

Enzymatic hydrolysis of starch in electrochemically activated aqueous solution

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Abstract. In this work, the hydrolysis of starch in an enzyme solution was studied, where softened water or fractions of a metastable electrochemically activated aqueous solution (ECAS) were used as a solvent. Extracts obtained after hydrolysis of food starch grains were analyzed using optical density spectrometry of the sample and micro-weighing with a quartz resonator of the dry residue contained in an aqueous solution. It is shown that the enzyme solution on the reduced fraction of ECAS (catholyte) contains the least amount of extracted substances, but it contains the highest concentration of oligosaccharides. This fact may mean the presence of a more efficient cleavage by the enzyme of water-insoluble polysaccharides to low molecular weight derivatives in the medium of the electrolyte. As a result of this synergistic effect, a relatively high content of oligosaccharides is observed even at a low level of primary hydrolysis on the surface of the starch grain. This assumption was investigated on modified starch soluble in water. For the compared aqueous solutions of the enzyme preparation, which initially contain the same concentration of amyloextrins, faster hydrolysis is observed in the enzyme medium on softened water.

1 Introduction

The use of enzyme preparations in food production technologies determines the importance of optimizing the conditions for their effective use. An electrochemically activated aqueous solution is a promising aqueous medium that influences the course of enzymatic reactions. As a result of the electrolysis of water, the corresponding metastable fractions of an aqueous solution with unique properties accumulate in the electrode region [1-2]. The selection of the electrolysis mode allows you to adjust the main parameters of the ECAS: acidity (pH) and redox potential (RP). The oxidized ECAS fraction (anolyte) is characterized by an acidic pH value and an abnormally high positive RP, while the reduced fraction (catholyte) – alkaline pH and negative RP. The chemical activity of ECAS is largely due to the presence of nano-bubbles of oxygen in the anolyte and hydrogen in the catholyte, stabilized by uncompensated electric charges, which are concentrated at the gas-

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liquid phase interface [3]. The presence of such bubbles causes a decrease in the surface tension of the ECAS [1, 4].

It is known that ECAS fractions stimulate biocatalytic processes in food technologies [5-6], increasing the yield of the target product during extraction [7-12]. ECAS accelerates seed germination by activating enzymes, primarily hydrolytic ones [13-17]. Soaking the grain in slightly acidic ECAS followed by rinsing with tap water or alkaline catholyte accelerated the germination of triticale malt by 25% [17]. Another example, the treatment of beer pellets during the day with a solution of an enzyme preparation on a catholyte, allowed to obtain extracts with an increased content of phenolic acids, aldehydes and the flavonoid rutin [9]. In general, electrochemical activation changes not only the properties of an aqueous solution, but also the substances dissolved in it. Such aqueous solutions can affect the activity of enzymes, for example, by changing the ionization of the active center, the degree of stability of the tertiary structure of the protein, lowering the activation energy of the enzymatic reaction.

Another strategy when using amylase complex preparations is the development of innovative methods for the preparation of starch-containing raw materials [18-22]. It has been experimentally confirmed that catholyte and anolyte obtained from distilled water [23] or softened tap water [24] themselves have an effect on starch hydrolysis. In other words, ECAS can be used both to stimulate enzymatic activity and for non-enzymatic modification of starch. Therefore, the combined action of ECAS fractions and amylolytic enzyme is of interest, despite the fact that the physico-chemical properties of anolyte and catholyte suggest their multidirectional influence on the hydrolysis process. The present study was conducted to investigate the effectiveness of an amylolytic preparation dissolved in water or ECAS fractions. Non-soluble (native) or soluble (modified) starch in water was taken as the target of exposure. The efficiency of starch hydrolysis was studied by UV-vis spectrometry, as well as by micro-weighting on a quartz resonator – a method for determining the dry residue content in solution samples with a volume of several microliters. The practical significance of the work is due to the need to develop starch modification technology in order to hydrolysis it under gentle conditions, as well as the desire to increase the yield of target starch derivatives.

2 Objects and methods

2.1 Electrochemically activated aqueous solutions

For the experiment, softened drinking water from the municipal water supply with a pH of 7.2 and an RP of +360 mV was used. Anolyte and catholyte were obtained by means of a commercial electrolyzer STEL-Universal. The parameters of the aqueous fractions are as follows: anolyte RP +800 mV and pH 2.2, catholyte RP -800 mV and pH 8.2. These parameters of the aqueous solution were measured using the Ecotest-120 ionomer (ECONIS, Russia), where RP is recorded with a platinum electrode EPV-1, and pH with an ion-selective glass electrode and for comparison silver chloride electrode with a saturated solution of KCl.

2.2 A solution of an enzyme preparation

For the study, a commercial enzyme amylolytic preparation Amylosubtilin (Sibbiopharm LLC, Russia) with an alpha-amylase activity of at least 1500 units/g, and glucoamylase activity of up to 100 units/g was taken. The preparation was dissolved in distilled water at a ratio of 20 mg/ml. During the preparation of the test sample, the solution of the preparation

was added to the aqueous starch medium. The operating conditions of the exposure, which are given by the manufacturer: pH 4.0÷8.5; temperature 30 °C÷80 °C.

2.3 An aqueous extract from starch grains

An aqueous extract from grains of water-insoluble edible potato starch (Skyfood LLC, Russia) was obtained at the rate of 7 g of starch in 24 ml of an aqueous medium, where softened water or ECAS fractions obtained from this water were taken for comparison. For hydrolysis, 35 µl of an enzyme preparation solution was added to the prepared starch suspension, the mixture was placed in a thermostat at 55 °C. After 1 hour or 3 hours of exposure, the enzyme activity was inhibited by adding hydrochloric acid at the rate of 2 ml of 1N HCl per 5 ml of starch suspension. After that, the sample was centrifuged for 20 minutes at 2000g in order to obtain an extract free from a suspension of starch particles, which contained substances dissolved in it, including oligosaccharides.

2.4 An aqueous extract from modified starch

At the beginning, 2.5% (by weight) was prepared with distilled water mother liquor of modified starch (Agat-Med LLC, Russia). For enzymatic hydrolysis, mixing 25 ml of mother liquor and 75 ml of solvent (softened water, anolyte, catholyte), a working solution was obtained, to which 35 µl of an enzyme preparation solution was added. The mixture was placed in a thermostat at 55 °C, after 60 minutes of exposure, the enzyme activity was inhibited by adding hydrochloric acid at the rate of 2 ml of 1N HCl per 5 ml of starch working solution.

2.5 Spectrometry of oligosaccharides

The effectiveness of hydrolysis was evaluated by analyzing the absorption spectra of aqueous extracts obtained after enzymatic treatment of starch in softened water or ECAS fractions. Using an iodine reaction, the absorption spectrum of oligosaccharides was recorded in the visible region for an aqueous extract. In this group of macromolecules, depending on the molecular weight, the characteristic peak in the absorption spectrum is in the wavelength range 550nm÷650nm. It should be noted that the coloring of oligosaccharides using this reaction is carried out with an excess of iodine in solution, as evidenced by the presence of an absorption peak at a wavelength of 355 nm. For spectrometry, a quartz cuvette was filled with 4 ml of the analyzed solution, the optical density of which was recorded on a Shimadzu UV-2401PC spectrophotometer (Japan). As a comparison, a quartz cuvette filled with water, anolyte or catholyte was used for samples prepared on water or the corresponding fractions of ECAS.

2.6 Micro-weighing of the dry residue in an aqueous solution

The micro-weighing (QCM – quartz crystal microbalance) method is based on recording changes in the resonant frequency of a quartz resonator after applying a substance to its surface. To determine the mass of the dry residue using quartz micro-weights, a drop (2 µl) of the test solution was applied to the surface of the crystal electrode. After evaporation of water, a dry residue remains, the weight of which linearly determines the decrease in the resonant frequency of vibrations of a quartz crystal. In a comparative experiment, samples of the extract were weighed, which was formed as a result of enzymatic hydrolysis of starch in softened water or ECAS fractions obtained from this

water. If necessary, the calculation of the content of substances in the initial solution is carried out on the basis of an empirical calibration line.

3 Results and Discussion

3.1 Hydrolysis of starch insoluble in water

The suspension of food starch granules was treated with an enzyme preparation in a medium of softened water or ECAS fractions. Then, in order to remove the suspension of starch particles, the suspension was centrifuged and, as a result, an extract containing water-soluble substances, including oligosaccharides, was obtained over the precipitate, which were stained with an iodite reaction. Specific absorption spectra were recorded for this extract (Figure1).

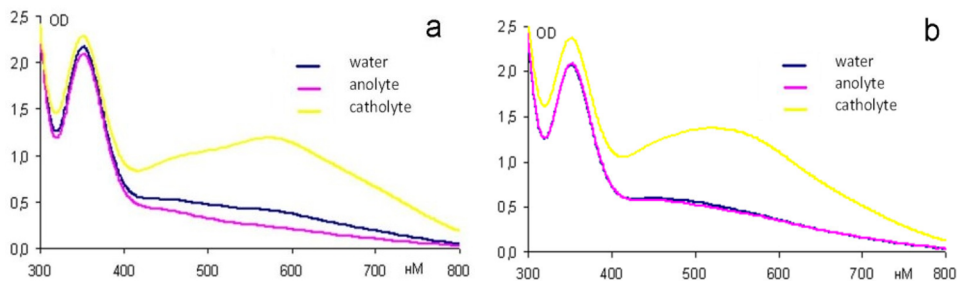


Fig. 1. Specific absorption spectra for oligosaccharides in aqueous solutions obtained as a result of enzymatic hydrolysis of starch grains at 55 °C in different media, where: (a) hydrolysis for an hour, (b) hydrolysis for 3 hours. Designations: (water) – softened water medium, (anolyte) – medium of the oxidized ECAS fraction, (catholyte) – medium of the restored ECAS fraction.

The effectiveness of hydrolysis of food starch grains under the action of an enzyme preparation was studied in solutions (water or ECAS fractions) stained with an iodite reaction, which is specific to the target product – oligosaccharides. A comparison of the optical density of such solutions shows a high concentration of oligosaccharides in the solution on the catholyte, after hydrolysis for 1 hour (Figure 1a). This trend persists for several hours (Figure 1b), when the aqueous solution remains in the metastable state of the reduced fraction. At the specified exposure time, a relatively low content of the target product is recorded for starch grain extract in water and anolyte. Unfortunately, the interpretation of the obtained spectra in terms of amylolytic activity is ambiguous, given the fact that two multidirectional processes occur in the situation under consideration, affecting the level of a specific absorption spectrum. Hydrolysis of starch polysaccharides causes an increase in the concentration of oligosaccharides in solution and, consequently, an increase in the intensity of the specific absorption spectrum. At the same time, the optical density of the solution in the region of the characteristic peak may decrease, due to the cleavage of complex sugars to low molecular weight derivatives that are not stained by the iodite reaction.

When discussing the effect of catholyte on increasing the optical density of the extract obtained by hydrolysis of starch grains in catholyte, the following should be taken into account. The main activity of Amylosubtilin is α -amylase with an endogenous mechanism of action. Therefore, it is possible that the hydrolysis of amylose and amylopectin macromolecules by α -1,4 bonds occurs first with the formation of large dextrans, which causes an increase in the intensity of the absorption spectrum in the region of 550-650 nm.

At the same time, the intrinsic hydrolytic effect of the catholyte manifests itself, leading to the cleavage of oligosaccharides from the ends of starch molecules or the resulting large polysaccharides. It cannot be excluded that the relative high concentration of oligosaccharides indicates an increase in the activity of Amylosubtilin in the medium of the electrolyte, for example, due to a change in the conformation of the enzyme molecule. It is also possible that this effect is due to a change in the spatial arrangement of amylose and amylopectin in the medium of the catholyte, which increases the availability of sites of α -1,4-glucoside bonds for enzyme attack.

Thus, the content of oligosaccharides in the solution is a consequence of the combined action of several factors. Among them is the rate of primary hydrolysis on the surface of starch grains, which may depend on the aqueous medium (water, ECAS fractions). In such a situation, the total amount of solutes in the compared enzyme solutions will be different. This assumption was studied by gravimetric method by measuring the weight of the dry residue in the analyzed extract (Figure 2).

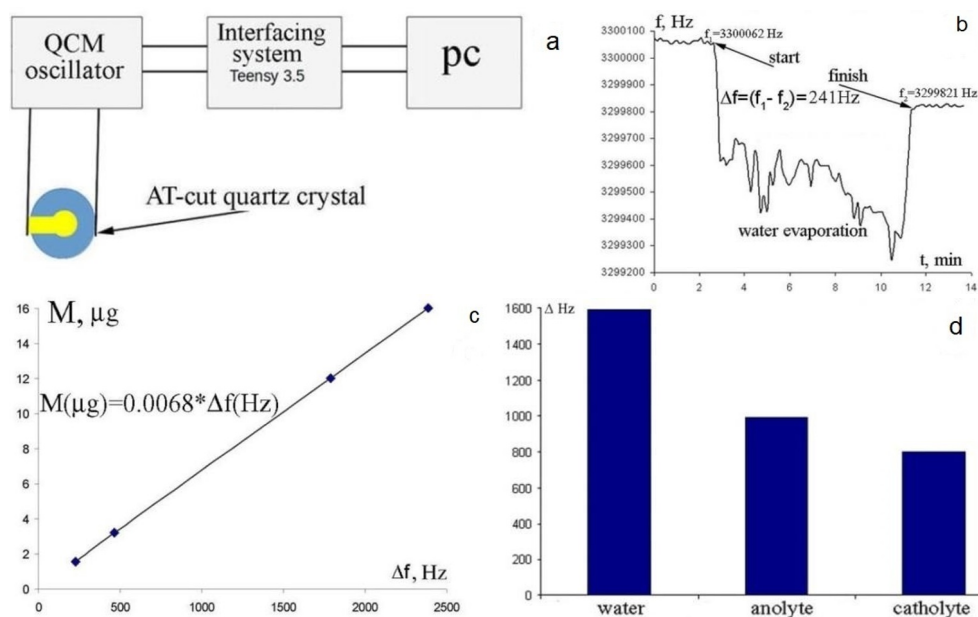


Fig. 2. An illustration of the approach for micro-weighing the dry residue in a drop (2 μ l) of an aqueous extract from starch granules: (a) a block diagram of the device used; (b) an example of a time change in the resonant frequency of a quartz crystal when a drop of aqueous solution applied to the surface of the crystal dries, the beginning and completion of water evaporation are shown; (c) calibration line of the dependence of the displacement (Δf , Hz) of the resonant frequency of a quartz crystal from a scale of dry residue containing an reference aqueous solvent in a drop; (d) a change in the frequency of the quartz resonator after drying of the applied drop of an oligosaccharide solution obtained after incubation of grain starch in an aqueous medium of an enzyme preparation.

Designations: (water) – incubation in the plant preparation in the water, (anolyte) – incubation in the plant preparation in the anolyte, (catholyte) – incubation in the plant preparation in the catholyte.

The dry residue content in the solution was recorded using a quartz resonator [25-26]. Figure 2 illustrates the principles of micro-weighing, which is based on the linear dependence of the change in the resonant frequency of a quartz crystal on the weight of the film deposited on the surface of the electrode (Figure 2a). When applying a drop of solution, the frequency decreases sharply, but after evaporation of the water returns to a stationary state (Figure 2b). The difference between the initial and final resonant frequency

is directly proportional to the mass of the dry residue in the drop. And this allows us to make quantitative measurements using an empirical calibration dependence (Figure 2c). The results of dry residue gravimetry were obtained for an enzyme solution after hydrolysis of grains in it (Figure 2d). It can be seen that the largest amount of dissolved substances contains a solution on water. There is the least dry residue in the solution on the catholyte, despite the fact that the highest concentration of oligosaccharides is recorded in it (Figure 1a,b). In other words, a high level of oligosaccharides remains in the enzyme solution on the catholyte even at a low rate of extraction of substances from starch grains. This contradiction may be explained by the more efficient hydrolysis of polysaccharides in the medium of the reduced fraction of water. And this maintains a relatively high concentration of low molecular weight sugars even with a relatively low content of polysaccharides.

Thus, for the synergistic effect of the catholyte and the enzyme preparation, the presence of polysaccharides in the medium is necessary. To verify this conclusion, the hydrolysis of modified starch, which is a mixture of water-soluble amyloextrins, was investigated. In a solution of such a preparation, only the cleavage reaction of amyloextrins to oligosaccharides and glucose takes place without replenishing the initial pool of high-molecular sugars, as in the case of hydrolysis of starch granules. In this case, the absorption spectrum allows us to judge the effect of the properties of the aqueous solution on the amylase activity of the enzyme preparation.

3.2 Hydrolysis of modified starch

The amylolytic effect of an enzyme preparation was experimentally studied in softened water or ECAS fractions, which initially contained the same concentration of modified starch. The obtained solutions were analyzed by spectrometry, comparing their absorption spectra (Figure 3).

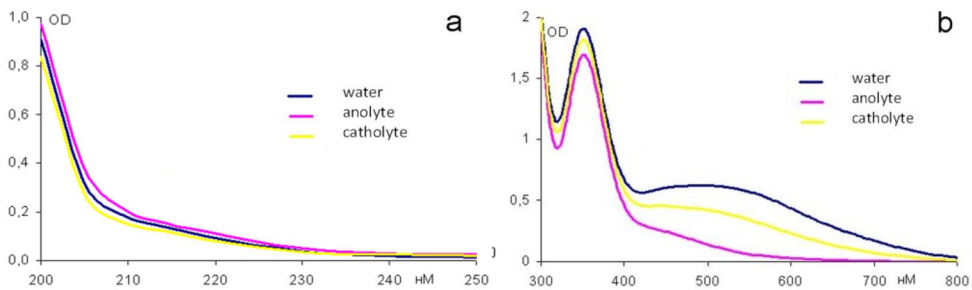


Fig. 3. Absorption spectra of an aqueous solution of modified starch, where: (a) non-specific spectra before the start of enzymatic hydrolysis, (b) specific spectra for oligosaccharides obtained after enzymatic hydrolysis for an hour at 55 °C. Designations: (water) – softened water medium, (anolyte) – medium of the oxidized ECAS fraction, (catholyte) – medium of the restored ECAS fraction.

The condition of the experiment was the same initial amount of modified starch dissolved in the compared aqueous media. This was achieved by using a mother starch solution in all cases and observing the proportions of the initial ingredients, and control was carried out by recording a non-specific absorption spectrum in the ultraviolet region (Figure 3a). The intensity of such a spectrum reflects the total amount of the organic component in an aqueous solution, therefore, for samples with the same composition it can serve as a measure of the concentration of substances. An analysis of the specific absorption spectra (Figure 3b) shows that after hydrolysis, the lowest content of oligosaccharides is recorded in the enzyme solution on the oxidized ECAS fraction. This result suggests that the activity

of glucoamylase in the anolyte increases, catalyzing the cleavage of oligosaccharides to glucose, which is not stained by the iodite reaction.

4 Conclusion

Thus, in a comparative experiment, the effectiveness of the enzyme preparation Amylosubtilin was analyzed in the splitting of starch grains in an aqueous suspension, as well as an aqueous solution of modified starch. Softened water or ECAS fractions (anolyte, catholyte) were used as an aqueous medium. It is shown that in the case of a suspension of starch grains, the least amount of extracted substances contains catholyte, despite the fact that the highest concentration of oligosaccharides is recorded in it. This fact may mean that the enzyme preparation has a high activity of the type of α -amylase in the catholyte, which ensures the rapid accumulation of oligosaccharides even at a relatively low level of extraction of substances from starch grains. In an experiment with modified starch, which contains only water-soluble amyloextrins, enzymatic hydrolysis proceeds relatively quickly in an anolyte medium.

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