DSC OF RESISTANT STARCH PRODUCED BY DIFFERENT METHODS

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ABSTRACT

Resistant starch (RS) refers to the sum of starch and starch degradation products that are not absorbed in the small intestine of healthy individuals. The fact that resistant starch escapes digestion confers to it many positive health effects like reducing the glycemic response and acting as a functional prebiotic. Resistant starch also interacts with dietary macronutritients such as fats and protein, and with micronutritient such as minerals. Resistant starch type 3 comprises retrograded starches.

In this study resistant starch type 3 was prepared by two different treatments. The samples obtained by suspending 10% and 20% (w/v) of maize starch in 1000ml of water were autoclaved at adequate conditions: pressure 1.1 bar, temperature 120°C, autoclaving time 30 min and volume 60 dm^3. After autoclaving the samples were stored at 4°C for 24h and two autoclaving–cooling cycles were applied. The enzyme treatment means debranching of starch samples with pullulanase after gelatinization process. The maize starch gels were exposed to different concentration of pullulanase, 2% and 4% calculated on dry starch weight, and the reaction time was varied (1, 3 and 5h). Adequate analysis was performed after the preparation of the samples and also after three weeks of keeping them at 4°C.

A method of differential scanning calorimetry (DSC) measurements was used for thermal analysis of the samples. Hermetically sealed aluminum standard crucibles contained 5mg of each sample (mixed RS and distilled water). Heating range was from 25 to 200°C at heating rate of 10 °C/min. From DSC curves the temperature of degradation *Td* and enthalpy ΔH could be obtained. DSC thermal analysis of examined resistant starch showed significant higher values of *Td* and ΔH for the samples that were stored for three weeks than samples analyzed after preparation. It could be concluded that during that period the content of retrograded fractions, which are nondigestible and designate RS3 as resistant starch, increased.

Key words: resistant starch, enzymatic treatment, autoclaving, DSC analysis

1. INTRODUCTION

Starch has a major contribute to the appearance, structure and quality of food products. Starches can be classified according to the retention time in the gastrointestinal tract and accessibility to the enzymes into three major groups, as rapidly digestive starch (RDS), slowly digestive starch (SDS) and resistant starch (RS). (1, 2)

Resistant starch is indigestible by body enzymes so escapes digestion and absorption in the small intestine and may be fermented in the large intestine, providing many physiological functions similar to those of dietary fiber and many positive health effects. Resistant starch affects reduction of the glycemic response, acting as a functional prebiotic for some probiotic microorganisms and increases the production of short fatty acids chains, primarily acetates, propionates and butyrate. It has indirect influence to the pH value of the large intestine, colonic blood flow and mineral absorption. (3)

Four types of resistant starch vary: physically inaccessible starch (RS1), raw starch (RS2) that is in a certain granular form and resistant to enzyme digestion, retrograded or recrystallised starch (RS3) and chemically modified starch (RS4). (4)
RS3 represents the most resistant starch fraction and is mainly retrograded amylose formed during cooling of gelatinized starch. Most moist–heated foods therefore contain some RS3. In the formation of RS3, the starch granule is completely hydrated. Amylose leaches from the granules into the solution as a random coil polymer. The polymer chains upon cooling begin to reassociate as double helices that are ordered into a crystalline structure over a particular region of the chain and interspersed with amorphous enzyme degradable regions. The conformation and stability of recrystallised starch is accomplished during processing and storage. Crystalline structure type A is formed in gelatinized starch stored at high temperature, it has dense structure and it is less open than B form, which has hexagonal symmetry and is processed and recrystallised at low temperature. (2) The conformation and physico–chemical properties determine functionality, nutritional value and the health implications of recrystallised starch. Structural stability affects interactions between the starch and intestinal microorganisms, thus recrystallised starch possess bifidogenic and butyrogenic properties. RS3 is structurally and thermally more stable than others RS types, thus may be used as a functional fiber ingredient in many processed food. (4, 5)

2. DIFFERENTIAL SCANNING CALORIMETRY OF THE RESISTANT STARCH

Thermal analysis of resistant starch type 3 obtained by different processes is suitable method for determining physical properties and structural changes of resistant starch during thermal treatments and storage. DSC (Differential Scanning Calorimetry) is based on measuring the thermal effects that occurs during heating of the examined sample and a reference sample exposed to the same temperature regime. (6)

2.1. Preparation of the resistant starch

Resistant starch type 3 was prepared by two different treatments.
The samples were prepared by suspending 10% and 20% (w/v) of commercial maize starch obtained from local producer („Jabuka", Pančevo, Serbia) in 1000 ml of water. The suspensions were heated in a boiling water bath for 15 min with stirring and then autoclaved at the adequate conditions: pressure 1.1 bar, temperature 120°C, autoclaving time 30 min and volume 60 dm^3. Two autoclaving–cooling cycles were applied and then the samples were stored at 4°C for 24h.
The enzyme treatments means debrancing of starch samples using pullulanase after gelatinization process. Commercial debranching enzyme, pullulanase (PromozymeBrewQ, 400 PUN/ml), from *Bacillus acidipullulyticus* was obtained from Novozymes (Bagsvaerd, Denmark). The enzyme concentrations were 2% and 4% (calculated on dry starch weight) and the reaction time varied (1, 3 and 5h). After that the samples were heated at 95°C for 20 min, cooled down to room temperature and stored for 24h at 4°C. Finally, the samples were dried at 40°C and then stored in closed glass containers.
A method of differential scanning calorimetry (DSC) was used for thermal analysis of the samples. Measurements were performed at DSC Q20 V23.10, TA Instruments, UK, Ltd. and data were analyzed using manufacturer's software Universal V4.3A TA Instruments. Resistant starch and distilled water were mixed in the aluminum crucibles that contained 5 mg of each samples (3 mg of resistant starch and 2 mg of distilled water) and then were hermetically sealed. The samples and the empty crucible used as a reference were heated

from 25 to 200°C at heating rate of 10°C/min. From DSC curves the temperature of degradation T_d and enthalpy ΔH could be obtained. Thermal properties of examined samples were analyzed after preparation of the resistant starch and after storage at refrigerator temperature of 4°C for three weeks.

2.2. DSC analysis

Samples treated by enzyme pullulanase were exposed to different enzyme concentration, 2% and 4%, and different reaction time, 1, 3 and 5h. Determined parameters from DSC curves, the temperature of degradation T_d are presented in Table 1.

Table 1. Degradation temperature of resistant starch obtained by enzyme treatment

Sample	T_d [°C]	$T_{d\ (after\ three\ weeks)}$ [°C]	*Sample*	T_d [°C]	$T_{d\ (after\ three\ weeks)}$ [°C]
I (1h,2%)	138.93	169.58	IV (1h,4%)	139.70	158.30
II (3h,2%)	139.40	159.25	V (3h,4%)	139.85	167.36
III (5h,2%)	139.53	169.21	VI (5h,4%)	136.62	163.74

Longer time of enzyme action for the same enzyme concentration caused slightly increase of degradation temperature. Applied enzyme, pullulanase, is debranching enzyme that rapidly hydrolyses only the α–1, 6–glycosidic bonds of amylopectin molecule. Longer reaction time caused an increase of debranching degree and appearance of more short side–chains from the initial amylopectin molecule. Residual side amylopectin chains aligned and by hidratic bonds aggregated and hence form crystalline structures. More released side–chains lead to the formation of broad range of crystalline forms, thus more energy is required for decomposition (dissociation) of the sample, accordingly degradation temperature increased.

The addition of the higher amount of enzyme did not result in complete debranching, thus higher pullulanase concentration of 4% has not significant influence on DSC parameters.

Storage of resistant starch type 3 obtained by enzyme treatment at 4°C for three weeks significantly increased parameters of thermal properties. During storage the retrogradation of amylopectin occurred. It is slow process that requires several weeks and depends of storage temperature and water availability (7). Required degradation energy is higher and confirmation of that was a significant higher value of degradation temperature T_d and enthalpy ΔH of stored samples compared to the samples measured after preparation. Significant increase of enthalpy for stored samples is presented at Figure 1.

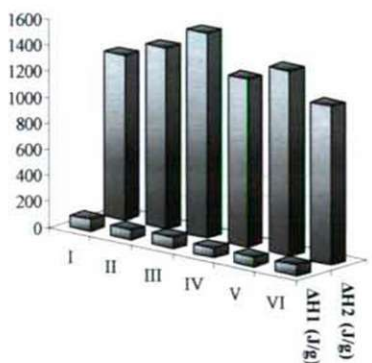

Figure 1. Enthalpy of resistant starch samples obtained by enzyme treatment: ΔH_1–enthalpy of samples measured after preparation, ΔH_2–enthalpy of samples stored for three weeks

Thermal characteristics of resistant starch samples obtained by autoclaving are presented in Table 2.

Table 2. Degradation temperature and enthalpy of resistant starch samples obtained by autoclaving

Sample	T_d *[°C]*	$T_{d\ (after\ three\ weeks)}$ *[°C]*	ΔH *[J/g]*	$\Delta H_{(after\ three\ weeks)}$ *[J/g]*
I (10%, I autoclav)	139.34	164.52	85.63	1771
II (10%, II autoclav)	139.44	163.10	52.27	1341
III (20%, I autoclav)	140.98	59.82	78.81	93.91
IV (20%, II autoclav)	138.56	48.31	43.09	104.2

Slight increase of degradation temperature points that increase of starch concentration in the suspensions caused higher resistant starch yield. The number of autoclaving cycles did not increase the number of released side–chains, thus amount of crystalline regions in the resistant starch structure was approximately equal, and differences between degradation temperatures were negligible. Storage of these samples at 4°C for three weeks led to retrogradation process. It affected the values of enthalpy and degradation temperature that were higher for stored samples.

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