

# Potato starch plasticization by natural deep eutectic solvent<sup>\*</sup>)

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**Abstract:** A natural deep eutectic solvent (NADES) based on choline chloride and malic acid was used to plasticize potato starch. The influence of starch gelatinization temperature, NADES content and the method of its incorporation (before and after gelatinization) on starch plasticization was investigated. Polymer-plasticizer interactions were studied using MDSC, DMTA, XRD and FTIR-ATR methods. The viscosity of starch solutions as well as the mechanical properties and water absorption of the obtained films were determined. Gelatinization conditions had a significant impact on the viscosity of starch solutions and the physicochemical properties of the obtained films. The cross-linking reaction between starch and malic acid was confirmed by MDSC, DMTA, and mechanical tests. Slight recrystallization of starch was observed after 12 months of storage.

**Keywords:** starch, natural deep eutectic solvent, malic acid, plasticization.

## Plastyfikacja skrobi ziemniaczanej naturalną cieczą głęboko eutektyczną

**Streszczenie:** W pracy do plastyfikacji skrobi ziemniaczanej użyto naturalnej cieczy głęboko eutektycznej (NADES) na bazie chlorku choliny i kwasu jabłkowego. Zbadano wpływ temperatury żelowania skrobi, zawartości NADES oraz sposobu jego wprowadzenia (przed i po żelowaniu) na plastyfikację skrobi. Oddziaływania polimer-plastyfikator badano przy użyciu metod MDSC, DMTA, XRD i FTIR-ATR. Oznaczono także lepkość roztworów skrobi oraz właściwości mechaniczne i nasiąkliwość otrzymanych folii. Warunki żelowania miały istotny wpływ na lepkość roztworów skrobi oraz właściwości fizykochemiczne otrzymanych folii. Reakcję sieciowania skrobi z kwasem jabłkowym potwierdzono za pomocą MDSC, DMTA i badań mechanicznych. Po 12 miesiącach przechowywania zaobserwowano nieznaczną rekrytalizację skrobi.

**Słowa kluczowe:** skrobia, naturalna ciecz głęboko eutektyczna, kwas jabłkowy, plastyfikacja.

The external plasticizers are the substances incorporated into a polymeric material to increase its elasticity, workability, or stretchability [1]. The plasticizer addition is necessary to process or obtain polymeric materials, i.e., thermoplastic starch. However, the common low-molecular weight starch plasticizers (e.g., water, glycerol, ethylene glycol) tend to migrate from the starch materials limiting their application [2]. Recently, deep eutectic solvents (DES) were selected as a promising group of starch plasticizers.

The deep eutectic solvents are mixtures of at least two components, where the individual component has a melting point higher than the mixture [3]. The DESs prepared by mixing solid quaternary ammonium halide salt (hydrogen bond acceptor) with solid or liquid car-

boxylic acid (hydrogen bond donor) are popular for processing biopolymers. The carboxylic acids with various chemical structures could be applied: mono- or multicarboxylic, aliphatic or aromatic acids, containing other (e.g., hydroxyl) functional groups [2–7]. The DESs made from choline chloride and citric acid monohydrate were used as media for pectin extraction [8], and plasticization [9]. The eutectic systems made from choline chloride and acetic, lactic, oxalic, or levulinic acid were successfully applied for lignin [10, 11] and cellulose extraction [12].

Malic acid is a solid crystalline substance with asymmetric carbon. The name comes from Latin for “apple”, as it was isolated for the first time from unripe apples [13]. Neat malic acid was used for compatibilizing of starch/guar gum system (resulted in improved stretch-

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ability) [14], for starch esterification (starch malate) [15], cross-linking of high amylose starch (obtained films exhibited higher thermal resistance and reduced swelling power) [16, 17], and plasticization (increased retrogradation resistance was observed) [18, 19]. The DES from choline chloride and malic acid is classified as a Natural Deep Eutectic Solvent (NADES) [3, 20], as it is prepared from natural components that are abundant, biodegradable, and exhibit low toxicity [5, 21]. NADES based on choline chloride and malic acid (1:1) was used for starch dissolution [22]. Moreover, it was applied (30 wt% on a basis of dry polysaccharide) to obtain starch-based composite (with sodium and calcium montmorillonite) [23]. However, to our best knowledge, there is no report on the starch plasticizing efficiency of this system.

In this study, natural deep eutectic solvents based on choline chloride and malic acid (molar ratio 1:1) were used to plasticize potato starch. The influence of temperature, NADES content and the method of its incorporation (before or after starch gelatinization) on the polysaccharides plasticization was evaluated. Techniques were used to determine starch-plasticizer interactions: microscopy, FTIR spectroscopy, X-ray diffraction, viscometry, DSC, and DMTA. Additionally, starch-based films with NADES were obtained and the effect of the plasticizer dosing method on their mechanical properties and water absorption was examined.

## EXPERIMENTAL PART

### Materials

Choline chloride ( $\geq 98\%$ ) and DL-malic acid ( $\geq 98\%$ ) were purchased from Aldrich Chemistry. Potato starch (S) was supplied by Zetpezet Pila (Poland) (ca. 20 wt% amylose content, 19 wt% moisture). To obtain a natural deep eutectic solvent, choline chloride and malic acid (molar ratio 1:1) were placed in a glass container and stirred with a magnetic stirrer in a water bath at 80°C until a homogeneous liquid was obtained. NADES was designated "CM".

### Samples preparation

Starch (S) and plasticizer (5, 10, 20, 30, 50, and 70 wt% based on dry polysaccharide) were mixed with pestle and mortar, and subsequently placed in the sealed glass vials. The samples were prepared 24 hours before the tests.

Starch films marked as "A" were prepared as follows: starch in distilled water was gelatinized while stirring for 30 minutes at 90°C. Then NADES was added, and the entire system was mixed for 10 minutes until homogeneity. Subsequently, the starch-based mixture was poured into Petri dishes and dried at 50°C.

Starch films marked as "B" were prepared as follows: starch with NADES and distilled water were stirred with

a mechanical stirrer for 30 minutes at 70, 80, or 90°C. Subsequently, the starch mixture was poured into Petri dishes and dried at 50°C.

All films were conditioned for at least 24 hours in a climate chamber (23°C, RH 50%) before testing (the starch film samples were labelled as "F").

## Methods

### Modulated differential scanning calorimetry (MDSC)

The thermal properties of the films were determined by modulated differential scanning calorimetry. The mixture of starch with NADES (ca. 10 mg sample) was placed in the aluminium hermetic pan. The tests were performed using DSC Q100 (TA Instruments, New Castle, DE, USA) in the temperature range from -70 up to 220°C, and a heating rate of 3°C/min (1 cycle).

### Viscometry

The viscosity of the aqueous starch-NADES solutions (containing 30 wt% CM based on dry polysaccharide) was evaluated with Brookfield viscometer model RVT (Brookfield Engineering, Middleboro, Massachusetts, USA) at 60°C. The letters: A and B in the systems' symbols were for CM added after and before starch gelatinization, respectively. The last number in the sample abbreviation was the gelatinization temperature value.

### Infrared spectroscopy with Fourier transformation (FTIR)

The FTIR spectra of starch/DES mixtures and starch-based films were obtained using Nexus spectrometer (Thermo Nicolet Corp., USA) equipped with ATR. Each sample was scanned 32 times in a wave number range from 4000 to 400  $\text{cm}^{-1}$ . The spectra were analysed with OMNIC software.

### X-ray diffraction (XRD)

The starch films were dried for 24 h at 60°C. Then they were ground with a mortar and pestle and sieved. The powders were analysed by Panalytical Empyrean X-ray (Malvern Panalytical, Malvern, England) diffractometer, with  $\text{Cu K}\alpha$  radiation.

### Dynamic mechanical thermal analysis (DMTA)

Dynamic mechanical thermal analysis tests were performed using DMA Q800 (TA Instruments, New Castle, DE, USA). The measurements were conducted using film tension mode, at a frequency of 1 Hz, a heating rate of 3°C/min, and a temperature range from 90 to 140°C.

## Mechanical properties

Tensile properties were performed in accordance with the PN-EN ISO 527-3 standard using Instron 5982 (Norwood, MA, USA). The initial gap spacing was 50 mm and the crosshead speed was 10 mm/min (with a 1 kN load cell). Seven replicas of starch film strips (5 × 100 mm, approximately 0.3 mm thick) were tested. Mechanical parameters (maximum tensile strength, elongation at break and Young's modulus) were determined using Bluehill 3 software.

## Moisture absorption

The moisture absorption tests were performed as follows. The samples (10 × 20 mm) were dried for 24 h at 60°C. Then, they were weighed ( $m_0$ ) and placed in a climate chamber (23°C, RH 50%). Subsequently, they were reweighed ( $m_w$ ) after 1, 3, 24, 48, and 168 hours. The moisture absorption was calculated from equation (1).

$$\text{Moisture absorption} = \frac{m_w - m_0}{m_0} \cdot 100\% \quad (1)$$

## RESULTS AND DISCUSSION

On the base of MDSC thermograms (non-reversing transitions), the values of gelatinization temperature ( $T_{\text{gel}}$ ) and enthalpy ( $\Delta H$ ) values were determined (Fig. 1). It was observed that with plasticizer content increase, the gelatinization temperature decreased to ca. 84°C for the mixture with 50 wt% NADES. The sample containing 20 wt% plasticizer did not fit this trend, and a slightly higher  $T_{\text{gel}}$  value was noted. This may indicate the so-called antiplasticization phenomena [24].

The viscometry results for aqueous starch-NADES solutions were presented in Fig. 2. Significant differences were observed depending on the temperature of starch gelatinization and the type of plasticizer addi-

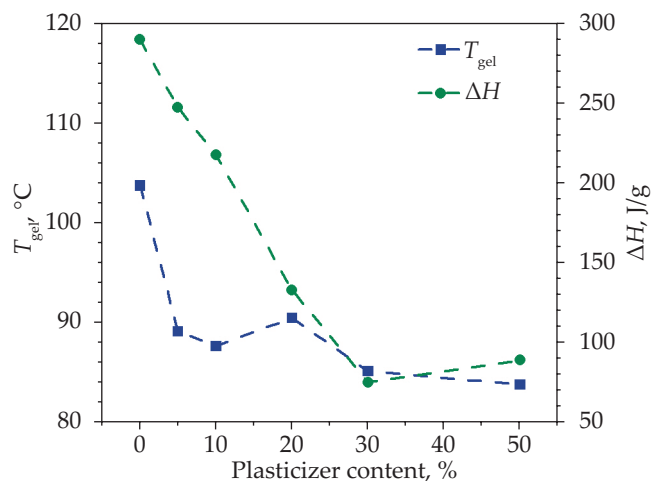


Fig. 1. The gelatinization temperature ( $T_{\text{gel}}$ ) and enthalpy ( $\Delta H$ ) as a function of plasticizer content

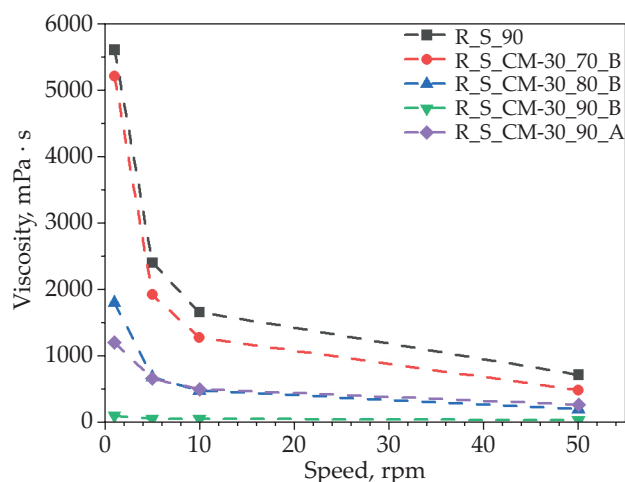
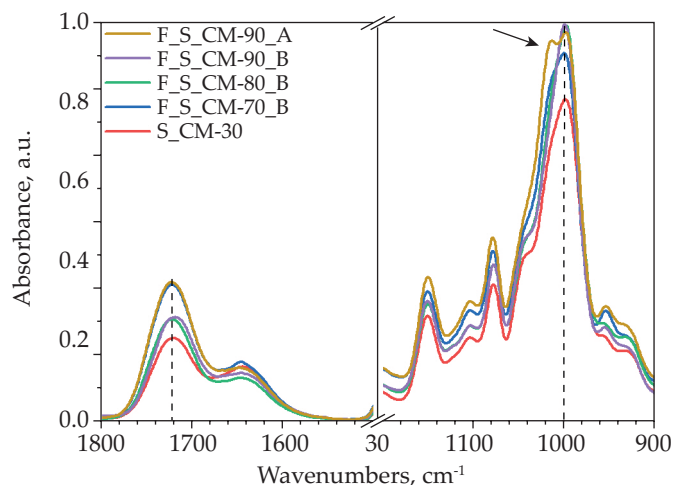


Fig. 2. Viscosity of starch-plasticizer (30 wt%) aqueous solutions, after gelatinization at 70, 80, or 90°C. The sample R\_S\_90 was the reference without NADES

tion. For all tested samples, the higher the processing temperature, the lower the viscosity of the final aqueous starch-NADES solution. The lowest viscosity value (ca. 40 Pa·s) was determined for the system prepared at 90°C, where CM was present during starch gelatinization (R\_S\_CM-30\_90\_B). Interestingly, the analogous system prepared at the same temperature and CM amount, but with NADES added after starch gelatinization, exhibited noticeably higher viscosity, i.e., 270 Pa·s (like the solution gelled at 80°C: R\_S\_CM-30\_80\_B). This may indicate that NADES present during starch gelatinization could lead to hydrolysis of polysaccharide (low pH and elevated temperature). However, even delayed CM addition resulted in lowering the viscosity, when compared to reference R\_S\_90, indicating the interactions between plasticizer and starch macromolecules.

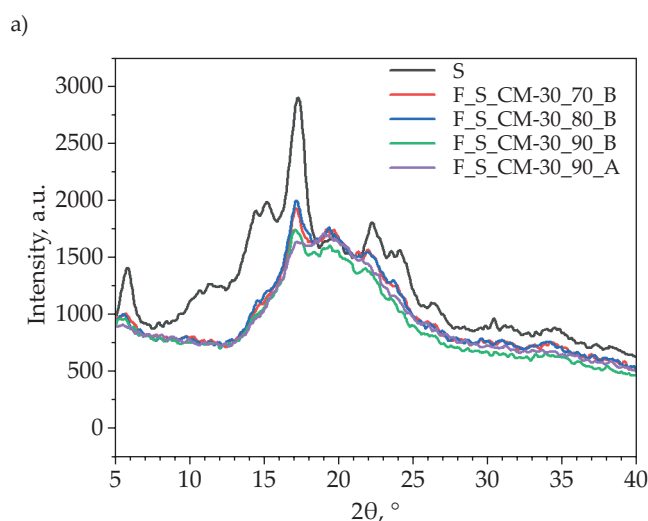
The FTIR-ATR spectra of the starch-based films showed bands typical for polysaccharide (3000  $\text{cm}^{-1}$  attributed to OH, 2900  $\text{cm}^{-1}$  attributed to  $\text{CH}_2$ , 1000  $\text{cm}^{-1}$  attributed to C-O). However, a band assigned to the carbonyl group (1750  $\text{cm}^{-1}$ ) from malic acid, a component of the plasticizer, was also observed. The differences in the intensity of the band about 1650  $\text{cm}^{-1}$  band representing water bound in amorphous regions were observed [25]: the lowest intensity for F\_S\_CM-30\_80\_B and slightly higher for F\_S\_CM-30\_90\_B (for both CM present during starch gelatinization). This may indicate a lower tendency for water accumulation on the surface of those films where starch was processed at higher temperature. Interestingly, a significantly lower intensity of the carbonyl bands (Fig. 3) was observed for the film where starch was gelatinized at 70°C and the system with delayed plasticizer addition. This could indicate a lower amount of malic acid on the sample surface, which may be due to the esterification reaction between starch hydroxyls and acid carboxylic groups (occurring more intensively at elevated temperatures), resulting in limited migration of the plasticizer. In the sample F\_S\_CM-30\_90\_A, the new band



**Fig. 3.** FTIR-ATR spectra of starch-DES mixture (S\_CM\_30) and films differing in the gelatinization conditions

about 1020  $\text{cm}^{-1}$  was observed, suggesting a higher content of the amorphous phase [26]. Thus, the type of plasticizer addition could significantly affect the crystallinity of starch materials. So, in the next step, XRD measurements were performed.

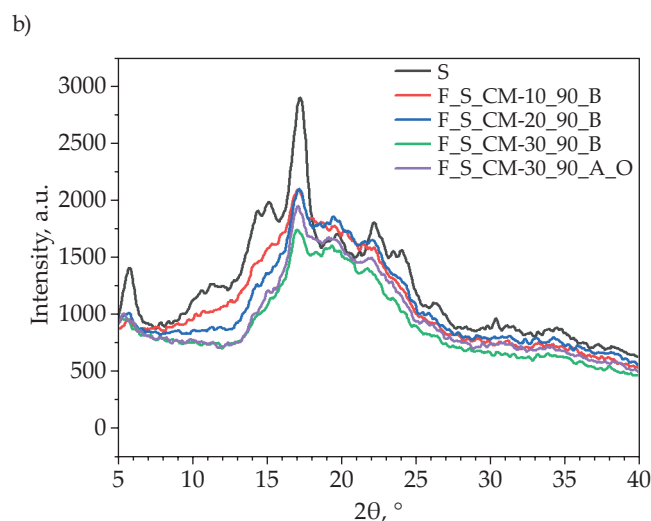
In Figure 4, the XRD diffractograms of native potato starch and starch/NADES films were presented. The native potato starch exhibits B-type crystallinity, typical for the tuber, with characteristic signals at ca. 6°, 15°, 17°, 20°, 22°, 24° and 26° [27]. For all the films with NADES evaluated, a decrease of the degree of crystallinity was observed. The films obtained with delayed plasticizer addition seemed to exhibit a slightly less crystalline phase. It could be noticed that the degree of crystallinity decreased with NADES content (Fig. 4b). Moreover, higher gelatinization temperature resulted in lower crystallinity (Fig. 4 a). To evaluate the retrogradation, diffractograms of “fresh” film (F\_S\_CM-30\_90\_B) and those stored for 12 months (F\_S\_CM-30\_90\_B\_O) were



compared. An increased intensity of signal at 17° was observed, indicating slight recrystallization of the material.

To determine the differences in material structure caused by the type of plasticizer addition, dynamic mechanical thermal analysis was performed (Fig. 5). However, the course of both loss and storage modulus curves of the starch-based films were similar. Two thermal transitions in  $\tan \delta$  thermograms were observed, at ca. 25°C corresponding to the starch glass transition, and at the lower temperature, ca. -25°C, referred to the plasticizer-rich phase and the glass transition of the plasticizer. In addition, a third transformation (ca. 70°C) was observed for sample A – Fig. 5b [28].

The effect of gelatinization conditions was evaluated by mechanical tests as well. An increase of starch gelatinization temperature had a beneficial impact on the mechanical properties of starch films (improved tensile strength, Young’s modulus) as can be seen in Figure 6. Interestingly, Figure 7 shows a significant difference in the value of these parameters noted for the materials with different type of plasticizer addition (samples marked A and B). The film containing 35 wt% CM added after starch gelatinization, and a film obtained with 20 wt% CM present during starch gelatinization were not tested due to “over-plasticization” and brittleness, respectively. Comparing the tensile strength and Young’s modulus of the films containing 30 wt% NADES, the materials with plasticizer added after starch thermal treatment, exhibited ca. 7 times and ca. 4 times lower values of this parameter, respectively, and slightly higher elongation at break. This confirmed the starch esterification, i.e., crosslinking of the polysaccharide with malic acid, as determined by FTIR measurements. Therefore, depending on the type of plasticizer addition, different plasticizer content was recommended. Zdanowicz et al. [29] reported the differences in the mechanical properties of starch-based films



**Fig. 4.** XRD patterns of native starch (S) and starch films with a) various parameters of starch gelatinization, and b) plasticizer content

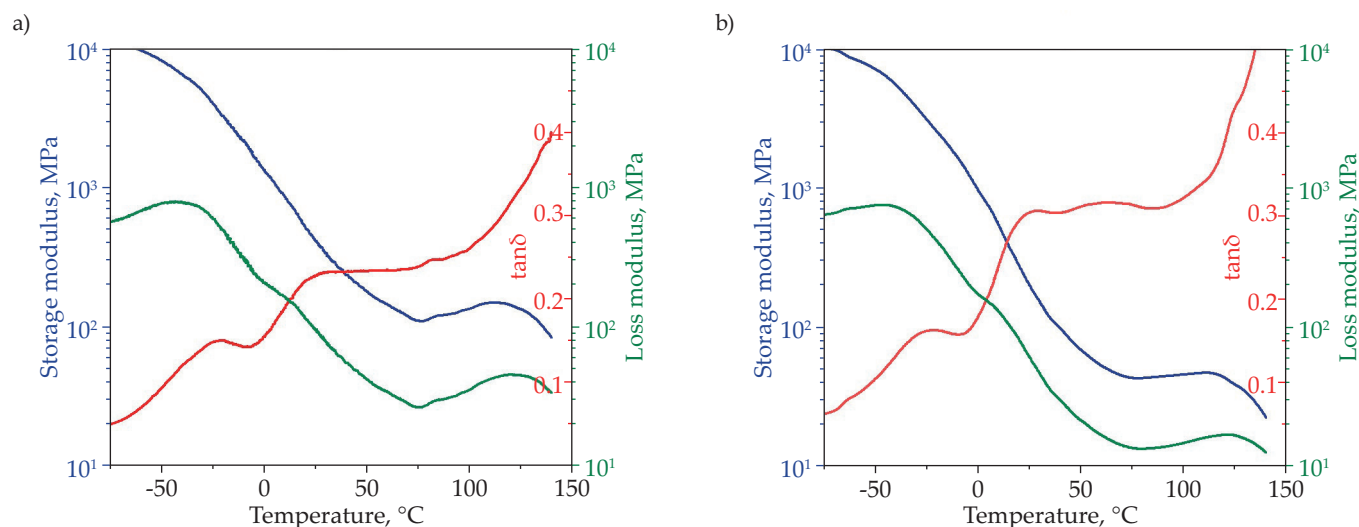


Fig. 5. DMTA curves of starch films plasticized at 90°C when: a) plasticizer was added during gelatinization, b) CM was added after thermal treatment

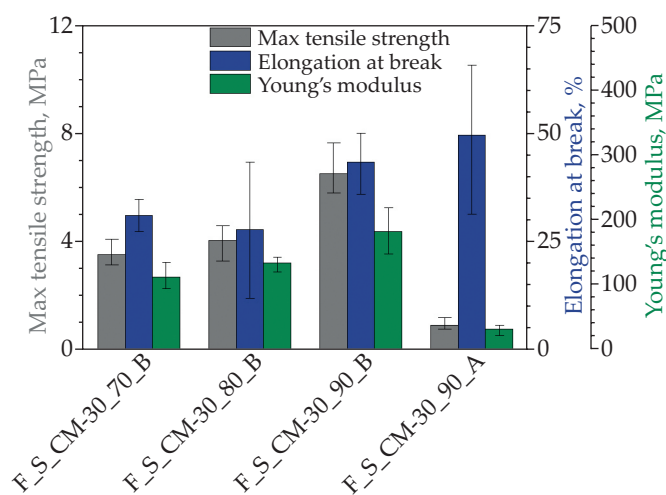


Fig. 6. Mechanical properties of starch films plasticized with CM obtained with various process parameters

plasticized with two- and three-component DES (based on choline dihydrogen citrate, glycerol, and urea), when the DES was added before and after starch gelatinization. However, observed differences in tensile strength were not as significant as for films plasticized with CM [29].

Determination of the moisture absorption of starch films can be seen in Figure 8. This parameter allows to determine the susceptibility of materials to external conditions. The moisture absorption strongly correlates with hygroscopic plasticizer content, the highest value (after 7 days) was reported for the films 30\_90\_A and 35\_90\_B, and the lowest one for 30\_80\_B (probably caused by incomplete starch gelatinization, similarly to 30\_70\_B). Lower moisture absorption noted for 90\_B (when compared to 90\_A) could be the result of consuming a part of the plasticizer in the cross-linking process. These observations correlate with mechanical measurement results.

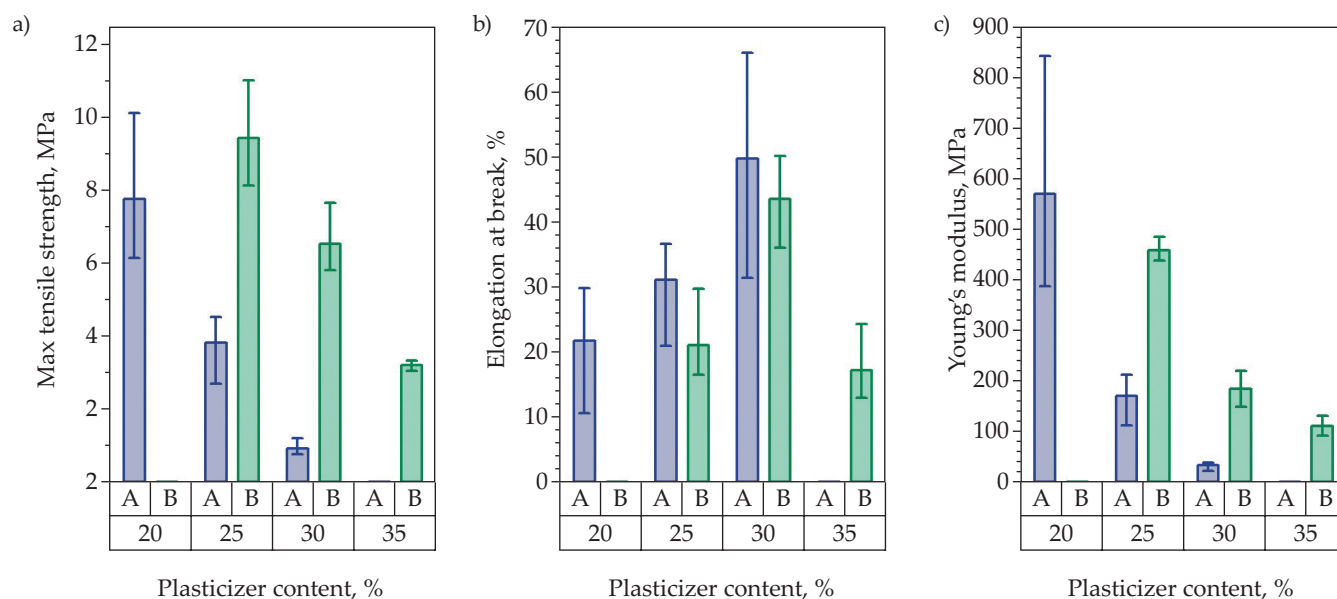


Fig. 7. Mechanical properties of starch films with various plasticizer content (starch gelatinized at 90°C). Samples A – CM added after gelatinization, samples B – CM was present during starch gelatinization

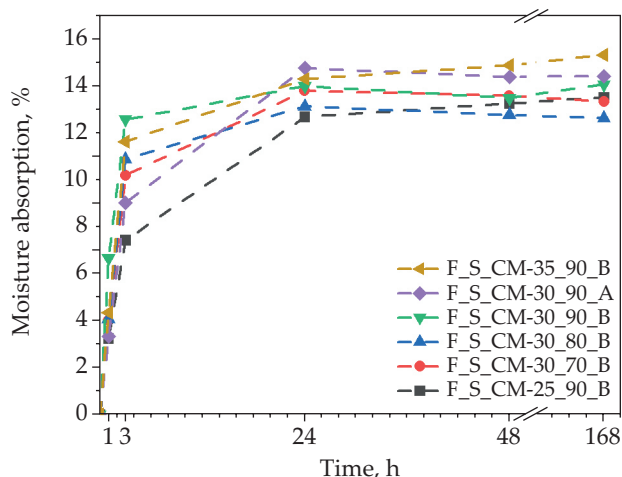


Fig. 8. Moisture absorption of starch-CM films

## CONCLUSIONS

NADES based on choline chloride and malic acid was used to plasticize starch. The effect of temperature, NADES content and the method of its incorporation (before or after starch gelatinization) on starch plasticization was evaluated. Gelatinization parameters (temperature and method of plasticizer addition) significantly influenced the viscosity of starch solutions and the physicochemical properties of starch-based films. The results of MDSC, DMTA, and mechanical properties showed that the cross-linking reaction between starch and malic acid occurred. XRD patterns showed slight recrystallization of starch after 12 months of storage. Taking into account the results described in this work, the applied NADES system can be used as an effective potato starch plasticizer.

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