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Enzyme modification of starch with amylomaltase results in increasing gel melting point

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ABSTRACT

Melting properties of gelatin-based gels are fundamental for their functionality. With the aim at generating gelatin-like starch-based systems, thermodynamic properties of 20% (w/w) gels of 51 amylomaltase-(AM) (4-α-glucanotransferase; E.C. 2.4.1.25) modified starches, 7 non-enzyme-modified starches and 2 gelatins were investigated using differential scanning calorimetry (DSC). AM modification generally increased gel peak temperature (T_p) and enthalpy of transition (ΔH). The increase in T_p for the potato starches was from 65 to 74 °C, whereas for the maize starches it was elevated from 57 to 70 °C. Only for the combined AM and branching enzyme (BE) modified pea starches decreased T_p (from 79 to 61 °C) was obtained. This effect was followed by a decreased gel formation and hence a fully gelatin comparable gel was not obtained. A two-component principal component analysis (PCA) model of the entire DSC dataset revealed the gross features indicating the ΔH information. The T_p was highly correlated to the amylopectin chain length distribution. In particular, T_p was found to be negatively correlated to short chains (DP 11–21) and positively correlated to long chains (DP 60–80).

1. Introduction

Starch is widely used in the food industry. The majority of the industrial starch is extracted from maize, tapioca, potato, and wheat sources. Most starches can form gels, but lack the property of reversible phase transition of a defined gel state to a liquid (solution) upon heating – a property termed thermoreversibility. A classic example of a thermoreversible polymer widely used in the food industry is gelatin that is an animal protein derived from skin, connective tissue and bones (Djagny, Wang, & Xu, 2001). A unique ability of gelatin gels is its melting around human body temperature making aroma release optimal and accountable for the appetizing mouth feeling of food products containing gelatin (Renard, van de Velde, & Visschers, 2006).

A plant and chemical-free alternative to gelatin is amylomaltase-(AM) (4-α-glucanotransferase; E.C. 2.4.1.25) modified starches that is expected to find application in the food industry (Euverink & Binnema, 2005). AVEBE launched in 2007 an AM-modified potato starch that is used as fat replacer and enhancer of creaminess in yoghurt (Alting et al., 2009). The search for a gelatin-replacer has been ongoing for many years, and several potential polysaccharide-based alternatives for the food industry have been reviewed (Karim & Rajeev, 2008). Modifying potato and rice starch with AM from *Thermus thermophilus*, *Pyrobaculum aerophilum* and *Thermus scotoductus* results in products with acquired thermoreversibility gel characteristics (Kaper et al., 2005; Lee, Kim, Park, & Lee, 2006; van der Maarel et al., 2005a). Gels of potato starch modified with AM from *P. aerophilum* was 50% melted at 37 °C and completely melted at 60 °C (Kaper et al., 2005). AM catalyzes the intermolecular transfer of a segment of a α-1,4-D-glucan to a new 4-position in another α-1,4-D-glucan in a disproportionation reaction. The basis behind the gained thermoreversibility and increased gel texture is supposedly a direct effect of the quite dramatic molecular changes catalyzed by AM including the disappearance of free amylose, broadened amylopectin chain distribution and decreased molecular weight (Hansen, Blennow, Pedersen, Norgaard, & Engelsen, 2008; Kaper et al., 2005; Lee et al., 2006; van der Maarel et al., 2005). In a recent study, maize starch was granular modified with AM from *Thermotoga maritima* causing amylose consumption, an almost unaltered amylopectin chain length distribution and products capable of forming gels with thermoreversible gelling property (Oh, Choi, Lee, Kim, & Moon, 2008).

DSC is a useful technique for monitoring thermal transitions of solids or gels. In a typical DSC experiment, the difference in energy input into the sample and reference is measured as a function of temperature (Watson, Justin, Brenner, & Oneill, 1964). For most gel systems, the phase transition is endothermic and the endotherm, ΔH, is

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Table 1
An overview of the starch and gelatin samples. Parent starches are marked in italics.

Sample No.	Enzyme modification[a]		Mean CL[b,c]	Gel texture[d,c]	App. M_w[c]	Spin–spin relaxation times[e]		Thermal data (this study)	
	(Enzyme activity, incubation time)		(DP)	(N s)	(10^6 Da)	Day 1 T_2 (ms)	Day 10 T_2 (ms)	T_p (°C)	ΔH (J/g starch)
Potato									
1	*No enzyme*	*0 h*	*32.0*	*0.02*	*6.8*	*516.3*	*461.0*	*65.2*	*0.23*
2	2 Unit AM/g starch	1½ h	35.0	2.32	25.9	672.0	229.5	68.7	2.94
3	2 Unit AM/g starch	3½ h	37.1	4.51	18.7	438.5	111.7	71.5	3.77
4	2 Unit AM/g starch	5½ h	37.6	4.43	14.8	289.0	95.4	72.9	4.72
5	2 Unit AM/g starch	22 h	36.8	3.88	8.8	173.5	87.5	73.0	4.43
6	10 Unit AM/g starch	15 min	34.8	1.20	18.0	657.7	245.2	68.8	4.06
7	10 Unit AM/g starch	45 min	36.0	4.14	11.2	307.3	95.6	71.2	3.79
8	10 Unit AM/g starch	1½ h	37.0	3.65	6.8	190.4	91.5	72.9	4.16
9	10 Unit AM/g starch	2½ h	37.7	3.73	4.7	158.5	89.5	73.4	3.27
10	10 Unit AM/g starch	3½ h	37.6	3.33	3.9	150.7	88.4	73.2	3.10
11	10 Unit AM/g starch	20 h	36.4	2.68	2.1	141.0	89.8	74.0	3.26
12	100 Unit AM/g starch	1½ h	36.2	1.84	0.4	132.4	91.7	74.0	3.42
13	100 Unit AM/g starch	3½ h	37.8	1.72	0.3	111.0	91.3	75.2	2.41
14	100 Unit AM/g starch	5½ h	37.8	1.65	0.2	90.9	75.5	73.9	3.93
15	100 Unit AM/g starch	22 h	37.0	1.46	0.1	100.8	86.1	73.2	3.38
16[f]	10 Unit AM/g starch	15 min	33.9	1.29	2.3	329.9	190.9	61.0	2.73
17[f]	10 Unit AM/g starch	45 min	35.6	3.50	19.7	471.2	137.1	70.2	4.46
18[f]	10 Unit AM/g starch	1½ h	35.8	4.98	22.8	260.0	98.6	72.5	4.81
19[f]	10 Unit AM/g starch	3½ h	38.0	4.63	13.4	176.1	92.7	73.1	3.95
20[f]	10 Unit AM/g starch	5½ h	37.6	4.30	9.6	138.3	88.9	73.7	3.83
21[f]	10 Unit AM/g starch	22 h	37.0	3.96	6.7	137.5	87.5	73.5	3.88
Gelamyl 120									
22	*No enzyme*	*0 h*	*33.3*	*0.87*	*0.5*	*404.8*	*196.7*	*60.7*	*3.49*
23	10 Unit AM/g starch	1½ h	34.7	0.86	0.6	373.9	172.2	69.6	2.71
24	10 Unit AM/g starch	3½ h	34.5	0.84	0.6	354.2	174.8	68.5	3.34
25	10 Unit AM/g starch	5½ h	34.3	0.84	0.6	315.6	164.9	68.6	3.69
26	10 Unit AM/g starch	22 h	34.6	0.72	0.6	281.5	155.9	69.2	3.70
HAP									
27	*No enzyme*	*0 h*	*35.0*	*9.05*	*1.0*	*113.4*	*98.0*	*76.9*	*0.49*
28	2 Unit AM/g starch	1½ h	37.9	11.52	5.7	90.6	88.0	82.0	0.39
29	2 Unit AM/g starch	3½ h	40.6	10.51	5.7	90.8	89.5	83.3	0.18
30	2 Unit AM/g starch	5½ h	41.2	10.43	5.8	90.2	89.2	82.6	0.17
31	2 Unit AM/g starch	22 h	39.5	10.09	5.2	88.8	88.3	85.9	0.17
Maize									
32	*No enzyme*	*0 h*	*28.1*	*0.62*	*3.9*	*246.7*	*229.0*	*56.8*	*0.53*
33	10 Unit AM/g starch	15 min	32.3	0.37	14.6	327.9	262.2	61.0	1.11
34	10 Unit AM/g starch	45 min	33.3	1.02	18.3	329.1	203.8	65.5	2.70
35	10 Unit AM/g starch	1½ h	33.5	2.72	22.4	304.4	153.8	66.4	3.24
36	10 Unit AM/g starch	3½ h	34.5	2.96	29.2	284.2	134.3	69.5	3.90
37	10 Unit AM/g starch	5½ h	34.1	2.91	26.8	294.7	127.7	70.3	3.74
38	10 Unit AM/g starch	22 h	34.5	3.26	29.2	286.6	125.5	70.1	4.03
Waxy maize									
39	*No enzyme*	*0 h*	*26.5*	*0.01*	*51.2*	*746.5*	*702.8*	*n.d.*	*n.d.*
40	2 Unit AM/g starch	1½ h	29.4	0.00	31.4	762.7	747.1	n.d.	n.d.
41	2 Unit AM/g starch	3½ h	30.1	0.01	17.0	774.1	760.6	n.d.	n.d.
42	2 Unit AM/g starch	5½ h	31.3	0.00	13.6	776.8	765.0	n.d.	n.d.
43	2 Unit AM/g starch	22 h	31.3	0.01	11.9	790.7	778.9	n.d.	n.d.
Pea									
44	*No enzyme*	*0 h*	*28.6*	*3.08*	*4.7*	*233.7*	*183.2*	*62.9*	*2.94*
45	10 Unit AM/g starch	1½ h	37.4	6.15	9.0	82.1	77.6	78.6	2.38
46	10 Unit AM/g starch	3½ h	37.9	4.93	4.6	82.0	77.5	78.1	2.85
47	10 Unit AM/g starch	5½ h	37.7	4.71	3.4	82.0	77.8	78.5	2.67
48	10 Unit AM/g starch	22 h	36.6	3.30	1.3	85.8	80.4	78.8	2.13
49[g]	AM/BE combined	0 h	37.4	6.87	12.5	82.4	78.9	79.4	2.42
50[g]	AM/BE combined	1½ h	34.3	2.61	1.5	101.4	81.1	74.7	2.31
51[g]	AM/BE combined	3½ h	29.5	0.37	1.0	285.1	135.7	69.4	4.11
52[g]	AM/BE combined	5½ h	27.3	0.02	0.8	392.3	274.9	61.0	1.10
53[g]	AM/BE combined	22 h	22.0	0.02	0.6	476.8	405.2	n.d.	n.d.
Wheat									
54	*No enzyme*	*0 h*	*25.4*	*2.27*	*17.1*	*168.5*	*164.6*	*49.0*	*0.28*
55	2 Unit AM/g starch	1½ h	28.6	0.05	19.4	260.7	227.1	61.3	0.31
56	2 Unit AM/g starch	3½ h	29.9	0.09	12.1	249.9	193.8	66.3	1.51
57	2 Unit AM/g starch	5½ h	30.7	0.15	10.0	236.8	176.3	68.6	0.91
58	2 Unit AM/g starch	22 h	30.2	0.40	5.3	239.9	155.8	69.4	2.34
Gelatin									
59	–	–	–	4.16	–	481.3	420.4	26.8	19.6
60	–	–	–	6.16	–	400.8	355.8	27.6	23.0

One unit of AM activity is defined as the release of 1 μmol of glucose per minute at pH 6.5, 60 °C with maltotriose as substrate, and one unit of BE is defined as the amount of enzyme that can decrease A_{660} of the amylo-iodine complex by 1% per minute at 60 °C pH 7.0. AM, amylomaltase; BE, branching enzyme; T_p, peak temperature; ΔH, enthalpy of transition; n.d., not detected.

(continued on next page)

Table 1 *(continued)*

[a] After autoclavation for 30 min at 140 °C, starches were modified at 85 °C.
[b] Amylopectin mean chain length (CL).
[c] From Hansen et al. (2008).
[d] Gel texture indicates the *force of deformation*, which is the area of compression during texture analysis of 12% gels (w/w) after storage at 4 °C for 24 h.
[e] Measured by LF NMR.
[f] Starches were modified at 70 °C.
[g] Pea starch was modified with 10 unit AM/g starch for 2 h in order to 'remove' amylose, resulting in sample # 49. Meanwhile maintaining the AM activity, # 49 was further modified with 1000 unit BE/g starch for 1½–22 h at 60 °C (resulting in # 50–53).

recorded as the peak area and peak temperature (T_p) is recorded as the temperature at maximum enthalpy. For starch, both these parameters are typically interpreted "crystallinity" (ΔH) and molecular perfection or order (T_p) (Kozlov, Blennow, Krivandin, & Yuryev, 2007). Native starches show complex thermal profiles that depend on their botanical origin. Moreover, the ratio between the amylopectin and amylose fractions affects the melting behavior considerably and the onset temperature of recrystallized amylopectin and amylose is above 40 and 90 °C, respectively (Creek, Benesi, Runt, & Ziegler, 2007; Sievert & Pomeranz, 1990). For starch gels, the main phase transitions originate mostly from disentanglement of double helical junction zones formed by adjacent chains (Gidley, 1989).

The purpose of this investigation was to study the thermal properties of gels prepared from starches of various botanical origin after being subject to enzyme modification with AM from *T. thermophilus* (Kaper, van der Maarel, Euverink, & Dijkhuizen, 2004; van der Maarel et al., 2005). Some starches were also subjected to combined modification with branching enzyme (BE) (1,4-α-D-glucan branching enzyme; EC 2.4.1.18) from *Rhodothermus obamensis* (Shinohara et al., 2001). Data was primarily analyzed using chemometrics (Daszykowski, Kaczmarek, Heyden, & Walczak, 2007) permitting extraction of applicable and/or hidden information in the data set, and providing relationships with data from previous studies (Hansen et al., 2008; unpublished results).

2. Materials and methods

2.1. Materials

Potato, high amylose potato (HAP), maize, waxy maize, wheat and pea starches and Gelamyl 120 (chemical oxidized potato starch) were enzyme-modified using mainly amylomaltase (AM) isolated from the hyperthermophilic bacterium *T. thermophilus* (Kaper et al., 2004; van der Maarel et al., 2005). In one time-course experiment branching enzyme (BE) isolated from the thermophilic bacterium *Rhodothermus obamensis* (Shinohara et al., 2001) was used in a combined modification with AM. Gelatin 1 (bloom 120) and gelatin 2 (bloom 300) were from Sigma–Aldrich (www.sigma-aldrich.com). An overview of the samples is given in Table 1.

2.2. Preparation of enzymes

AM and BE used in this study was expressed in the *Bacillus subtilis* high secretor strain A165Δ5 as described (Widner et al., 2000). Expressed and excreted protein was concentrated by ultrafiltration and used directly as enzyme source. Transferase kinetics for these highly concentrated starch systems and lack of significant hydrolytic activity was confirmed for both enzyme preparations used in this study (Hansen et al., 2008).

2.3. Differential scanning calorimetry (DSC)

Gel melting temperatures were examined by DSC using a Pyris Diamond DSC instrument (PerkinElmer, www.perkinelmer.com). Approximately 10 mg of a sample (dry weight basis) was weighed in high-pressure stainless steel pans (24 atmospheres; 60 μl; operating in the range from minus 40 to plus 300 °C; PerkinElmer). Phosphate buffer (50 mM, pH 7.0) was added to make a suspension of 20% (w/w). The pans were sealed and heated for 15 min at 120 °C in the instrument, and subsequently stored at 4 °C for 24 h allowing formation of a gel network. The samples were scanned from 1 to 150 °C at 10 °C/min. Experiments were performed in duplicates. An empty stainless steel pan was used as reference. The amylose melting information in the 90–150 °C region is very dependent on the presence of lipids as helical amylose–lipid inclusion complexes having higher endothermic transition than free amylose (Creek et al., 2007). However, no data was recorded in this region, and hence only melting of amylopectin segments was recorded. Thermal profiles were analyzed using Pyris Software version 7.0 software (PerkinElmer, www.perkinelmer.com) and the peak temperature (T_p) and the enthalpy of transition (ΔH) were calculated (see Table 1).

2.4. Data mining

Principal component analysis (PCA) (Hotelling, 1933; Wold, Esbensen, & Geladi, 1987) and partial least squares (PLS) regression (Geladi & Kowalski, 1986) was carried out by use of MatLab 7.2 (The Mathworks Inc., USA, www.mathworks.com) installed with the PLS toolbox version 3.5.3 (Eigenvector Research, www.eigenvector.com). Data was either mean centered or autoscaled (Bro & Smilde, 2003) prior to building PCA models. All PLS models were mean centered and leave-one-out cross validated (Stone, 1974; Wold, 1978). The optimal number of PLS components was determined from the root mean square error of cross-validation (RMSECV). Figs. 2 and 5 were created using LatentiX (Latent5, Copenhagen, Denmark, www.latentix.com).

2.4.1. Principal component analysis (PCA)

PCA is a technique for data compression, extraction of the main variation (information) and data visualization (Hotelling, 1933; Wold et al., 1987). The systematic variation is described by the principal components (PC 1, PC 2 etc.). In PCA, the original data **X** (samples × variables) is decomposed into a score matrix (**T**) and a loading matrix (**P**): $\mathbf{X} = \mathbf{T}\mathbf{P}^{T}$. The PCA is visualized with score plot (describes the relationship between samples) and loading plot (describes the relationship between variables). Samples and variables located very close to each other in the score and loading plot, respectively, are highly correlated.

2.4.2. Partial least squares (PLS) regression

PLS regression (Geladi & Kowalski, 1986) is used to model the relationship between two data sets: the predictor matrix **X** (e.g. amylopectin chain length distribution) and a property of interest **Y** (e.g. gel melting point). PLS performs a simultaneous decomposition of **X** and **Y** in such a way that the information in the **Y** is directly used as a guide for the decomposition of **X**. The linear regression model is defined as $\mathbf{Y} = \mathbf{X}b + E$, where b is the regression

coefficient and E is the residuals (model errors). The model performance is given by the cross-validated correlation factor (R^2), which describes the correlation between the measured **Y** and the predicted **Y**, and by the prediction error RMSECV.

3. Results

3.1. Thermal data for 20% (w/w) gels

The endothermic peaks for melting of recrystallized amylopectin appeared over a broad temperature range. The widths of the melting transition as deduced from the difference between the onset and conclusion temperature of both AM-modified potato (Fig. 1A) and maize (Fig. 1B) starches appeared over a ~45 °C range (from about 40 to 85 °C), whereas melting of the AM-modified pea starches was narrower (~25 °C range spanning from 65 to 90 °C) (Fig. 1C). Likewise, the melting range for gels of AM-modified HAP starches was wide (75–90 °C, data not shown).

The peak temperature (T_p) of recrystallized amylopectin of all the starches ranged from 49.0 to 85.9 °C, and the enthalpy of transition (ΔH) ranged from 0.17 to 4.81 J/g starch. For the waxy maize starches (# 39–43) and extended combined AM/BE-modified pea starch (# 53), the T_p and ΔH were not detectable even at a starch concentration as high as 20% (w/w).

The effects of AM modification caused a general increase in both T_p and ΔH as compared to its corresponding parent starch gels. For example, modification of parent potato starch (# 1) using 2 unit AM/g for 1½ h starch increased T_p from 65.2 to 68.7 °C. Additional

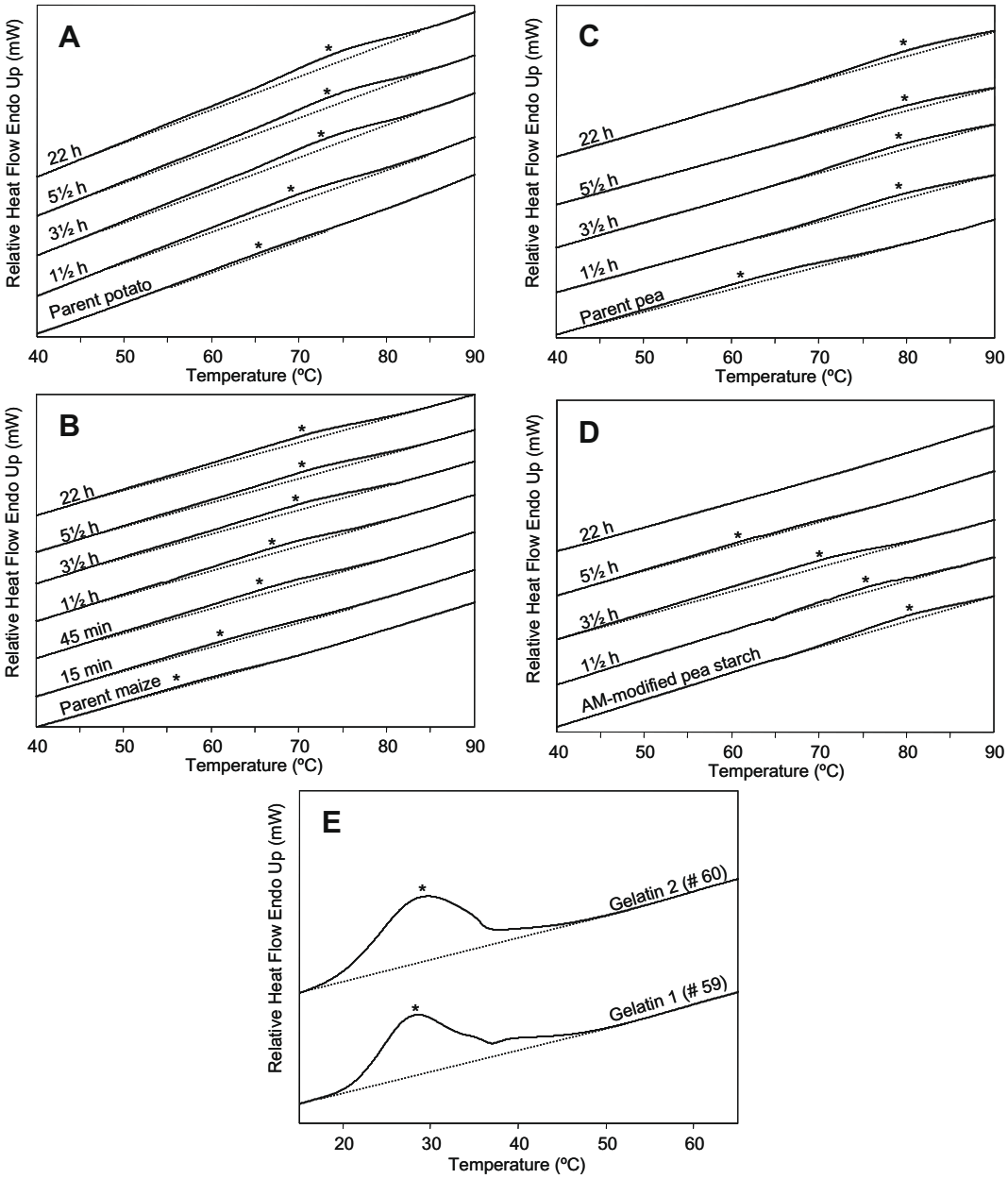

Fig. 1. Differential scanning calorimetry (DSC) traces showing the thermal profiles of potato starch modified with 2 unit AM/g starch for 0–22 h (A), maize starch modified with 10 unit AM/g starch for 0–22 h (B), pea starch modified with 10 unit AM/g starch for 0–22 h (C), pea starch combined AM (10 unit/g starch) and BE (1000 unit/g starch) modified (D) and gelatins (E) at 20% (w/w). The gels were prepared directly in the DSC pans by heating for 15 min at 120 °C. After 24 h storage at 4 °C, the samples were scanned from 1 to 150 °C at a scan rate of 10 °C/min. Note that temperature scale in *E* is different as compared to A–D.

treatment increased T_p to 73 °C and resulted in a 19-fold increase in ΔH (Fig. 1A). Extensive modification did not significantly change the T_p (Table 1). Comparable result was found for the maize system, although the parent maize starch (# 32) had a lower T_p of 56.8 °C as compared to the potato system. Modified maize starch also gradually increased both T_p and ΔH with enzyme treatment (Fig. 1B). Extended treatment of maize starch increased T_p from 56.8 to 70.1 °C and ΔH nearly 8 times. Similarly, for the pea starch system, products with T_p ranging from 62.9 to 78.8 °C were generated after extended enzyme treatment (Fig. 1C). Only for combined modification of pea starch with AM and BE generating shorter chains, it was possible to suppress T_p as function of enzyme treatment (Fig. 1D). After 5½ h combined AM/BE treatment, T_p decreased by nearly 20 °C from 79.4 to 61.0 °C, and additional treatment result in a product with no detectable transition even at 20% (w/w). Hence, the CL of the amylopectin is clearly important for the generation of crystalline segments in the gels.

As compared to the starch samples the two gelatins had extreme transitions about 22–64 °C lower T_p (26.8–27.6 °C) and 5–100 times higher ΔH (19.6–23.0 J/g gelatin) (Fig. 1E and Table 1).

3.2. Data mining

Analysis of systematic variations in the raw DSC dataset of all starches was performed using PCA modeling (Hotelling, 1933; Wold et al., 1987). Gelatin data were excluded as they are extremes with regards of having very low T_p and high ΔH compared to the starches. A PCA score plot for a model based on the first two principle components (PCs) describing 95.9% of the total variation clearly demonstrates the thermal relation between the samples (Fig. 2). Samples that cluster have comparable thermal properties, whereas disperse samples are dissimilar. The PC 1 in the PCA score plot describes well the variation in ΔH. The PCA also demonstrates the similar thermal properties between the majorities of the AM-modified potato starches. The entire AM-modified pea and all the HAP starches group in separate clusters. The discrimination of the AM-modified potato starches located in upper right corner from the samples located in the lower right corner, both having comparable ΔH, is the slightly higher T_p (~5 °C), which partly described by PC 2.

To examine possible correlation between the thermal data and previously obtained data (Hansen et al., 2008), a PCA model was

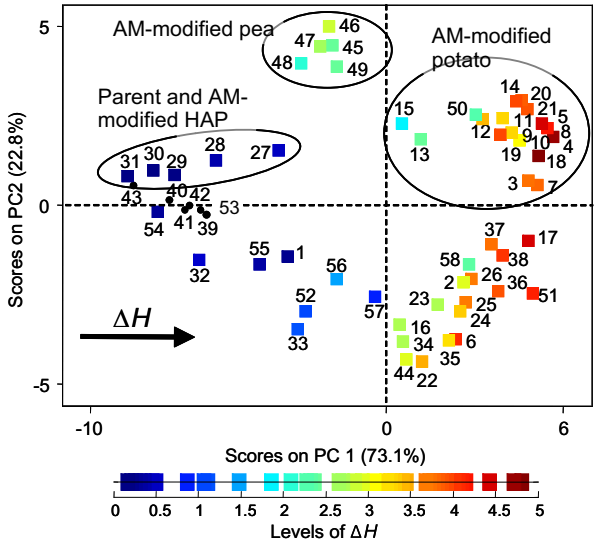

Fig. 2. PCA score plot colored according to levels of ΔH for the first two PCs of the raw DSC data from 40 to 90 °C of all starches. Prior to PCA calculation, data was detrended (Barnes, Dhanoa, & Liu, 1989) then mean centered, in order to get a better representation of the data. The two PCs explain 95.9% of the total variation. Waxy maize starches (# 39–43) and pea starch modified with combined AM/BE treatment for 22 h (# 53) having no detectable ΔH are marked with black dots.

calculated using DSC descriptors (ΔH and T_p), gel-texture descriptors (*adhesion I–II*, *hardness* and *force of deformation*), amylopectin mean CL, apparent molecular weight (M_w) and spin–spin relaxation time constants (T_2) day 1 and day 10 data (Fig. 3). Data for waxy maize starches (# 39–43) and pea starch modified with AM/BE for 22 h (# 53) were excluded in the model as the T_p and ΔH could not be determined for these starches. The first two PCs in this model explain 74.1% of the total variation. Also in this score plot, AM-modified potato, pea and HAP starches separate from the rest of the samples (Fig. 3A). As evident from the close clustering in the loading plot of the HAP starches scoring high in PC 2 and low in PC 1 these starch types are characterized by having high gel texture, high amylopectin mean CL and high T_p, (Fig. 3B). Moreover, they have low ΔH and low T_2 as judged from the clear discrimination in the plot. The PCA

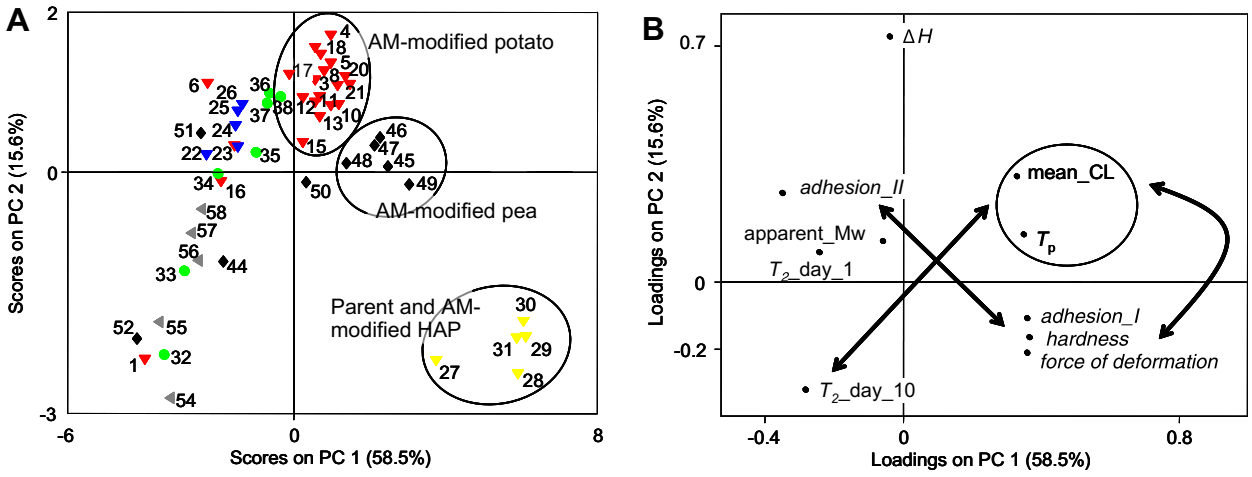

Fig. 3. PCA model of the autoscaled descriptors: T_2 relaxation times (day 1 and 10), gel texture (*force of deformation*, *hardness*, *adhesion I–II*), amylopectin mean CL, apparent M_w and DSC data (ΔH and T_p) for all the starches except for the high AM/BE-modified pea starch (# 53) and waxy maize starches (# 39–43). Score plot (A) for PC 1 vs. PC 2 and corresponding loading plot (B). The two PCs explain 74.1% of the total variation. Starch samples are colored according to botanical origin: potato (red ▼); Gelamyl 120 (blue ▼); HAP (yellow ▼); maize (light green ●); pea (black ♦); wheat (gray triangle). Black arrows indicate the correlations between the descriptors. (For interpretation of color mentioned in this figure legend the reader is referred to the web version of the article.)

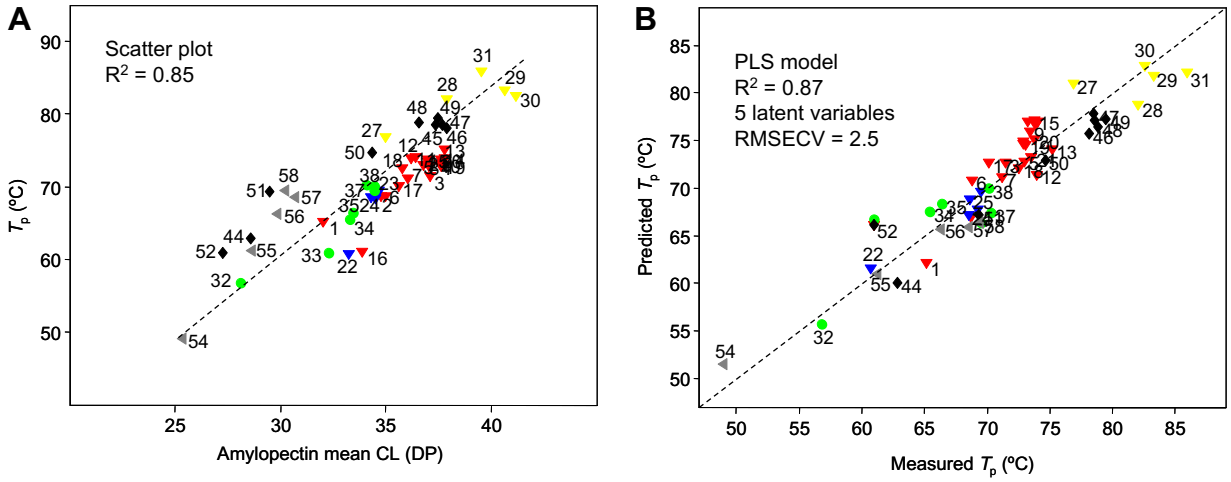

Fig. 4. Correlations between amylopectin CL and T_p data. Scatter plot of amylopectin mean CL vs. T_p (A). Five-component PLS model predicting the T_p from the amylopectin CL distribution profiles (B). Waxy maize starches (# 39–43) and pea starch modified with AM/BE for 22 h (# 53) were excluded. Starch samples are colored according to botanical origin: potato (red ▼); Gelamyl 120 (blue ▼); HAP (yellow ▼); maize (light green ●); waxy maize (dark green ●); pea (black ♦); wheat (gray triangle). (For interpretation of color mentioned in this figure legend the reader is referred to the web version of the article.)

loading plot reveals multiple relations between the variables. Correlations between amylopectin CL and gel texture and T_2 relaxation data have previously been discussed (Hansen et al., 2008). These effects are again well visualized (indicated by black arrows) in the loading plot. The ΔH variable is described by PC 2 and does not show any correlation to the rest of the variables. A high positive correlation between T_p and amylopectin mean CL was found, as they are located in close proximity to each other. Consequently, the effect of increased average chain length in amylopectin molecules cause an increase in T_p in gels. The correlation is linear having a correlation factor (R^2) of 0.85, which is depicted in the scatter plot of amylopectin mean CL vs. T_p (Fig. 4A). The correlation between amylopectin CL and T_p was further analyzed by partial least squares (PLS) regression (Geladi & Kowalski, 1986). Instead of using an average chain length number, PLS is able to analyze correlation between the entire dataset of amylopectin CL distribution and T_p. A five-component PLS model gave a correlation of R^2 = 0.87 between actual T_p and T_p predicted from all the amylopectin CL distribution data, and gave a root mean square error of cross-validation (RMSECV) of 2.5 °C (Fig. 4B). Although using 5 components (latent variables), a slightly improved model was obtained using PLS. Using this model, it was possible to predict T_p of waxy maize starches (# 39–43) and pea starch modified with AM/BE for 22 h (# 53), which was not included in the model. The parent waxy maize starch is predicted to have a T_p of ~56 °C, and extended AM modification for 22 h is predicted to increase T_p to ~68 °C. Extended combined AM/BE-modified pea starch is predicted to have T_p to ~57 °C, which seem reasonable when comparing to the rest of the starches from the enzyme modification time-course (compare # 53 with # 49–52 in Table 1).

Fig. 4 shows that the whole distribution of chain in amylopectin molecules is correlated to the T_p. With the aim of exploring the relationship of individual chains in the amylopectin molecule, a correlation factor distribution was calculated. Fig. 5 shows the amylopectin CL distribution of all starches overlaid with the R^2 distribution (in red) for each DP going from 4 to 80. High correlation was found for chains with a specific length. Fig. 5 shows that T_p was highly correlated to short chains (DP 11–21) as well as to long chains (DP 60–80) both having R^2 around 0.81–0.85. The short chains are found to be negatively correlated to T_p, whereas long chains (DP 60–80) are positively correlated. No relationship between T_p and very short chains (below DP 9) and middle length chains (DP 26–35).

4. Discussion

In this study, we have used DSC to analyze and compare the thermal profiles of 20% (w/w) gels prepared from amylomaltase- (AM) modified starches from various botanical origins and gelatins. AM-modified starches are expected to find application in the food industry because products obtain thermoreversible character with gelatin-like properties (Euverink & Binnema, 2005). Even though 20% gels are rather concentrated in most food applications this concentration allowed us to directly compare gelation and crystallization over the range of very different structures. There are several factors important for the perception of gelled food products – the two most important are gel texture and flavor release (Renard et al., 2006). A previous study demonstrated that modification of potato and maize starches with AM result in products with

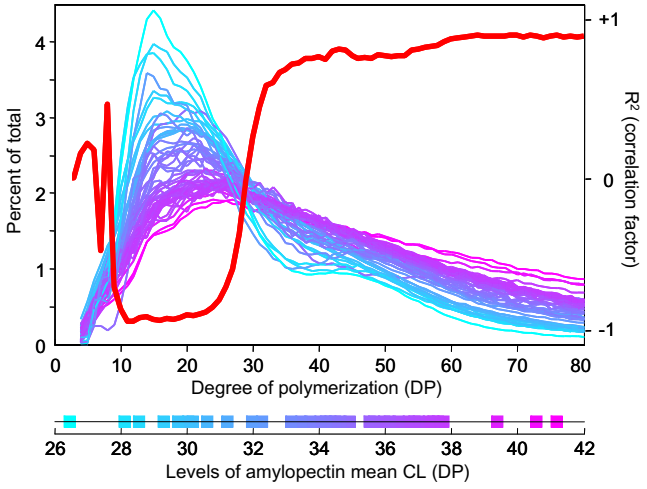

Fig. 5. The amylopectin CL distribution from DP 4 to 80 of all starch samples colored according to the levels of amylopectin mean CL. Red line indicate the correlation factor (R^2) between T_p and each individual chain length in amylopectin. Waxy maize starches (# 39–43) and pea starch modified with AM/BE for 22 h (# 53) were not included in the CL distribution and excluded in the calculation of R^2. (For interpretation of color mentioned in this figure legend the reader is referred to the web version of the article.)

increasing gel texture as a result of increasing longer chains (DP 60–80) in amylopectin molecules (Hansen et al., 2008). Even though AM-modified starches acquire thermoreversible character, the melting temperatures obtained in this study are too high for applications of gelled food product which should be around (or below) body temperature in order to obtain optimal organoleptical properties and aroma release in the mouth. The low melting point is one of many unique ability of gelatin gels. In gelatin gels the molecules resume an ordered triple helix conformation upon cooling by forming a cross-linked network held together predominantly by hydrogen bonded junction zones (Stainsby, 1977). The much higher melting enthalpy of the gelatin gels is due to the stronger interaction and higher content of triple helix structures as compared to the presence of dispersed double helical α-glucan junction zones in starch (Gidley, 1989).

In this study it was found that modifying potato and maize starches with AM resulted in products with increasing peak temperature (T_p) during enzyme treatment (Fig. 1A–B). Typically, AM-modification of parent starches resulted in products with increasing T_p (Table 1), which actually is in the opposite direction of what is preferable to achieve an enzyme-modified starch with gelatin-like melting point. The high amylose potato (HAP) starches had the highest T_p around 90 °C and at the same time they had the lowest enthalpy of transition (ΔH) (below 0.4 J/g starch), whereas parent wheat starch (not enzyme-modified) had the lowest T_p at 49 °C (Table 1). These transition temperatures are 30–70 °C higher than the gelatin samples, which melts at 27–28 °C (Fig. 1E). However, pea starch modified with a combined AM and branching enzyme (BE) strategy resulted in products with lower T_p (Fig. 1D). As an effect of the combined AM/BE modification, ΔH and as well as gel texture decreased and after extended modification, the resulting product was not able to form a gel even at 20% (w/w) (Table 1). Hence, combined modification of starch with AM and BE is certainly ambiguous since it results in the acquired thermoreversibility and decreased T_p, but the decreased gel texture is certainly undesirable. As a result, in food application, AM-modified starches will need additional modification e.g. chemical modification to overcome the drawback of high melting point.

The reason for the increased T_p of AM-modified starches was revealed by PCA (Fig. 3B) which demonstrates that T_p is highly correlated to amylopectin mean chain length (CL). Correlation is linear (Fig. 4A) and interestingly, when examining the individually chains, it was found that short chains around DP 11–22 were negatively correlated to T_p, whereas long chains above DP 60 were positively correlated to T_p (Fig. 5). The opposite correlation between the short and the long chains demonstrates that during AM modification, the sum of long chains increase on the expense of a specific pool of short chains. The chain transfer is amylose-independent as shown previously for AM-modified waxy maize starch (Hansen et al., 2008) demonstrating that chains are transferred within the amylopectin molecules. The data cannot show whether T_p increases as a result of increase in long chains or decrease in short chains. However, short chains can disturb crystallization of the longer chains so the effect can very well be combined. Combined AM/BE-modified pea starches, which was the only treatment which resulted in decreasing T_p, cannot help to clarify this as long chains is converted into short chains (Hansen et al., 2008). Given that AM-modification of starches results in disappearance of amylose, broadened amylopectin CL distribution from a bimodal to an unimodal long chain profile (Hansen et al., 2008), and given that recrystallized amylose melts in the region of 90 to 150 °C (Creek et al., 2007), the most reasonable explanation for the increasing T_p during AM-treatment is that the newly formed longer chains in amylopectin molecules exhibit an amylose-like behavior. The increase in longer chains results in a higher content of longer double helices formation becoming more heat resistant. This is in good agreement with the decrease in T_p during combined AM/BE-modified pea starches. The general broad range of melting of AM-modified HAP starches suggests the presence of a wide distribution of recrystallized chain segments in amylopectin molecules having varying stability. The narrower melting range of the HAP and pea starches indicates a more homogeneous crystal distribution for these type of starches. The ΔH, which is a measure of the overall crystallinity of the amylopectin molecules, was 3–80 times lower compared to the granule/native starch (Blennow et al., 2005; Karlsson, Leeman, Björck, & Eliasson, 2007). Hence, only a fraction of the original molecular order of the starch granules can be recovered after enzyme treatment and re-crystallization (Cooke & Gidley, 1992).

In contrast to our results, Kaper et al. (2005) reported that gels made of potato starch modified with AM from *P. aerophilum* had melting temperature around 37 °C and that the gel was completely melted at 60 °C. No analysis of the thermal profile of the parent potato starch was performed, but we conclude that the T_p must have decreased during this treatment, which is opposite of what we observed and about 30 °C lower than our AM-modified potato starches. They also reported that modifying potato starch with the *P. aerophilum* AM resulted in broadened amylopectin CL distribution that is in accordance with our data. The difference cannot readily be explained from existing data. Comparing the two enzymes, the *P. aerophilum* enzyme shares 43% amino acid identity with the *T. thermophilus* enzyme, is 30 amino acids smaller and has higher temperature optimum. They both share the four conserved short motifs comprising the catalytic residues, including two aspartate and one glutamate (Takaha & Smith, 1999), which is highly conserved in the α-amylase superfamily (MacGregor, Janecek, & Svensson, 2001). The production of enzyme-modified potato starches using the AM from *P. aerophilum* only slightly differed from our study (Hansen et al., 2008; Kaper et al., 2005) and the preparation of the gels prior to DSC analysis was the same. This indicates that very minor differences in the molecular structure of the glucan products are detrimental for gel formation and more detailed studies of the products at the molecular level e.g. the positioning of branch points and existence of longer chains than analyzed here, are required.

5. Conclusion

The effect of modifying starch with amylomaltase (AM) resulted in products generating aqueous gels with increasing peak temperature (T_p) and enthalpy of transition (ΔH) as compared to their corresponding parent samples. Starches modified with combined AM and BE catalysis had the opposite effect i.e. reduced T_p and ΔH. Using PCA, detailed phenomena of the produced gel systems was provided. The T_p was negatively correlated to the short (DP 11–22) and positively correlated to long (DP 60–80) amylopectin chains. Thermoreversible AM-modified starches are expected to have potential for many applications in the food industry. However, to serve as true plant-derived alternatives to gelatin, further suppression of the melting point has to be achieved.

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References

Alting, A. C., van de Velde, F., Kanning, M. W., Burgering, M., Mulleners, L., Sein, A., et al. (2009). Improved creaminess of low-fat yoghurt: The impact of amylomaltase-treated starch domains. *Food Hydrocolloids, 23*, 980–987.

Barnes, R. J., Dhanoa, M. S., & Liu, H. J. (1989). Standard normal variate transformation and detrending of NIR spectra. *Applied Spectroscopy, 43*, 772–777.

Blennow, A., Wischmann, B., Houborg, K., Ahmt, T., Jorgensen, K., Engelsen, S. B., et al. (2005). Structure function relationships of transgenic starches with engineered phosphate substitution and starch branching. *International Journal of Biological Macromolecules, 36*, 159–168.

Bro, R., & Smilde, A. K. (2003). Centering and scaling in component analysis. *Journal of Chemometrics, 17*, 16–33.

Cooke, D., & Gidley, M. J. (1992). Loss of crystalline and molecular order during starch gelatinization – Origin of the enthalpic transition. *Carbohydrate Research, 227*, 103–112.

Creek, J. A., Benesi, A., Runt, J., & Ziegler, G. R. (2007). Potential sources of error in the calorimetric evaluation of amylose content of starches. *Carbohydrate Polymers, 68*, 465–471.

Daszykowski, M., Kaczmarek, K., Heyden, Y. V., & Walczak, B. (2007). Robust statistics in data analysis – A review basic concepts. *Chemometrics and Intelligent Laboratory Systems, 85*, 203–219.

Djagny, K. B., Wang, Z., & Xu, S. Y. (2001). Gelatin: A valuable protein for food and pharmaceutical industries [Review]. *Critical Reviews in Food Science and Nutrition, 41*, 481–492.

Euverink, G. J. W., & Binnema, D. J. (2005). Use of modified starch as an agent for forming a thermoreversible gel. US Patent 6864063.

Geladi, P., & Kowalski, B. R. (1986). Partial least-squares regression – A tutorial. *Analytica Chimica Acta, 185*, 1–17.

Gidley, M. J. (1989). Molecular mechanisms underlying amylose aggregation and gelation. *Macromolecules, 22*, 351–358.

Hansen, M. R., Blennow, A., Pedersen, S., Norgaard, L., & Engelsen, S. B. (2008). Gel texture and chain structure of amylomaltase-modified starches compared to gelatin. *Food Hydrocolloids, 22*, 1551–1566.

Hotelling, H. (1933). Analysis of a complex of statistical variables with principal components. *Journal of Educational Psychology, 24*, 417–441, 498–520.

Kaper, T., Talik, B., Ettema, T. J., Bos, H., van der Maarel, M. J. E. C., & Dijkhuizen, L. (2005). Amylomaltase of *Pyrobaculum aerophilum* IM2 produces thermoreversible starch gels. *Applied and Environmental Microbiology, 71*, 5098–5106.

Kaper, T., van der Maarel, M. J. E. C., Euverink, G. J. W., & Dijkhuizen, L. (2004). Exploring and exploiting starch-modifying amylomaltases from thermophiles. *Biochemical Society Transactions, 32*, 279–282.

Karim A. A., & Rajeev B. (2008). Gelatin alternatives for the food industry: Recent developments, challenges and prospects. *Trends in Food Science & Technology*. doi: 10.1016/j.tifs.2008.08.001.

Karlsson, M. E., Leeman, A. M., Björck, I. M. E., & Eliasson, A. C. (2007). Some physical and nutritional characteristics of genetically modified potatoes varying in amylose/amylopectin ratios. *Food Chemistry, 100*, 136–146.

Kozlov, S. S., Blennow, A., Krivandin, A. V., & Yuryev, V. P. (2007). Structural and thermodynamic properties of starches extracted from GBSS and GWD suppressed potato lines. *International Journal of Biological Macromolecules, 40*, 449–460.

Lee, K. Y., Kim, Y. R., Park, K. H., & Lee, H. G. (2006). Effects of alpha-glucanotransferase treatment on the thermo-reversibility and freeze–thaw stability of a rice starch gel. *Carbohydrate Polymers, 63*, 347–354.

MacGregor, E. A., Janecek, S., & Svensson, B. (2001). Relationship of sequence and structure to specificity in the alpha-amylase family of enzymes. *Biochimica et Biophysica Acta – Protein Structure and Molecular Enzymology, 1546*, 1–20.

Oh, E. J., Choi, S. J., Lee, S. J., Kim, C. H., & Moon, T. W. (2008). Modification of granular corn starch with 4-alpha-glucanotransferase from *Thermotoga maritima*: Effects on structural and physical properties. *Journal of Food Science, 73*, C158–C166.

Renard, D., van de Velde, F., & Visschers, R. W. (2006). The gap between food gel structure, texture and perception. *Food Hydrocolloids, 20*, 423–431.

Shinohara, M. L., Ihara, M., Abo, M., Hashida, M., Takagi, S., & Beck, T. C. (2001). A novel thermostable branching enzyme from an extremely thermophilic bacterial species, *Rhodothermus obamensis*. *Applied Microbiology and Biotechnology, 57*, 653–659.

Sievert, D., & Pomeranz, Y. (1990). Enzyme-resistant starch. 2. Differential scanning calorimetry studies on heat-treated starches and enzyme-resistant starch residues. *Cereal Chemistry, 67*(21), 7–221.

Stainsby, G. (1977). The physical chemistry of gelatin in solution. In A. G. Ward & A. Courts (Eds.), *The Science and Technology of Gelatin* (pp. 109–135). London: Academic Press.

Stone, M. (1974). Cross-validatory choice and assessment of statistical predictions. *Journal of the Royal Statistical Society Series B – Methodological, 36*, 111–147.

Takaha, T., & Smith, S. M. (1999). The functions of 4-alpha-glucanotransferases and their use for the production of cyclic glucans. *Biotechnology and Genetic Engineering Reviews, 16*(16), 257–280.

van der Maarel, M. J. E. C., Capron, I., Euverink, G. J. W., Bos, H. T., Kaper, T., Binnema, D. J., et al. (2005). A novel thermoreversible gelling product made by enzymatic modification of starch. *Starch/Starke, 57*, 465–472.

Watson, E. S., Justin, J., Brenner, N., & Oneill, M. J. (1964). Differential scanning calorimeter for quantitative differential thermal analysis. *Analytical Chemistry, 36*, 1233–1238.

Widner, B., Thomas, M., Sternberg, D., Lammon, D., Behr, R., & Sloma, A. (2000). Development of marker-free strains of *Bacillus subtilis* capable of secreting high levels of industrial enzymes. *Journal of Industrial Microbiology & Biotechnology, 25*, 204–212.

Wold, S. (1978). Cross-validatory estimation of number of components in factor and principal components models. *Technometrics, 20*, 397–405.

Wold, S., Esbensen, K., & Geladi, P. (1987). Principal component analysis. *Chemometrics and Intelligent Laboratory Systems, 2*, 37–52.