqueous solution, since binding to aqueous amylose is possible in both anions and cations (Li et al., 2014; Saenger, 1984). However, this apparent paradox can be resolved by taking into account the following effect, on each ion, of the structural properties of starch granules (Brouillet-Fourmann, Carrot, & Mignard, 2003; Ratnayake & Jackson, 2006):

* Extruded amylose “needles” have been observed for granules in solution, which has been referred to as the “hedgehog effect” (Lii, Tomasik, Hung, & Lai, 2002).
* Channels of amorphous starch, which are more easily penetrable upon gelatinization, extend into the hilum (centre) of the starch granule (Buléon et al., 1997; Chen et al., 2009; Manca et al., 2015).
* Starch granules exhibit semi-permeable membrane-like behaviour on cations and whole salts, preventing them from entering the granule. This is likely present until the granule is fully diaphanous(Rockland, Jones, & Hahn, 1977; Wang, Kuo, Wang, & Patindol, 2007). (Note that this effect is present in the endotherm for the initial stage of gelatinization, which we focus on in this paper (Biliaderis, 2009)).

These features in the granule’s structure lead to exclusion behavior in both anions and cations at low salt concentration. Exclusion is used here to refer to the case where has a negative value (Shimizu & Matubayasi, 2014a; Stenner et al., 2016). This can be interpreted in terms of the definition of KBIs given in Eq. (4), which signify the distributions of salt ions or water molecules around starch. Indeed, any reduction in the distribution of co-solute molecules around the solute or surface is due to changes in their binding strength or access to the solute (Shimizu & Matubayasi, 2014b; Shimizu & Smith, 2004).

The effect of the semi-permeable membrane-type behavior of starch granules requires further discussion. It prevents cations from interacting with the majority of the constituent molecules of starch inside the granule. Large negative at this concentration is consistent with this experimental observation. It should be noted that this “semi-permeable membrane” (Lii et al., 2002; Wang et al., 2007) is not strictly a semi-permeable membrane as defined in thermodynamics text books (Prigogine & Defay, 1954). It excludes cations but not anions, which suggests that this phenomenon is an ion-interaction induced ion concentration difference mediated by the presence of the granule envelope (Wang et al., 2007). Anions are able to penetrate into the granule from aqueous solution (Lii et al., 2002). Therefore, some mechanistic barrier to their entry at low concentration must exist, leading to this reduction in access to the solute. These anion exclusion effects could be due to cation – anion pairing with cations unable to enter the starch structure (Lii et al., 2002). This is supported for low salt concentration as Figure 4 shows that there is minor salt self-accumulation at low concentration for all salts, as calculated by Eq. (9).

We can conclude that kinetic effects are likely not the cause of ’s negative value (Kohyama & Nishinari, 1991), even though heating rates are generally higher than responses in starch systems (Blanshard, 1987). However, the relatively slow heating rate in the main source of our data (0.1 K min-1 for all salts except KI) (Ahmad & Williams, 1999) shows little difference at infinite dilution from the comparatively faster heating rate for KI (10 K min-1) (Jane, 1993). This factor of 100 change in heating rate does not lead to a change in sign of , providing an evidence against the kinetic interpretation.

 Hence, when transitioning from granule gelatinized starch we do not see a marked increase in binding of ions at low concentration, we instead see salt exclusion in effect. These contributions from both anions and cations both lead to a negative . In contrast to the previous hypothesis that the ion binding on the granule state stabilizes this state and hence hinders gelatinization (Jane, 1993), it is the difference in ionic affinity between the gelatinized and granular states as well as its dependence on ionic strength that is crucial in our theory.

It is interesting to note that the weaker co-solute binding on the gelatinized state can also be observed for sugars (Table 4), which also exhibit dominant, negative values as the driving force for the suppression of gelatinization. (Sugars in fact show no decrease (Ahmad & Williams, 2000)). Sugars are widely known to be preferentially excluded from the biomolecular surfaces, through which more compact conformational states (native protein structures, aggregates or gels) are stabilized (Shimizu et al., 2017). The parallel roles of sugars and salts on gelatinization suggests that the structural properties of the granule in its gelatinized state may play a key role in how co-solutes modulate gelatinization.

**4. Starch gelatinization under increased salt concentration**

**4.1. The origin of the maxima**

We have shown that the salt effect on starch gelatinization at is dominated by a large negative change of , which originates from a significant reduction of salt-starch interaction when the starch gelatinizes. Here we investigate how changes with the concentration of salts, aiming at drawing a molecular picture from the calculated KBIs. Because of the lack of literature data on along salt concentrations of different salts, we cannot use Eqs. (3) and (4) to separate and . However, it is possible to calculate the difference via Eqs. (4) and (11) from for all salts. Figures 2 and 3 show that goes through a maximum in all salts studied except Na2SO4. Consequently, at the corresponding salt concentration, changes its sign from negative to positive. This KBI sign change will be rationalized in this section on a molecular basis.

Figure 3 demonstrates that preferential salt-starch binding, signified by a positive value, promotes gelatinization. Preferential salt-starch binding occurs when changes in salt ion binding overcomes the contributions from changes in exclusion and starch-water interaction. Changing the anions significantly effects the dependence of on concentration, whereas changing the cation does not, as demonstrated by the grouping of anions in Figure 3. This corroborates the evidence in the literature suggesting that anion binding changes are most important in determining overall binding changes (Ahmad & Williams, 1999; Jane, 1993; Lii et al., 2002), and can also inform our understanding of the mechanism:

* Salts with I- anions’ values start negative and quickly become positive with addition of salt. These anions are capable of forming inclusion complexes with starch and overcome the exclusion forces to pass more easily into the granule (Saenger, 1984). Therefore, penetrated anions promote gelatinization to occur from the hilum outward, as it does in pure water (Chen et al., 2009; Lii et al., 2002; Manca et al., 2015).
* Salts with Cl- anions require a higher concentration to overcome the exclusion forces as demonstrated by Figure 3. This suggests that they would be more likely to bind externally or just inside the channels. Salt ions therefore have less access to starch molecules within the granule and promote gelatinization from the periphery. This peripheral gelatinization has been seen in LiCl and CaCl2 most notably (Jane, 1993; Wang et al., 2007).
* Finally SO42- anions, within the data range studied (Ahmad & Williams, 1999), do not overcome the exclusion forces and, from Figure 2, do not reduce the at any salt concentration. This suggests that their inability to penetrate the granule, or to access enough starch for significant binding (due to exclusion), prevents them from assisting in gelatinization.

**4.2. Salt accessibility to and association with starch**

The changes that occur in can therefore be attributed to the salt’s accessibility to the starch rather than water’s, which is always present in the hilum. Indeed, the increasing indicates that salt-gelatinized affinity becomes stronger than salt-granular at higher salt concentrations. Supporting this with the following spectroscopic, microscopic and swelling data in the literature can give us a stronger interpretation of what is occurring.

Firstly, increased salt accessibility to the gelatinized state at higher salt concentrations is possible when there is a significant salt-dependent structural change that opens up the gelatinized surface or strong salt ion binding. Experimental evidence for this comes from the salt-dependent swelling change observed by DSC (Zhu, Gayin, Chatel, Dewettinck, & Van der Meeren, 2009) and optical microscopy (Liu, Lelievre, & Ayoung-Chee, 1991; Takahashi, Shirai, & Wada, 1981). Secondly, the strength of conjugation of anions with their cations reduces the anion binding in gelatinized starch (Lii et al., 2002). This discovery, which is based on short range NMR measurements, still holds true for longer ranges as quantified by FAST, via , in Figure 4.

This term signifies preferential salt self-association for the bulk solution in this system, thereby supporting this relationship between bulk phase ion self-association and the binding strength of the anion. As increases, in Figure 4, we can observe this relationship:

- Na2SO4 shows strong self-association, and consequently does not demonstrate dominant binding in the gelatinized state.

- NaCl and NaI show minor self-association with very minor dissociation at higher c2. Their gelatinized state binding increase requires more salt ions than potassium salts.

- KCl and KI initially show a change from minor self-association to minor self-dissociation. They demonstrate faster increases in gelatinized state binding.

This further supports the dominance of salt-starch binding effects and helps to explain the mechanism behind .sign changes. It should be noted that salt accumulation itself is a secondary effect and does not directly change the sign of .

**5. Advantages over the current polymer theory**

**5.1. Quantifying co-solute exclusion**

We have shown that the change from co-solute exclusion from the gelatinized state at lower concentration to co-solute binding is responsible for the existence of the peak in . Indeed, all salts in dilution studied in this paper counteract gelatinization because they are more excluded from the gelatinized state than from the granular state. Such exclusion can be quantified as the negative contribution to KBIs. In contrast, the approaches based on polymer theory relies on the Flory parameter (Clark, 2000; Habeych et al., 2009), which is the difference in the contact energies. Because parameters are based solely on contact energies in the lattice model, it cannot easily deal with the free energy contribution arising from the exclusion of co-solutes, as has been recognized in biophysics for a long time in terms of the crowding effect. The theory of co-solutes based on KBIs can naturally handle crowding and binding alike.

**5.2. Other processes involving starch**

The advantages of the approach proposed here for gelatinization can naturally be extended to other processes involving starch, such as retrogradation and the salt effect thereupon. The prevalent approach in the literature is to start from the starch-water system as the reference state, and to consider how salt affects starch hydration (Fu, Chen, Luo, Liu, & Liu, 2015). According to this view, salt affects retrogradation by predominantly disturbing hydrogen bonding and water structure, which is a view similar to gelatinization (Katsuta, Miura, & Nishimura, 1992). Still, as we have shown in this paper, a presumed link between salt-induced hydration changes and the state change in starch have never been linked in a quantitative manner. However, a potential difficulty in extending our current approach to retrogradation would be the two-step characteristics of the retrogradation process due to the different crystallization behaviours of amylose and amylopectin (Alcázar-Alay & Meireles, 2015).

**6. Conclusion**

Controlling starch gelatinization by salts, despite its central importance in our culinary life, has been poorly understood on a molecular basis. The path towards understanding was hampered by the lack of a theoretical framework that can extract microscopic information from experimental data in a quantitative manner. However, a recent development in statistical thermodynamics (Fluctuation Adsorption-Solvation Theory) has made it possible to extract starch-water and starch-salt affinity changes that accompany gelatinization directly from calorimetry (Shimizu et al., 2017).

Our theory has attributed the suppression of gelatinization at low salt concentrations to the exclusion of salts from the gelatinized state. Such an exclusion effect, recognized in biophysics as one of the dominant roles of some co-solutes, has been beyond the reach of the polymer theory (Habeych et al., 2009), for whom the interaction is defined in terms of the contact energy differences.

The gelatinization temperature goes through maximum at higher salt concentrations, whose cause has been identified as the increase of salt binding on the gelatinized state, as has been inferred by experiments (Jane, 1993). The increase of salt binding is tied to salt accessibility to the inside of the starch granule. This accessibility has been shown to inform whether gelatinization progresses from the hilum or the periphery of the starch granule, in support of evidence presented by Jane. The negligible contribution of changes in water effects demonstrated here does not agree with the traditional approaches that have attempted to attribute the salt effect to the change of starch hydration.

The methodology from the present study could also be applied to the other important processes involving starch, such as retrogradation, when the process complexity has been sufficiently addressed. This is possible due to the general nature of this theory and its application to transition processes.

**Appendix**

Based on a simple order-of-magnitude argument, here we show that the granule-granule interaction is likely to be negligible, despite the seemingly large concentration of starch (100 g dm-3 or 333 g dm-3) used in the DSC experiments (Ahmad & Williams, 1999; Jane, 1993). Starch takes a granular form, and the granules are dispersed throughout the aqueous solution. The average diameter of a sago starch granule is approximately 30 (Ahmad et al., 1999). Hence the granular volume, , is

 (A1)

Amylose is generally taken as the amorphous component in starch, whereas amylopectin forms crystallites which contribute to the crystalline component in starch (Bertoft, 2017; Waigh et al., 1997). Using this information, along with composition measurements of sago starch (Ahmad et al., 1999; Donald, Kato, Perry, & Waigh, 2001), we can approximate that a granule consists of the following:

1. Crystalline layers, which account for about 50 – 65 % of the mass and have a density of ca. 1.2 g cm-3;
2. Amorphous layers, which account for about 25 – 30 % of the mass with the density of ca. 0.5g cm-3.
3. The remaining proportion of water, density *ca.* 1 g cm-3.

Based on the above, the mass of a single granule , can be roughly estimated as:

 (A2)

In the case of the most concentrated solution (333 g dm-3), the number of granule particle per dm3 is:

 (A3)So distance between the granule particles are in the order of:

 (A4)which is much larger than the average diameter of the granule particle. Granule molecules can therefore be considered isolated.

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