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Effect of the red and green linearly polarized light upon polysaccharide depolymerization-repolymerization in starch granules

Summary — Aqueous suspensions of normal corn, waxy corn and potato starches were illuminated for 5—50 h with linearly polarized light (LPL) of 480—560 nm (green) and 600—680 nm (red). Similarly as with white LPL, depolymerization followed by repolymerization of starch polysaccharides, that is amylose and amylopectin, resulted from such illumination. Quantitative effects caused by illumination with the green and red LPL depended on the botanical origin of starch but qualitative effects were similar to those observed on illumination of those starches with white LPL.
Key words: linear polarized light, colour light, starch, botanical origin, depolymerization, repolymerization.

WPŁYW LINIOWO SPOLARYZOWANEGO ŚWIATŁA CZERWONEGO I ZIELONEGO NA PROCES DEPOLIMERYZACJA-REPOLIMERYZACJA POLISACHARYDÓW W ZIARNIE SKROBIOWYM
Streszczenie — Wodne zawiesiny skrobi kukurydzianej, kukurydzianej woskowej i ziemniaczanej naświetlano w ciągu 5—50 h liniowo spolaryzowanym światłem (LPL) długości 480—560 nm (zielonym) i 600—680 nm (czerwonym). Na podstawie wyników oceny produktów metodami DSC (kinetyka żelowania), spektrofotometrycznie oznaczanego zabarwienia w reakcji z jodem (*BV*), granicznej liczby lepkościowej ([η]), dyfraktometrii rentgenowskiej oraz badania kinetyki α-amylolizy (tabele 1 i 2, rys. 1 i 2) ustalono, że podobnie jak zastosowane we wcześniejszych pracach białe LPL — barwne LPL powodowało depolimeryzację, a następnie repolimeryzację polisacharydów, tj. amylozy i amylopektyny. Ilościowe efekty naświetlania zielonym i czerwonym LPL są na ogół niezbyt wielkie i zależą od botanicznego pochodzenia skrobi, natomiast jakościowy charakter zmian pod wpływem kolorowego LPL jest taki sam jak w przypadku białego LPL.
Słowa kluczowe: liniowo spolaryzowane światło, barwa światła, skrobia, pochodzenie botaniczne, depolimeryzacja, repolimeryzacja.

Even in rich in resources, and industrially advanced countries, polysaccharides become more and more appreciated as sustainable, versatile, biodegradable, cheap sources for chemical industry. They are supposed to subside fossils to a considerable extent, and their application seems to be a remedy for economical [1] and environmental [2] problems. Among polysaccharides, starch attracts most attention. It is useless in a native state and has to be modified on either physical, for instance [3, 4], chemical, for instance [3, 5, 6], and enzymatic, for instance [7], ways. Also chitosan [8, 9], alginate [10] and cellulose [8] received certain attention as polysaccharides of technical value. Currently, for relatively high costs of processing, physical treatment of polysaccharides seems to attract more attention. There are several energy sources useful in physical modifications of starch [11].

Recently, it has been found that white, linearly polarized light (LPL) decomposed starch suspended in aqueous solutions and that process was non-enzymatic in its character [12—14]. In that process, mainly amylopectin suffered scission of the branches which subsequently, on prolonged illumination with LPL, repolymerized into longer linear amylose-like polysaccharide. The optimum illumination time for both steps depended on the botanical origin of starch. Because depolymerization occurred as a result of the absorption of the light in granule crystallites, the crystallinity of the granules and not the amylose-to-amylopectin ratio was the factor controlling that process. Varieties of the B- and V-crystallographic type were more susceptible to the changes [15]. Regardless botanical origin, illumination of granules made them more susceptible to alpha-amylolysis [16, 17].

In this paper effect of red and green LPL upon polysaccharide depolymerization-repolymerization in corn, waxy corn, and potato starch granules is described.

EXPERIMENTAL PART

Materials

Normal and waxy cornstarch were products of National Starch and Chemicals (Neustadt, Germany), and

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potato starch was isolated in a Potato Enterprise (Łomża, Poland).

α-Amylase from porcine pancreas, ~ 250 U/mg of solid at pH 6.9 and 25 °C, (EC.3.2.1.1), was the product of Merck, Darmstadt, Germany.

Illumination

Illuminations of starch samples with LPL were carried out following the method of Fiedorowicz *et al*. [12]. Aqueous 30 % suspensions of normal corn, waxy corn and potato starches were exposed to light from a distance of 30 cm. An illuminator of the type "57-400 Optel" (Opole, Poland), equipped with a 150 W xenon arc lamp "XBO 150" (Oriel Ltd., Latherhead, Surrey, England) was used. The "HN 22" linear polarizing filter (Polaroid, USA), cutting out wavelengths from green (480—560 nm) or red (600—680 nm) ranges was fixed between the illuminator and a sample. The light source emitted the light of continuous intensity in the visible range. Its energy flux, when it passed through either red or green glass filter, at the place of the sample was 0.03 mW/cm^2 and 0.04 mW/cm^2, respectively, as checked by "Fieldmaster FM" (Coherent, USA). The exposition with agitation of starch suspensions to LPL lasted 5, 15, 25 and 50 h at 25 °C. Starch samples illuminated with non-LPL for the same period served as control. After the illuminations, all samples were filtered off and dried at 50 °C for 24 h. Experiments were run in duplicates.

Methods of testing

Gelatinization kinetics

Differential scanning calorimetry (DSC) measurements of samples of native and illuminated starch were performed with a "DSC-2" instrument constructed in the Department of Physics, Agricultural University of Cracow. The samples (8 mg) blended with distilled water in the 1:3 w/w ratio were sealed in capsules and allowed to stand for 1 h at 25 °C followed by heating at the 10 °C/min rate from 20 to 90 °C. Distilled water was used as reference. The measurements were run in triplicates. DSC thermograms were used to establish onset (T_o), peak (T_p), conclusion (T_c) temperatures together with enthalpy (ΔH) of starch gelatinization.

Iodine staining

The blue value (*BV*) and λ_{max} of starch samples were determined at room temperature based on the procedure of Morrison and Laignelet [18] with modifications described by Klucinec and Thompson [19]. The starch samples (40 mg) were dispersed in DMSO (10 mL) containing 10 % of 6 M urea. An aliquot of each sample (1 mL) was weighed into a 100 mL volumetric flask, to which deionised water (95 mL) and the iodine solution (100 mL solution contained 200 mg I_2 and 2 g KI) (2 mL) were added. The mixture was filled up to 100 mL with deionised water and mixed immediately. Blank solutions that were prepared following the identical procedure did not contain starch. Absorption spectra of the samples were measured in the wavelength range of 500—800 nm with "Shimadzu 2101 PC" UV-VIS spectrophotometer (Tokyo, Japan). The blue value was defined as the absorbance at 640 nm. The measurements were run in triplicates.

Intrinsic viscosity

The intrinsic viscosity of starch samples was measured with a modified Zimm rotary viscometer of own construction [20]. Solutions for measurements were prepared in 100 mL volumetric flask by dispersing appropriate native or illuminated starch (100 mg) in distilled water (10 mL). The dispersions were gently homogenized on a magnetic stirrer at 30 rpm. The stirring was continued and DMSO (60 mL) was added. Then the solution was within 20—30 min gradually heated to 80 °C, followed by maintaining samples for 3 h at that temperature, and cooling to 25 °C. Further volume of DMSO was added to obtain 100 mL solution. This stock solution was diluted by the addition of DMSO:water (90:10) solvent to achieve a desired concentrations. For each sample, seven different solutions at concentrations ranging from 0.0 to 0.1 g/dm^3 were measured. Measurements were performed at 25.0 ± 0.1 °C. Intrinsic viscosities were obtained by plotting the reduced viscosity versus concentration, and then by extrapolation to the zero concentration by a linear regression.

Powder X-ray diffractometry

X-ray patterns of samples of native and illuminated starch were obtained with copper, K_α radiation using a Philips diffractometer "X' pert-type" (Almelo, The Netherlands). The diffractometer was operated at 30 mA and 40 kV. Signals of the reflection angle of 2θ from 5.0° to 60.0° with a scanning step 0.02° were recorded.

α-Amylase hydrolysis

Native and illuminated starch samples were analyzed for resistance to porcine α-amylase hydrolysis following the method of Fiedorowicz and Chaczatrian [17]. Porcine pancreas α-amylase from porcine pancreas (0.01 g) was reconstituted in 0.9 % NaCl (10 mL) to obtain a stock solution with an enzyme activity of 250 units mg^{-1}. The samples (40 mg) of native and illuminated starch were suspended in distilled water (38 mL) and aliquots of the enzyme stock solution (2 mL) were added to achieve a 1 mg ml^{-1} final concentration of starch and a 12.5 units mg^{-1} final enzyme activity. The mixtures were incubated at 37 °C in a water bath shaker. Aliquots (2 mL) were withdrawn at appropriate time intervals and the amount of solubilised starch by the 3,5-dinitrosalicylic acid method [21] was determined. The experiments were run in triplicates.

RESULTS AND DISCUSSION

In contrast to white LPL, color LPL, regardless its energy, produced only subtle changes in gelatinization characteristics recorded with DSC (Table 1).

It could be rationalized in terms of lower energy put on illuminated samples. In case of cornstarch, green LPL slightly more than red LPL decreased onset, peak and conclusion temperatures causing subtly stronger disorder in granules as manifested by slightly higher melting enthalpy values. Approximately the same effect could be found in case of potato starch, whereas based on changes in the melting enthalpies, red LPL more efficiently damaged matrix of waxy cornstarch. Changes of those parameters not necessarily were linear in time of illumination.

Such irregularity was observed by us also in case of illumination of starches with white LPL. Based on changes of molecular weight distribution in illuminated starches, that observation was interpreted in terms of preliminary depolymerization of starch crystallites followed by subsequent repolymerization of abstracted fragments, usually short chains of amylopectin into a linear amylose-like linear polysaccharide [12, 14]. The subtle irregularities appeared between 15th and 25th hour of illumination, although in potato starch such irregular changes could be noted on the beginning and the end of the illumination period. The thermal properties of potato starch exposed to white LPL remained unaffected. The phosphate moieties could contribute to the rigidity of the amylopectin chains and their macroscale organization, making the matrix more rigid and, therefore, more susceptible to illumination. As the LPL was absorbed in clusters of the helical amylopectin side-chains, one could assume that the depolymerization involved mainly amylopectin molecules [13].

The maximum absorption wavelength (λ_{max}), blue value (*BV*), defined as E_{640}, and E_{640}/E_{525} ratio for native and illuminated starches are summarized in Table 2. Observed changes caused by illumination were also subtle but, frequently, significant. The λ_{max} of starch iodine complexes depended on the length of the glucan helices and asymptotically approached 640 nm for polymerization degree >200 [12, 13, 22—24]. For that reason, the extinction ratio measured at 640 and 525 nm (E_{640}/E_{525}), could be considered as a measure of the ratio of amylose-type non-branched/long-chain branched (nb/lcb)--glucans and amylopectin-type short-chain branched (scb) glucans. According to Bailey and Wehen [25] and Pfannemüller *et al*. [26], values of the iodine binding capacity, expressed as E_{640}/E_{525} ratio, exceeding 1.5 clearly indicated amylose-type, nb/lcb starch glucans.

The significant rise in the blue value and the E_{640}/E_{525} ratio was observed for waxy cornstarch illuminated with LPL for 5 h [14]. Further illumination led to gradual decrease in the blue value with the lowest value observed for samples illuminated for 50 h. Differences between the blue value and the E_{640}/E_{525} ratio, depending on the illumination time of the samples of iodine--normal cornstarch and iodine-potato starch complexes,

T a b l e 1. Results of the illumination of starches with red and green LPL observed by means of differential scanning calorimetry

Illumination time, *h*	T_0, °C		T_p, °C		T_c, °C		ΔH, J/g	
	red	green	red	green	red	green	red	green
	Cornstarch							
0[a)]	69.1±0.5		73.0±0.4		75.5±0.8		11.9±0.5	
5	68.0±0.4	68.8±0.8	72.1±0.6	72.7±0.5	75.5±0.3	75.8±0.7	11.4±0.4	11.4±0.9
15	67.2±0.1	67.4±0.5	72.4±0.1	72.0±0.4	75.5±0.4	75.2±0.5	11.8±0.2	12.1±0.3
25	66.6±0.6	66.7±1.2	72.2±0.7	72.1±0.4	75.8±0.6	75.2±0.9	10.4±0.8	10.7±0.3
50	65.5±0.9	65.4±0.4	71.1±0.2	71.3±0.5	74.6±0.3	75.1±0.9	11.0±0.6	10.6±0.5
	Waxy cornstarch							
0[a)]	67.0±0.7		70.1±0.8		73.2±1.0		13.2±0.7	
5	65.2±0.3	64.5±0.6	68.4±0.7	68.3±0.8	71.8±0.7	71.8±1.0	10.7±0.5	10.9±0.3
15	65.0±0.8	63.8±0.8	684±0.6	67.7±0.5	72.3±0.9	71.6±0.5	11.4±0.7	11.8±0.8
25	64.0±0.3	62.0±0.5	679±0.5	66.9±0.9	71.9±0.7	71.8±0.4	10.4±0.5	9.3±0.1
50	61.7±0.4	61.8±0.6	65.5±0.2	66.2±0.5	70.2±0.9	70.8±0.7	7.0±0.5	8.3±0.5
	Potato starch							
0[a)]	64.9±0.5		68.6±0.5		72.0±0.4		17.2±0.5	
5	60.0±0.6	60.8±0.6	64.3±0.5	65.1±0.8	67.8±0.6	68.5±0.7	14.3±1.2	15.0±1.4
15	60.4±0.4	60.9±0.6	64.5±0.3	65.0±0.5	67.5±0.2	68.2±0.3	14.4±0.4	15.0±1.0
25	61.7±0.3	60.8±0.3	65.3±0.4	65.0±0.5	68.2±0.2	68.2±0.4	13.7±1.1	15.4±0.4
50	60.0±0.7	60.1±0.9	64.4±0.8	64.3±0.8	67.8±0.4	67.5±0.7	15.1±0.4	15.1±0.9

[a)] Data for original unprocessed starch.

had a similar character, with the largest differences against the native starch occurring for the samples illuminated for 15 h [13, 27].

T a b l e 2. Iodine binding properties, blue value (*BV*) and intrinsic viscosity values of corn starch, waxy corn starch and potato starch illuminated with red and green LPL

Illumination time, h, and wavelength	λ_{max}, nm	*BV*	E_{640}/E_{525} *)	Intrinsic viscosity [η], dl/g
	Cornstarch			
0	596.5±0.3	0.328+0.003	1.21	3.96±0.08
5 red	596.6±2.8	0.313±0.008	1.26	4.01±0.04
green	595.2±0.7	0.311±0.005	1.18	4.30±0.10
15 red	600.1±1.0	0.295±0.004	1.29	3.29±0.10
green	596.1±0.6	0.314±0.003	1.20	3.73±0.17
25 red	595.8±0.3	0.303±0.003	1.25	3.74±0.10
green	596.4±0.3	0.306±0.002	1.21	3.64±0.14
50 red	596.3±1.0	0.331±0.001	1.21	5.22±0.12
green	596.7±0.1	0.315+0.002	1.22	4.47±0.13
	Waxy cornstarch			
0	530.2±0.4	0.028±0.006	0.41	1.63±0.08
5 red	526.0±0.2	0.026+0.003	0.39	2.22±0.10
green	530.1±0.2	0.031±0.004	0.41	1.2.1±0.06
15 red	528.8±0.6	0.024±0.001	0.40	2.07±0.10
green	525.7±0.2	0.026±0.004	0.42	1.62±0.08
25 red	528.1±0.5	0.019±0.001	0.33	1.76±0.04
green	531.9±0.9	0.027±0.006	0.44	1.20±0.04
50 red	538.5±0.7	0.024±0.001	0.44	2.30±0.10
green	537.8±0.4	0.035±0.008	0.45	1.93±0.07
	Potato starch			
0	600.2±0.1	0.345±0.006	1.34	6.81±0.08
5 red	600.2±0.2	0,.318±0.001	1.38	6.37±0.16
green	600.0±0.1	0.337±0,020	1.33	6.05±0.15
15 red	600.3+0.1	0.327±0.018	1.37	4.78±0.19
green	600.2±0.1	0.318±0.018	1.35	6,83±0.19
25 red	600.3±0.1	0.336±0.001	1.33	5.94±0.20
green	600.3±0.1	0.356±0.001	1.35	8.10±0.16
50 red	600.2±0.1	0.350±0.002	1.38	5.91±0.15
green	600.2+0.1	0.355±0.019	1.38	6.88±0.20

*) Explanation see text.

In case of corn and potato starch, illumination with either red or green LPL had practically no effect upon the absorption wavelength of the complexes with KI_5 (Table 2). These results confirmed known fact [17] that amylose responsible the complex formation did not suffer any damage on the exposure to LPL. However, blue value (*BV*) providing information on behaviour of both starch polysaccharides significantly changed on illumination. In the first 5 hour illumination, of corn starch green LPL slightly more efficiently decreased *BV* whereas in potato starch red LPL was significantly more efficient in that respect. After 15 hour illumination changes of *BV* for iodine complexes with corn and potato starches pointed, respectively, to red and green LPL as more efficient. After 25 hour illumination of both starches red LPL more decreased *BV* than did it green LPL. After 50 hour illumination of both starches, *BV* values exceeded these for original starches and in cornstarch red LPL, was clearly more efficient in repolymerization than the green LPL whereas in potato starch the effect of both LPLs was similar. The examination of the E_{640}/E_{525} ratio suggested that as that ratio preliminarily increased, amylopectin suffered the damage rather than amylose. Red LPL caused more efficient depolymerization. Final values of that ratio increased above these for original starches suggesting that repolymerization produced linear, amylose-like polysaccharides. The illumination with green LPL was slightly more efficient (compare results after 50 h of illumination of cornstarch and 25 h illumination of potato starch). Potato starch better and faster reacted to illumination than did cornstarch.

Changes of intrinsic viscosity initially decreased in order to increase above original values for unprocessed samples. In the case of cornstarch, red LPL provided higher final intrinsic viscosity whereas in the case of potato starch, illumination with green LPL offered gels of slightly higher intrinsic viscosity.

As shown by absorption wavelength of the blue complex (Table 2), waxy cornstarch was more sensitive to illumination than two other starches. As the illumination time was prolonged, λ_{max} initially decreased in order to increase gradually from the 25th h of illumination. There was no particular preference for either red or green LPL. Changes in *BV* and the E_{640}/E_{525} ratio were almost negligible but, evidently, the green LPL provided more pronounced changes than did the red LPL. Throughout the whole period of illumination, the red LPL provided gels of higher intrinsic viscosity. It suggested that red LPL more efficiently depolymerized and repolymerized this kind of starch.

Unchanged X-ray diffraction patterns for illuminated waxy corn starch suggested that visible LPL did not affect crystallinity of that starch, unlike potato starch whose changes in the diffraction patterns indicated the decrease in the crystallinity of samples illuminated for 5 and 25 h [17]. The X-ray diffraction patterns of native cornstarch and illuminated with both red and green LPL were almost identical and indicated the typical A-type pattern (Fig. 1a).

Similarly, illuminated waxy cornstarch showed characteristic A-type pattern but with lower peak intensities as compared to that of native starch (Fig. 1b). Waxy cornstarch illuminated with red as well as green LPL for 50 h showed the lowest peak intensities.

The diffractograms of native and illuminated potato starch displayed typical B-type pattern (Fig. 1c). In the diffractograms of potato starch illuminated for 5 and 25 h with red as well as green LPL, the reflection bands centered around 5, 15 and also 23 deg 2θ were low and diffused. However, in the diffractograms of samples illuminated for 15 and 50 h these same bands were distinct.

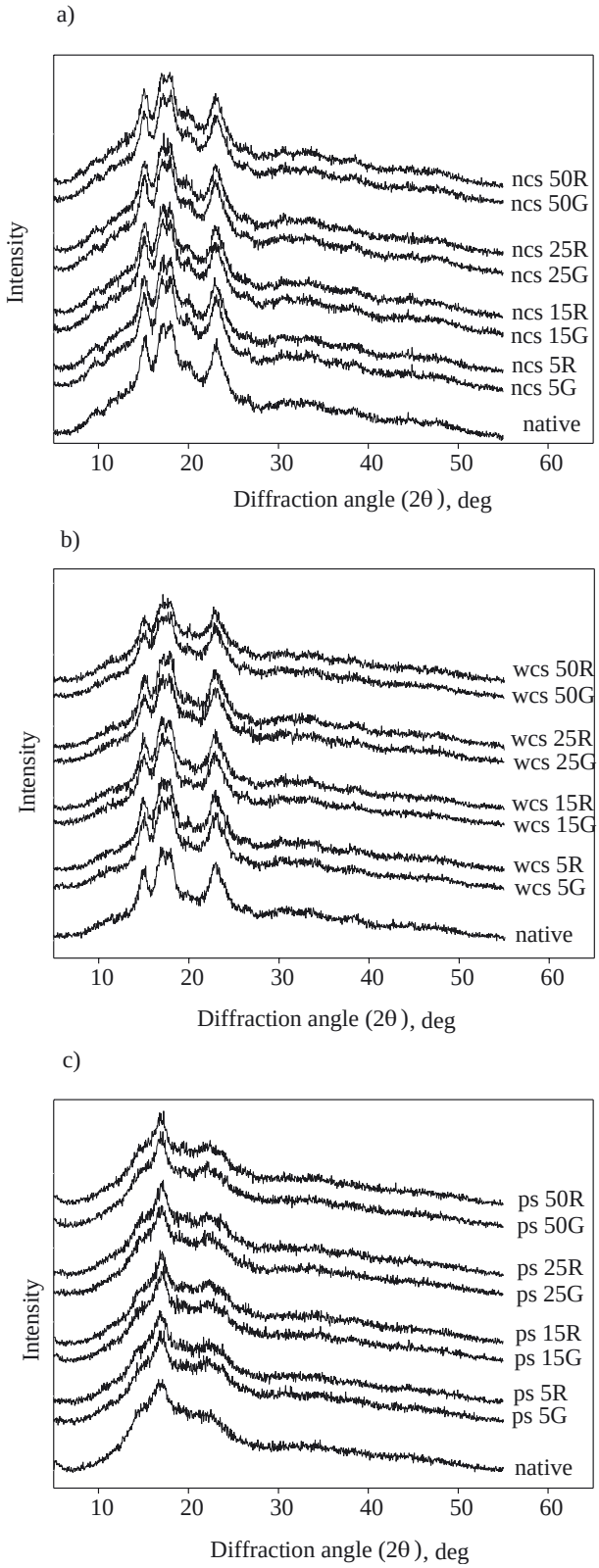

Fig. 1. X-ray diffraction patterns of native and illuminated: (a) normal cornstarch (ncs), (b) waxy cornstarch (wcs), (c) potato starch (ps); capital letter indicates either red (R) or green (G) light applied, and number denotes the illumination time (h)

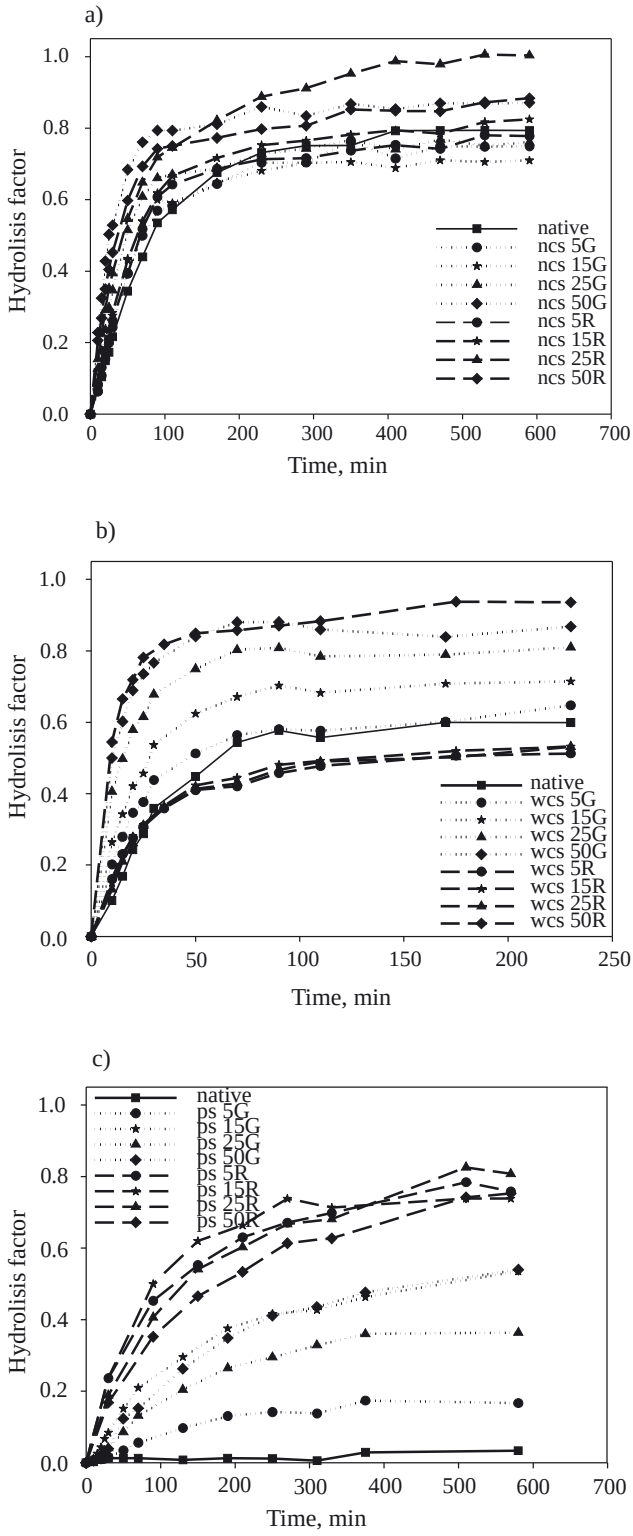

Fig. 2. α-Amylolysis kinetics of native and illuminated: (a) normal cornstarch (ncs), (b) waxy cornstarch (wcs), (c) potato starch (ps); capital letter indicates either red (R) or green (G) light applied, and number denotes the illumination time (h)

All these data indicated that neither red nor green LPL significantly affected the crystalline structure of normal cornstarch granules. The lowest X-ray diffraction intensities of waxy cornstarch illuminated for 50 h were in agreement with the lowest gelatinization enthalpy values recorded for those samples, indicating lower granule crystallinity of waxy cornstarch.

Susceptibility of waxy cornstarch to α-amylolysis after illumination with white LPL did not change, but in potato starch a gradual, illumination time-dependent increase in the amylolysis (*i.e.*, hydrolysis with α-amylase) rate took place [17]. The rate of α-amylolysis of native cornstarch and waxy cornstarch exceeded considerably the rate of that process for native potato starch (Fig. 2) which, commonly, was highly resistant to the hydrolysis with α-amylase.

However, the degree of the hydrolysis and the hydrolysis rate of potato starch illuminated with both red and green LPL were significantly higher than those of native, non-illuminated starch. All potato starch samples (Fig. 2c) illuminated with red LPL exhibited time-independent, higher susceptibility to the enzyme than the samples illuminated with green LPL, which exhibited gradual, time-dependent increase in the rate of hydrolysis. The susceptibility to the enzyme of normal cornstarch (Fig. 2a) illuminated with green and red LPL for 5 and 15 h was similar to that found for non-illuminated starch. Prolonged illumination led to an increase in the susceptibility to amylolysis. Similarly as in the case of potato starch, illumination of waxy cornstarch (Fig. 2b) with green LPL led to gradual, time-dependent increase in the rate of hydrolysis. Waxy cornstarch illuminated with red LPL for 5, 15 and 25 h was less susceptible to the enzyme than native starch, but illuminated with this light for 50 h was more readily hydrolysed than all illuminated samples of that starch.

Different response of particular starch varieties to illumination with green and red LPL reflected ability of their granule crystalline networks to absorb the waves of different frequencies to resonate with them, and, in consequence, to depolymerize.

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