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Carbohydrate Polymers 56 (2004) 157–168
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Annealing of normal, low and high amylose starches extracted from barley cultivars grown under different environmental conditions

V.I. Kiseleva[a], N.K. Genkina[a], R. Tester[b,*], L.A. Wasserman[a], A.A. Popov[a], V.P. Yuryev[a]

[a]*Institute Biochemical Physics RAS, 4, Kosygin Street, 199991 Moscow, Russia*
[b]*Department of Biological and Biomedical Sciences, Food Research Laboratories, Glasgow Caledonian University, Cowcaddens Road, City Campus, Glasgow G4 0BA, UK*

Received 28 July 2003; revised 8 January 2004; accepted 28 January 2004

Available online 19 March 2004

Abstract

Annealing studies were conducted on waxy, normal and high amylose barley starches extracted from cultivars grown at different temperatures. Starches grown at relatively low temperatures comprised a greater proportion of crystalline defects and were hence less 'perfect' than starches grown at the higher temperatures. Annealing of the starches caused very significant increases in gelatinisation temperatures, a small increase in the gelatinisation enthalpies and little change with respect to crystal size. It is proposed that the annealing process diminishes amylopectin crystalline defects by improving double helix registration and optimising the length of the double helices within crystallites.

Keywords: Growth-temperature; Starch; Gelatinisation; Melting; Crystallinity

1. Introduction

It is well known that annealing procedures when applied to semi-crystalline synthetic polymers are accompanied by structural reorganisation and particularly, a decrease of defects within the crystalline structures. As a rule, the process leads to an increase in the melting temperature of polymers and a narrowing of their calorimetric peaks due to the formation of more perfect crystals (Bershtein & Egorov, 1994). According to established polymeric theory (Bershtein & Egorov, 1994) the reasons for such behaviour are: (i) an increase of the cooperative melting units reflecting the thickness of crystalline lamellae (i.e. an increase of cooperativity in the melting system) and (ii) a decrease in size distribution of crystallites. Naturally, these events are interconnected.

At present it is believed by some authors that maturation of starch containing plants and/or a decrease of environmental temperature is accompanied by an accumulation of starch crystallite defects generated by amylose 'tie chains', amylopectin molecular ordered structures (double helices not participating in a formation of starch crystallites and, possibly, amylopectin B-chains) with no changes in the thickness of crystalline lamellae for waxy or normal starches (Kiseleva et al., 2003; Protserov, Karpov, Kozhevnikov, Wasserman, & Yuryev, 2001; Protserov et al., 2002; Wasserman et al., 2001). Recently it has been shown that a correlation exists between the gelatinisation temperature of native waxy barley starches and the amount of starch crystallinity (Qi et al., 2004). For high amylose starches different phenomena are apparent with respect to the formation of starch crystallinity.

During maturation of high amylose pea starches, an accumulation of amylose tie chains may promote formation of amylose B-type crystalline structures where the thickness of crystalline lamellae ($L_{crl} = 9.1$ nm) exceeds that for starches at milky stages of development ($L_{crl} = 6.3$ nm), as proposed by some authors (Kozhevnikov et al., 2001; Yuryev, Wasserman, Andreev, & Tolstoguzov, 2002) or mature normal pea starches ($L_{crl} = 5.1–4.3$ nm) (Kozhevnikov et al., 2001; Yuryev et al., 2002). In contrast to high amylose pea starches, an accumulation of defects in high amylose barley starches grown at low soil temperatures, leads to non-uniform distribution of defects in starch

* Corresponding author. Tel.: +44-141-331-8514; fax: +44-141-331-3208.
E-mail address: r.f.tester@gcal.ac.uk (R. Tester).

0144-8617/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2004.01.006

granules with formation of two types of crystalline structures ('perfect' and 'imperfect') which melt at different temperatures (Kiseleva et al., 2003).

It is believed that the annealing of native starches results in an improvement in the structural organisation of granules (Genkina, Wasserman, & Yuryev, 2004; Qi et al., 2004). Existing concepts regarding annealing mechanisms, in general, are based on:

(i) polymorphic modifications of crystalline regions, namely conversion of B- to A-type conformation representing a more energetically stable crystalline structure and/or V-type complex formation (Donovan, Lorenz, & Kulp, 1983; Knutson, 1990; Zobel, 1988a,b);
(ii) changes within amorphous regions, specifically conversation of amorphous amylose to structured helical forms, increased order (rigidity), decreased mobility with restricted plasticisation and destabilisation effects on crystallites (Donovan et al., 1983; Hoover & Vasanthan, 1994; Jacobs & Delcour, 1998; Kulp & Lorenz, 1983; Larsson & Eliasson, 1991; Tester, Debon, & Karkalas, 1998);
(iii) improvement of crystallinity and perfection of starch crystallites due to increased crystallisation and recrystallisation (Donovan et al., 1983; Krueger, Knutson, Inglett, & Walker, 1987a; Krueger, Walker, Knutson, & Inglett, 1987b) or enhanced registration of double helices (Jacobs, Eerlinger, Rouseu, Colonna, & Delcour, 1998; Knutson, 1990; Larsson & Eliasson, 1991; Marchant & Blanchard, 1978; Tester et al., 1998; Tester & Morrison, 1990; Yost & Hoseney, 1986);
(iv) lengthening of amylopectin double helices without an increase of number and without increasing of glucan chain length (Jacobs & Delcour, 1998a; Tester et al., 1998; Tester, Debon, & Sommerville, 2000).

Physico-chemical approaches used to determine the molecular basis of the annealing mechanism(s) within potato starches (B-type polymorphs) grown at different soil temperatures has shown that an improvement in structural organisation of native granules may be due to lengthening of amylopectin A-chains forming double helices (Genkina et al., 2004). However, it has been shown that annealing of waxy barley starches leads to the increase in the crystalline registration of starches without changes in the content or length of double helices (Qi et al., 2004). The accumulation of amylopectin defects in starch granules during deposition, depends on many factors, which include the amylose and lipid content (Protserov et al., 2001) and environmental conditions (Kiseleva et al., 2003; Protserov et al., 2002).

For a better understanding of macromolecular organisation in starch granules caused by the interplay of different compositional and environmental factors, the following work was undertaken. The specific focus of the work was designed to test if annealing caused a greater structural improvement to starches grown at relatively low temperatures and relate this to the presence of structural defects. Organisational effects induced by annealing are considered in terms of changes with respect to the thickness of crystalline lamellae and thermodynamic parameters defining crystalline faces. This is modelled with reference to the role of amylose to amylopectin ratios and how these molecules interact during starch deposition at different temperatures.

2. Materials and methods

2.1. Materials

Barley cultivars (waxy Oderbrucker, Golden Promise, Triumph and Glacier Pentlandfield) were grown in constant environment chambers until maturity, whereupon their starches were extracted and quantified in terms of amylose and lipid composition as previously described (Tester, South, Morrison, & Ellis, 1991).

Annealing temperatures were chosen as a function of the gelatinisation onset temperatures (T_o, Fig. 1) of the native starches at 2–3 K lower than T_o as shown in Table 1. Starch to water ratios of 1:333 or 1:200 were chosen to provide excess water for the annealing process.

2.2. Methods

Calorimetric investigations of starch dispersions in water (0.3–0.5% dry matter, sample volume 0.5 cm^3 in sealed cells) were performed using a high sensitivity differential scanning microcalorimeter DASM-4 (Puschino, Russia) from 10 to 130 °C with a heating rate of 2 K min^{-1} and excess pressure of 2.5 bar. The term 'melting' is used in preference to gelatinisation of starch ordered structures, in analogy to the term usually applied to the process describing melting of semi-crystalline synthetic polymers during heating. Heat capacity was calibrated using the Joule–Lenz effect for each endotherm. Corrections for dynamic lag and residence of the samples in calorimetric cell were not necessary under these conditions (Andreev et al., 1999; Danilenko et al., 1994). The average values of the thermodynamic parameters were used as described elsewhere (Andreev et al., 1999; ; Danilenko et al., 1994; Matveev et al., 2001), employing five measurements at 95% significance level and converted to dimensions per mole anhydroglucose unit (162 g mol^{-1}).

Values for van't Hoff enthalpy (ΔH^{vH}) were calculated according to Andreev et al. (1999), Danilenko et al. (1994), Privalov and Khechinashvili (1974), and Matveev et al. (2001). Values for the melting of cooperative units (ν) and the thickness of crystalline lamellae (L_{crl}) of starches with symmetrical DSC endotherms, were calculated according to others (Andreev et al., 1999; Danilenko et al., 1994;

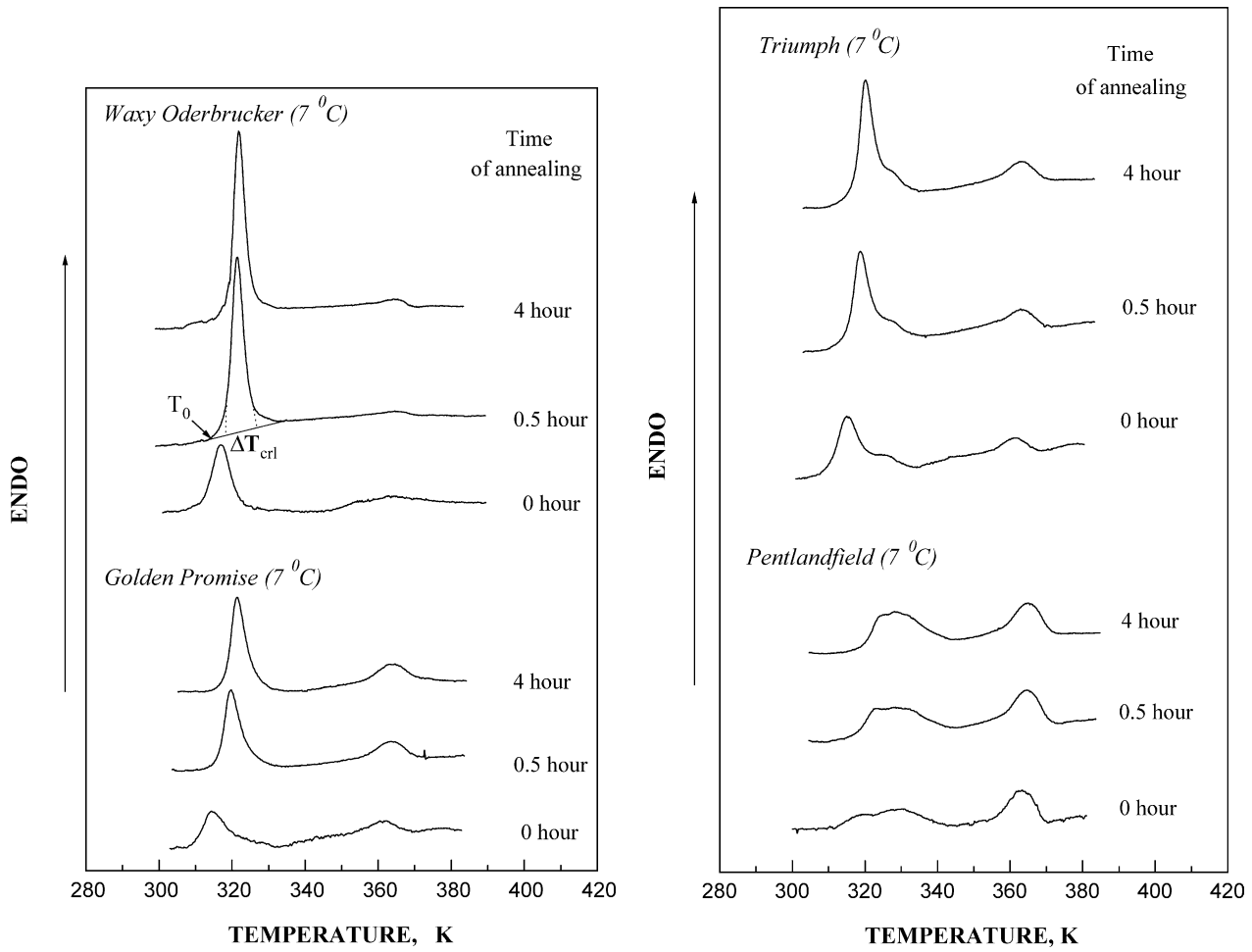

Fig. 1. DSC endotherms of annealed barley starches (Waxy Oderbrucker, Golden Promise, Triumph and Glacier Pentlandfield) grown at 7 °C.

Matveev et al., 2001; Protserov et al., 2001, 2002; Wasserman et al., 2001), and are presented in Eqs. (1) and (2) as follows

$$\nu = \Delta H^{vH}/\Delta H_m \quad (1)$$

where ΔH_m is the experimental melting enthalpy of crystalline lamellae

$$L_{crl.} = 0.35\nu \quad (2)$$

and according to Gernat et al. (1993), there is pitch of 0.35 nm per anhydroglucose residue in the double helices.

To calculate the thermodynamic parameters characterising surfaces of crystalline lamellae of the starches, symmetrical DSC endotherms were used applying the Thomson–Gibbs' equation (3) (Bershtein & Egorov, 1994)

$$T_m = T_m^o[1 - 2\gamma_i/(\Delta H_m^o \rho_{crl} L_{crl})] \quad (3)$$

where T_m^o and ΔH_m^o are the melting temperature and enthalpy, respectively, of a hypothetical crystal with unlimited size (a perfect crystal), γ_i is the free surface energy of faces of crystalline lamellae, while ρ_{crl} and L_{crl} are the density and the thickness of the crystals, respectively.

The parameter q_i may be calculated as follows (4,5)

$$q_i = [(\Delta H_m^o - \Delta H_{exp})L_{crl}\rho_{crl}]/2.5 \quad (4)$$

and

$$\gamma_i = q_i - T_m s_i \quad (5)$$

where q_i is the surface enthalpy of crystalline lamellae.

Since specific values for the melting temperature (T_m^o) and enthalpy (ΔH_m^o) for a perfect crystal are not available, for calculations of the thermodynamic parameters the values of T_m^o (366.5 K) and the ΔH_m^o (35.5 J g^{-1}) for A-type spherolitic crystals (Whittam, Noel, & Ring, 1991) were used. In addition, values of ρ_{crl} for A-type structures (1.48 g cm^{-3}) were used as described elsewhere (Wasserman et al., 2001; Whittam et al., 1991) together with L_{crl}, ΔH_m and T_m values for the starches investigated (Table 2).

Because of the occurrence of asymmetrical melting endotherms for some normal and high amylose starches, a peak fit programme (AISN Software Incorporated, Version 4) was used for the deconvolution of the melting endotherms and for the calculation of the thermodynamic parameters obtained as a result of the deconvolution.

Table 1
Melting parameters of native and annealed barley starches grown at different temperatures

Genotype	Growth temperature (°C)	Annealing time (h)	Annealing temperature (K)	$T_{o\ crl}$ (K)	T_{crl} (K)	ΔH_{crl} (kJ mol^{-1})	ΔT_{crl} (K)	T_{alc} (K)	ΔH_{alc} (kJ mol^{-1})
Waxy Oderbrucker	7	0	303.0	305.1	316.8	1.9	10.4	363.0	0.8
		0.5			321.3	3.3	6.9	365.9	0.1
		1.5			321.3	3.0	6.3	366.3	0.3
		4			321.9	3.5	6.5	365.1	0.3
		10			322.4	2.9	6.3	366.3	0.3
	10	0	308.5	310.5	324.3	2.5	12.7	366.3	0.3
		0.5			327.1	3.0	10	367.2	0.3
		1.5			327.5	3.0	9.4	366.8	0.3
		4			327.2	3.2	9.2	367.1	0.4
		10			328.0	3.4	7.5	365.9	0.4
	13	0	312.5	314.5	327.5	2.7	10.7	365.3	0.4
		0.5			330.1	3.5	9.2	367.4	0.2
		1.5			330.5	3.9	8.8	367.6	0.4
		4			330.7	3.3	7.9	367.8	0.3
		10			331.5	3.8	7.7	367.3	0.4
	15	0	315.5	317.6	328.0	2.6	9.3	365.0	0.4
		0.5			331.3	3.3	7.1	366.1	0.8
		1.5			332.0	3.1	6.7	367.2	0.6
		4			332.6	3.5	6.2	367.7	0.4
		10			333.4	3.6	5.9	368.4	0.3
	16	0	317.5	319.7	329.7	2.8	8.8	366.2	0.3
		0.5			333.0	4.2	6.9	367.6	0.2
		1.5			333.4	4.7	6.7	367.2	0.1
		4			334.0	4.1	6.1	367.2	0.1
		10			334.7	5.2	6.0	368.2	0.2
	20	0	319.0	321.3	333.0	2.8	7.1	366.8	0.3
		1.5			335.9	3.5	7.7	368.6	0.4
		10			337.0	4.0	6.2	368.8	0.2
Golden Promise (normal)	7	0	304.5	306.8	314.7	1.2	12.7	362.6	0.9
		0.5			319.7	1.7	9.4	363.6	0.5
		1.5			321.3	1.6	7.9	364.3	0.4
		4			321.3	1.8	8.3	364.0	0.6
		10			322.6	1.7	8.3	363.5	0.4
	10	0	306.0	308.0	320.9	1.9	14.2	365.9	0.7
		0.5			325.5	2.5	8.5	367.8	0.4
		1.5			326.1	2.3	8.1	366.9	0.7
		4			326.3	2.6	7.5	367.2	0.7
		10			326.8	2.7	7.1	365.9	0.7
	13	0	305.0	307.6	323.5	2.1	13.1	366.8	0.7
		0.5			326.8	2.3	9.7	368.0	0.6
		1.5			327.2	2.9	7.8	367.8	0.4
		4			327.2	2.7	7.3	368.7	0.4
		10			328.2	2.8	6.7	368.4	0.8
	15	0	310.5	312.6	325.5	2.0	11.2	366.8	0.8
		0.5			327.8	2.8	8.3	368.4	0.8
		1.5			329.3	3.4	7.1	368.2	0.9
		4			329.7	3.0	6.2	368.2	0.6
		10			330.3	2.7	6	368.0	0.6
	16	0	312.5	314.7	327.2	2.2	8.96	367.4	0.7
		0.5			330.7	2.8	6.4	368.8	0.5
		1.5			331.1	3.0	5.9	368.6	0.6
		4			331.3	2.7	5.7	368.6	0.6
		10			332.5	3.2	5.4	368.8	0.6
	20	0	318.5	320.9	330.9	2.1	9.0	367.6	0.8
		0.5			328.0	2.3	7.9	368.4	0.5
		1.5			334.7	2.5	6.5	368.8	0.5
		4			335.7	2.9	5.4	368.8	0.6
		10			336.8	3.1	5	368.8	0.6
Triumph (normal)	7	0	302.0	304.3	315.0	2.3	21.9	361.4	0.5
		0.5			318.6	2.3	17.9	363.0	0.6

Table 1 (*continued*)

Genotype	Growth temperature (°C)	Annealing time (h)	Annealing temperature (K)	$T_{o\ crl}$ (K)	T_{crl} (K)	ΔH_{crl} (kJ mol^{-1})	ΔT_{crl} (K)	T_{alc} (K)	ΔH_{alc} (kJ mol^{-1})
		1.5			319.3	2.4	16.7	363.4	0.6
		4			320.1	2.6	16.2	363.0	0.6
		10			321.1	2.6	14.8	363.2	0.6
	10	0	306.0	308.0	320.8	2.4	16.9	365.2	0.3
		0.5			323.6	2.5	10.2	365.9	0.8
		1.5			324.3	2.6	9.2	367.0	0.7
		4			325.3	1.8	7.7	366.6	0.6
		10			325.9	2.6	6.5	365.9	0.7
	13	0	305.0	307.2	325.0	2.8	11.8	365.1	0.4
		0.5			328.0	2.8	8.5	366.4	0.8
		1.5			328.1	2.9	7.9	366.1	0.3
		4			328.4	3.1	7.4	367.0	0.4
		10			329.0	2.7	6.7	365.9	0.4
	15	0	313.0	315.1	326.1	2.6	9.8	365.3	0.4
		0.5			329.3	2.9	6.9	367.8	0.6
		1.5			329.7	2.7	6.4	367.6	0.4
		4			330.9	2.3	5.4	366.5	0.4
		10			331.8	3.0	5	367.4	0.5
	16	0	313.0	315.1	327.7	3.0	9.6	366.3	0.3
		0.5			330.9	3.0	6.5	367.6	0.4
		1.5			330.9	3.2	6.6	367.6	0.6
		4			331.5	2.6	5.6	367.6	0.3
		10			332.6	3.2	5.2	368.0	0.5
	20	0	309.5	311.8	332.0	3.2	9.1	366.7	0.4
		0.5			333.8	3.8	9.2	368.0	0.9
		1.5			333.8	3.8	8.7	368.0	0.9
		4			334.0	3.8	8.3	367.2	0.8
		10			334.5	3.5	7.7	368.2	0.5
Glacier Pentland-field (high amylose)	7	0	307.0	309.3	–	1.3	30.0	363.4	0.9
		0.5			–	1.5	24.3	364.7	0.9
		1.5			–	1.7	23.6	364.9	0.9
		4			–	1.5	21.8	364.9	1.0
		10			–	1.7	20.4	364.7	1.0
	10	0	306.5	308.8	320.7	1.8	26.7	364.6	0.7
		0.5			323.8	2.0	22.5	366.3	1.0
		1.5			324.3	2.0	22.1	366.5	0.9
		4			325.1	2.0	19.9	366.3	0.9
		10			325.9	2.2	19.2	366.3	0.8
	13	0	308.0	310.1	323.6	2.1	24.0	366.4	0.5
		0.5			326.1	2.3	20.8	367.2	0.8
		1.5			326.8	2.1	19.4	367.2	0.8
		4			327.3	2.2	18.5	367.0	0.8
		10			328.1	2.2	17.7	367.4	0.6
	15	0	312.0	314.5	325.0	2.0	22.0	367.0	0.6
		0.5			328.0	2.0	17.7	368.0	0.8
		1.5			328.6	1.9	16.4	368.6	0.9
		4			329.7	2.1	15.4	368.8	0.9
		10			330.3	2.3	11.3	367.2	1.0
	16	0	316.5	318.4	332.1	2.1	19.6	367.2	0.7
		0.5			333.4	2.2	14.6	369.3	0.9
		1.5			334.0	2.1	12.3	369.3	1.0
		4			335.1	2.1	10.4	369.3	0.7
		10			335.9	2.5	8.3	369.3	0.8
	20	0	318.0	320.3	334.3	2.3	16.3	366.8	0.5
		0.5			335.7	2.2	13.3	368.8	0.8
		1.5			336.1	2.2	10.8	368.8	0.9
		4			337.2	2.5	8.8	368.8	0.9
		10			338.0	2.7	7.5	368.8	1.0

Where $T_{o\ crl}$, T_{crl} and ΔH_{crl} represent the onset temperature, peak temperature and enthalpy of amylopectin crystallite gelatinisation (melting), respectively; ΔT_{crl} represents the normalised/extrapolated gelatinisation (melting) range of amylopectin crystallites (see Fig. 1); T_{alc} and ΔH_{alc} represent the peak temperature and enthalpy of amylose–lipid complex dissociation, respectively.

Table 2
Values for the melting of cooperative units (ν_{crl}), thickness (L_{crl}), free surface energy (γ_i), enthalpy (q_i) and entropy (s_i) of crystalline lamellae faces

Genotype	Growth temperature (°C)	Annealing time (h)	ν_{crl}	$L_{crl.}$ (nm)	γ_i (J cm^{-2}) $\times 10^7$	q_i (J cm^{-2}) $\times 10^7$	s_i (J cm^{-2} K^{-1}) $\times 10^7$
Waxy Oderbrucker	7	0	16.0	5.6	17.83	71.25	0.169
		0.5	13.9	4.9	16.85	46.58	0.093
		1.5	14.4	5.0	16.85	52.27	0.110
		4	13.1	4.6	16.62	42.79	0.081
		10	16.2	5.7	16.44	54.18	0.117
	10	0	13.6	4.7	15.14	59.41	0.137
		0.5	13.3	4.7	13.56	48.25	0.106
		1.5	13.5	4.7	13.42	48.25	0.106
		4	13.7	4.8	13.52	44.76	0.095
		10	14.5	5.1	13.25	41.23	0.085
	13	0	13.1	4.6	13.99	55.74	0.127
		0.5	12.2	4.3	12.00	48.25	0.106
		1.5	12.0	4.2	11.87	48.25	0.106
		4	14.7	5.1	11.80	44.76	0.095
		10	13.0	4.6	11.54	41.23	0.085
	15	0	14.7	5.1	13.80	57.57	0.133
		0.5	15.1	5.3	13.88	49.26	0.107
		1.5	16.8	5.9	13.60	53.27	0.119
		4	15.9	5.6	13.36	45.26	0.096
		10	16.0	5.6	13.05	43.24	0.091
	16	0	14.5	5.1	13.19	53.75	0.123
		0.5	12.0	4.1	10.81	25.49	0.044
		1.5	12.0	4.2	10.68	17.29	0.020
		4	14.6	5.1	10.48	27.15	0.050
		10	11.7	4.1	10.26	9.06	-0.004
	20	0	14.1	4.9	12.07	54.40	0.127
		1.5	13.7	4.8	10.97	41.14	0.090
		10	14.9	5.2	10.57	32.00	0.064
Golden Promise (normal)	7	0	18.7	6.6	21.16	94.75	0.234
		0.5	18.1	6.3	22.48	99.20	0.240
		1.5	19.9	7.0	21.71	101.62	0.249
		4	18.5	6.5	21.71	96.74	0.234
		10	19.8	6.9	21.08	99.20	0.242
	10	0	14.0	4.9	18.63	80.18	0.192
		0.5	15.1	5.3	15.87	64.16	0.148
		1.5	16.4	5.7	15.64	68.09	0.161
		4	15.5	5.4	15.56	62.18	0.143
		10	15.8	5.5	15.37	60.20	0.137
	13	0	14.2	5.0	17.57	76.67	0.183
		0.5	15.9	5.6	15.08	66.83	0.158
		1.5	14.1	4.9	14.93	55.22	0.123
		4	16.2	5.7	14.93	57.17	0.129
		10	15.8	5.5	14.55	57.17	0.130
	15	0	15.9	5.6	16.75	79.30	0.192
		0.5	15.3	5.3	15.53	60.40	0.137
		1.5	13.9	4.9	14.93	48.10	0.101
		4	15.7	5.5	14.77	56.29	0.126
		10	18.9	6.6	14.53	62.43	0.145
	16	0	16.9	5.9	16.06	73.76	0.176
		0.5	16.8	5.9	15.65	65.80	0.152
		1.5	16.2	5.7	15.48	61.32	0.138
		4	19.3	6.8	15.39	65.80	0.152
		10	18.3	6.4	14.87	56.88	0.126
	20	0	17.5	6.1	14.54	75.52	0.184
		0.5	17.3	6.1	17.66	81.69	0.195
		1.5	19.0	6.6	14.59	76.04	0.184
		4	19.0	6.6	14.13	66.68	0.157
		10	18.8	6.6	13.62	61.98	0.144

Table 2 (*continued*)

Genotype	Growth temperature (°C)	Annealing time (h)	ν_{crl}	$L_{crl.}$ (nm)	γ_i (J cm^{-2}) $\times 10^7$	q_i (J cm^{-2}) $\times 10^7$	s_i (J cm^{-2} K^{-1}) $\times 10^7$
Triumph (normal)	7	nd	nd	nd	nd	nd	nd
	10	0	12.0	4.2	14.73	55.12	0.126
		0.5	14.2	5.0	15.26	58.97	0.135
		1.5	14.6	5.1	15.46	58.84	0.134
		4	22.3	7.8	23.03	112.59	0.275
		10	16.9	5.9	17.21	68.11	0.156
	13	0	12.2	4.3	13.39	48.11	0.107
		0.5	14.1	4.9	13.66	53.38	0.121
		1.5	13.8	4.8	13.33	50.47	0.113
		4	14.6	5.1	13.93	49.39	0.108
		10	16.2	5.7	15.27	63.32	0.146
	15	0	13.7	4.8	13.02	51.81	0.119
		0.5	15.7	5.5	14.65	57.25	0.129
		1.5	18.0	6.3	16.65	70.35	0.163
		4	22.2	7.8	19.86	98.15	0.237
		10	18.8	6.6	16.39	66.25	0.150
	16	0	13.1	4.6	12.52	45.23	0.099
		0.5	17.1	6.0	15.26	60.13	0.136
		1.5	16.2	5.7	14.48	52.90	0.116
		4	19.9	7.0	17.46	80.12	0.189
		10	18.3	6.4	15.53	59.59	0.132
	20	0	13.4	4.7	11.14	41.61	0.092
		0.5	11.6	4.1	11.02	33.50	0.067
		1.5	11.9	4.2	11.02	33.50	0.067
		4	12.3	4.3	10.95	33.50	0.068
		10	18.0	6.3	10.78	38.68	0.083
Glacier Pentlandfield (high amylose)	7	nd	nd	nd	nd	nd	nd
	10	nd	nd	nd	nd	nd	nd
	13	nd	nd	nd	nd	nd	nd
	15	nd	nd	nd	nd	nd	nd
	16	0	11.5	4.0	10.11	55.39	0.136
		0.5	13.4	4.7	11.16	61.04	0.150
		1.5	15.6	5.5	12.75	73.04	0.181
		4	16.2	5.7	12.75	75.61	0.188
		10	15.2	5.3	11.65	63.08	0.153
	20	0	12.1	4.2	9.46	51.7	0.126
		0.5	14.6	5.1	11.28	66.31	0.164
		1.5	15.8	5.5	12.01	71.53	0.177
		4	15.9	5.6	11.69	66.12	0.161
		10	16.7	5.9	11.96	65.27	0.158

The procedure for deconvolution has been described previously (Matveev et al., 2001).

3. Results and discussion

Specific DSC thermograms of annealed waxy, normal and high amylose barley starches are presented in Fig. 1. The thermograms are typical for cereal starches with different amylose contents (Yuryev et al., 2002) where the low-temperature endotherm is attributed to gelatinisation of amylopectin crystalline lamellae while the high-temperature endotherm represents the dissociation of amylose-lipid complexes (Yuryev et al., 2002). As can be seen from Fig. 1 and Table 1, irrespective of the amylose content, annealing time is accompanied by an increase of the transition temperature and a narrowing of the endotherm for crystalline lamellae. Such changes are typical for native starches irrespective of their polymorphic structure, concentration and amylose content (Hoover & Vasanthan, 1994; Jacobs & Delcour, 1998a; Knutson, 1990; Tester et al., 1998, 2000).

As noted earlier, according to established polymeric theory, the annealing of semi-crystalline polymers leads to an improvement of crystalline structure due to a decrease of crystalline defects. Taking into consideration that: (i) starches are comparable to semi-crystalline polymers and (ii) the starches investigated in this study contain different

kinds of defects (Kiseleva et al., 2003), the observed changes both in the thermodynamic melting parameters and the shape of DSC thermograms are not surprising.

Analysis of the changes in gelatinisation enthalpy as a consequence of annealing shows (Table 1) that irrespective of amylose content and growth history, an increase of annealing time tends to make no difference or leads to a slight increase of the amylopectin crystalline melting enthalpy. This should be viewed against a background knowledge base where it is understood that:

(i) melting enthalpy of starches is the sum of contributions from the melting enthalpies of double helices within crystalline regions and those not participating in a formation of crystals which may be considered as defects (Cooke & Gidley, 1992; Kiseleva et al., 2003; Protserov et al., 2002) especially if located within crystalline lamellae (Gidley, 1992; Gidley & Bociek, 1985);

(ii) an increase of gelatinisation enthalpy might be due to an increase in double helix length if, as reported elsewhere (Safford et al., 1998), during starch biosynthesis this is not optimised (but is generally too small to easily differentiate by for example NMR),

(iii) annealing of waxy barley starches is accompanied by an increase of crystallinity whereas the content of double helices remains constant (Qi et al., 2004),

(iv) annealing of synthetic polymers leads to a decrease of internal defects (Bershtein & Egorov, 1994), where it can be assumed that an increase in the melting enthalpy during annealing of the investigated barley starches here (Table 1) is due to an improvement of structural

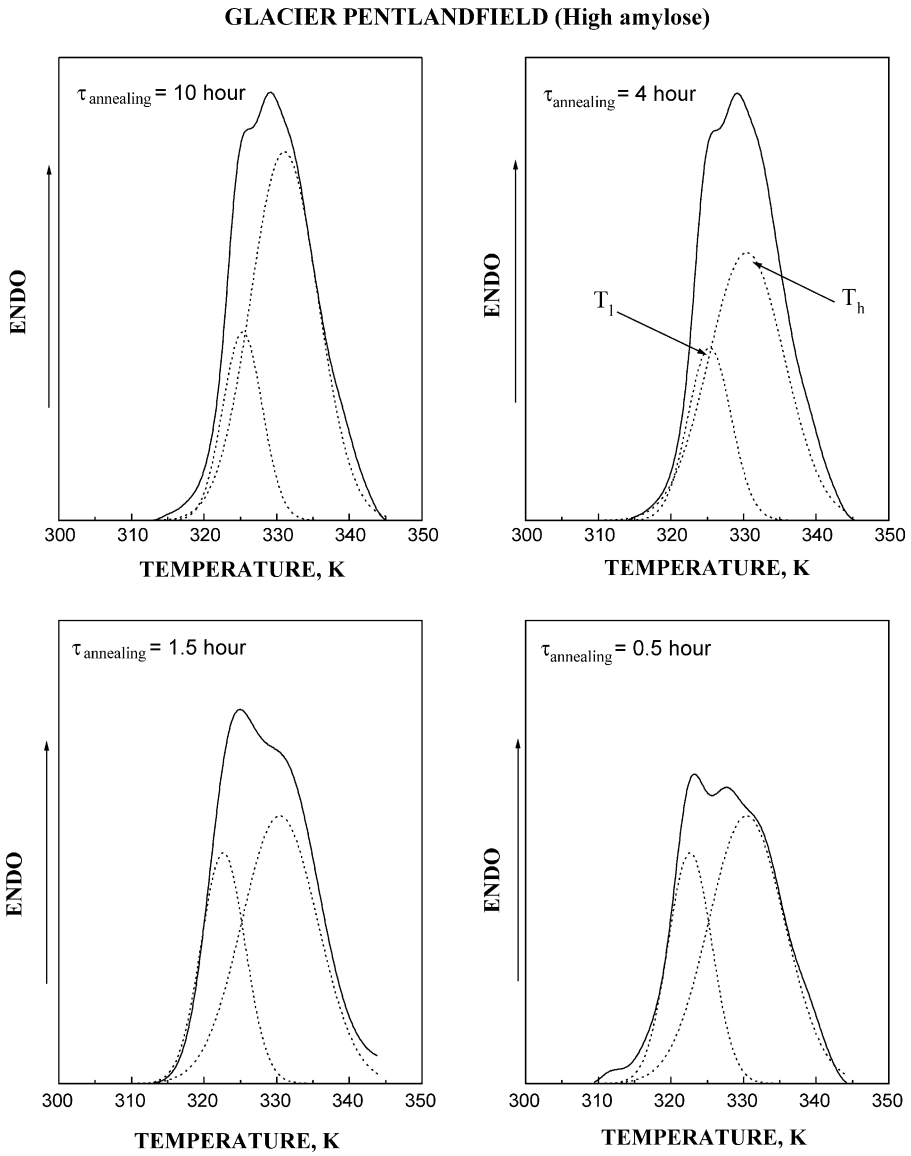

Fig. 2. DSC endotherms (—) of annealed some barley starches (Triumph and Glacier Pentlandfield) grown at 7 °C and results of their deconvolution (- - -). T_l and T_h are the melting temperature of low- and high-temperature structures, respectively.

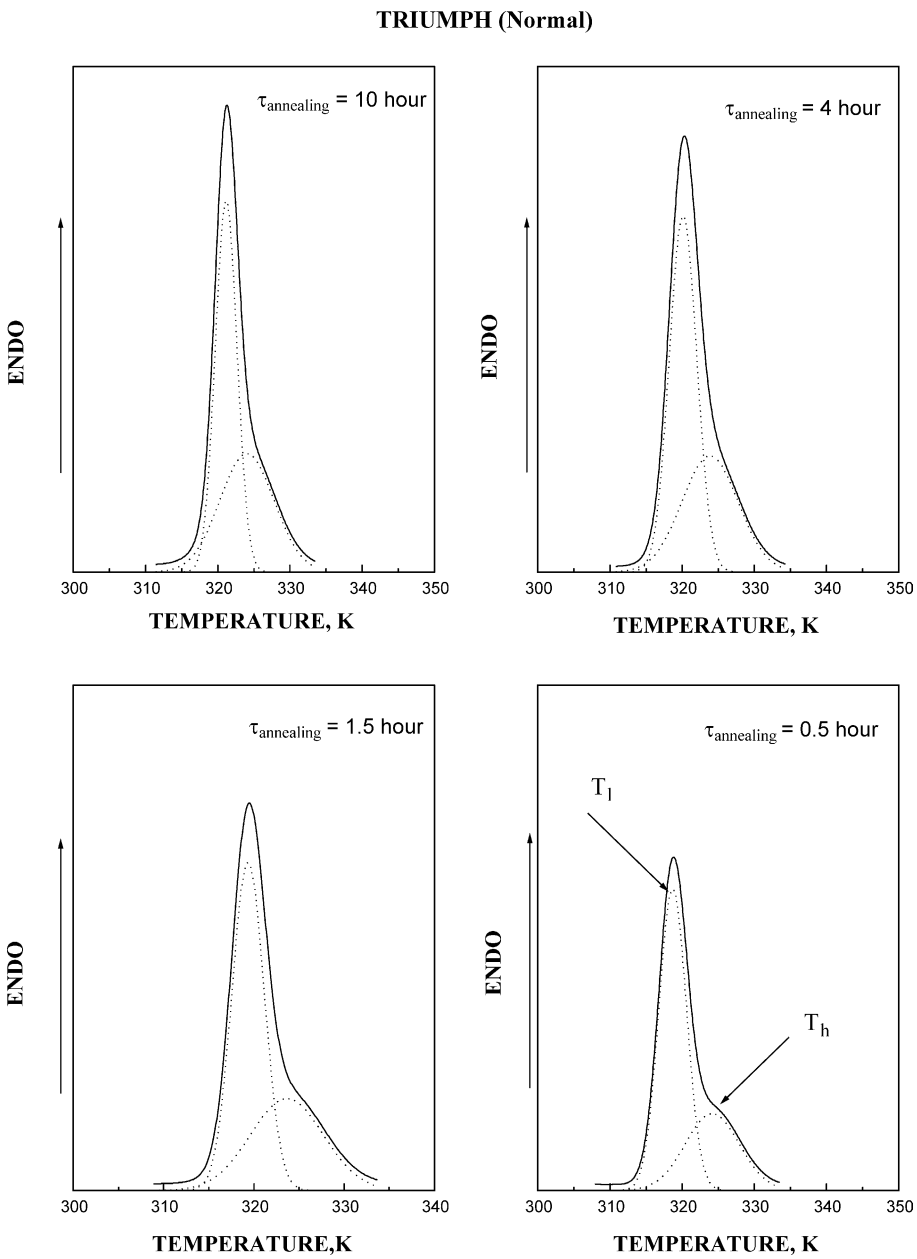

Fig. 2 (*continued*)

organisation in crystalline lamellae caused by an increase of crystallinity. The content of defects in granules correlates also with the melting temperature of starches (Qi et al., 2004).

According to the Thomson–Gibbs' equation (3) and Eqs. (4) and (5), the melting temperature of semi-crystalline synthetic polymers and native starches depends on the structure of the polymer system, thickness of crystalline lamellae and free energy of crystal faces (Bershtein & Egorov, 1994). Taking into consideration that an A- to B-type crystalline transformation of starch structures is not possible, T_m of annealed starches is determined by only two parameters, namely: the thickness of crystalline lamellae and the free energy of crystal faces. The last parameter is proportional to the content of defects.

The enthalpy of the annealed waxy Oderbrucker starches tended to increase with annealing time (Table 1) whilst for Golden Promise, Triumph (both normal) and Glacier Pentlandfield (high amylose) (Table 1) the enthalpy was much more constant and comparable to the control (un-annealed) starches (Table 2). This indicates that crystalline reorganisation is occurring in terms of optimisation of double helix registration within crystallites rather than increases in the number of double helices. Hence, the origin for increases of both the gelatinisation temperatures and enthalpies (Fig. 1, Tables 1 and 2) do not represent the formation of new larger crystallites (Kiseleva et al., 2003).

This is supported by determinations of the thickness of crystalline lamellae (L_{crl}, Table 2) which remain approximately constant for waxy Oderbrucker, Golden Promise and Triumph starches grown at 20 °C. Also, it is apparent from Table 2 that an increase of annealing time is generally accompanied by a decrease in surface entropy of the faces of the crystalline lamellae. In other words, an increase of annealing time is accompanied by a decrease of the proportion of crystalline defects and, correspondingly, by an increase of the gelatinisation temperatures.

In contrast to starches from waxy Oderbrucker and Golden Promise cultivars, the behaviour of starches extracted from Triumph after annealing is more complex and dependent on growth temperature (Tables 1 and 2). As can be seen from Tables 1 and 2, annealed starch extracted from Triumph grown at 20 °C functions similarly as starches extracted from Oderbrucker and Golden Promise cultivars grown at any temperature. An increase of annealing time is not accompanied by changes in the proportion of cooperative melting units or thickness of crystalline lamellae, but a decrease in s_i is observed. In other words, annealing of the starch leads to a decrease of 'unsoundness' in the structural organisation within granules without a change in the thickness of crystalline lamellae. Different behaviour is observed for Triumph starches grown at lower temperatures (16, 15, 13 and 10 °C) which represent starches containing greater amounts of defects (Kiseleva et al., 2003). As can be seen from Table 2, an increase of annealing time leads to an increase of the cooperative melting unit content and the thickness of crystalline lamellae—especially for short annealing times. Apparently an increase in the thickness of crystalline lamellae is the reason for the observed increase in s_i (Table 2). This means that a contribution to s_i caused by an increase in lamellar thickness exceeds the corresponding contribution caused by a decrease of unsoundness. It is important to note that in general the same situation is observed for high amylose starches extracted from Glacier Pentlandfield grown at 20 or 16 °C.

Taking into consideration that: (i) annealing leads to a decrease in a content of molecular ordered structures (Qi et al., 2004) and; (ii) the minimal length of double helices for a formation of A-type polymorphic structures is approximately 10 anhydroglucose residues (Pfannemuller, 1987), it can be seen that the origin of any additional double helical material within crystallites is derived from ends of double helices where the helical structures were not optimised during biosynthesis. According to Kiseleva et al. (2003), normal and high amylose starches extracted from

Table 3
Thermodynamic characteristics and proportions of low- and high-temperature structures in annealed Triumph and Glacier Pentlandfield barley starches

Genotype	Growth temperature (°C)	Annealing time (h)	Annealing Temperature (K)	Structure			
				Low temperature		High temperature	
				T_l (K)	Amount (%)	T_h (K)	Amount (%)
Triumph (normal)	7	0	–	315.2	69.9	323.9	30.1
		0.5	302	318.7	66.7	324.2	33.3
		1.5	302	319.4	61.3	323.7	38.7
		4	302	320.2	59.3	324.2	40.7
		10	302	321.2	55.3	324.0	44.7
Glacier Pentlandfield (high amylose)	7	0	–	314.9	63.7	326.6	36.3
		0.5	307	322.6	33.4	330.6	66.6
		1.5	307	323.7	34.5	331.0	65.5
		4	307	324.4	24.3	330.5	75.7
		10	307	325.4	22.4	331.1	77.6
	10	0	–	321.1	72.2	331.1	27.8
		0.5	306.5	323.7	51.2	330.1	48.8
		1.5	306.5	324.4	49.6	330.5	50.4
		4	306.5	325.3	59.0	331.6	41.0
		10	306.5	326.1	56.3	331.2	43.7
	13	0	–	323.6	52.6	331.0	47.4
		0.5	308	326.1	52.7	331.8	47.3
		1.5	308	326.8	41.6	330.8	58.4
		4	308	327.4	43.1	330.7	56.9
		10	308	328.3	43.9	331.1	56.1
	15	0	–	324.7	34.8	330.9	65.2
		0.5	312	328.0	31.7	332.1	68.3
		1.5	312	328.7	31.7	332.5	68.3
		4	312	329.6	26.6	332.6	73.4
		10	312	330.4	24.1	333.0	75.9

Triumph and Glacier Pentlandfield cultivars grown at low soil temperatures (< 15 °C) contain the greatest amount of defects. The DSC thermograms of such starches are asymmetric (Fig. 2, no annealing). Here, the low-temperature peak may be attributed to gelatinisation of crystalline lamellae containing the greatest proportion of defects whilst the high-temperature peak can be related to the gelatinisation of crystalline lamellae with the smallest proportion of defects. Therefore, it would be expected that an increase of annealing time for such starches will lead to a decrease in the proportion of defective structures. Assuming that these structures melt independently, deconvolution applied to the asymmetric calorimetric peaks provides relative enthalpic contributions (percent) of each structure for the overall melting enthalpy of the starches. The results of the deconvolution of the calorimetric peaks are presented in Fig. 2 and Table 3. As can be seen from Table 3, irrespective of environmental temperature and barley cultivar, an increase of annealing time leads to a decrease of the proportion of defects and, correspondingly, to an increase of the proportion of perfect structures (especially in granules of the high amylose Glacier Pentlandfield starches).

It is well known that if V-type amylose–ethanol complexes are annealed at 65 or 70 °C this gives rise to large increases in crystal thickness (Welland & Donald, 1991). A decrease of heating rate from 3 to 0.5 K min^{-1} or re-heating of normal and high amylose barley starches after annealing (of crystalline lamellae) is not accompanied by the changes in the thermodynamic melting parameters of amylose–lipid complexes (Wasserman, Misharina, & Yuryev, 2002; Yuryev et al., 2002), and hence it is difficult to extrapolate that annealing of native barley starches in the temperature range of 302–319 K leads to significant increases in the thermodynamic melting parameters of amylose–lipid complexes within cereal starches. Indeed as can be seen from Table 1, the dissociation temperature of amylose–lipid complexes is not increased significantly ($< \Delta 3$ K). More likely, any increase in the dissociation enthalpy is due to a shift of surface lipid during heating of starch dispersions into granules with the formation additional complexes with the same thermostability (Wasserman et al., 2002; Yuryev et al., 2002).

4. Conclusions

Analysis of annealing data for the barley starches studied here, shows that an increase of thermodynamic parameters of waxy and some normal starches during annealing is caused by a slight lengthening of amylopectin exterior chain regions forming double helices with a little increase in crystal thickness. Similarly, annealing of high amylose starches grown at relatively low environmental temperatures leads to an increase in the proportion of perfect crystallites. Annealing of starches does not give rise to increases in the dissociation temperature of amylose–lipid complexes although in some starches changes in the melting enthalpy of these structures are observed.

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