Biocatalytic esterification of common polysaccharides.
Starch modification using lipases.

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1. Abstract

Presented work focuses on some detailed studies on enzymatic polysaccharide esterification. As a substrate a maize starch was used. Carboxylic acids with different aliphatic chains were the acylation agents and the process was carried out at different temperature, solvents and substrate: enzymes ratio. Obtained esters were analyzed according to the degree of substitution, degradation of polymer chains and changes in topography of modified starch granules. Obtained results shows that investigated method may be used for obtaining high substituted starches; however the DS strongly depends on the aliphatic chain of the acid.

2. Keywords

microwave, polysaccharide, esterification, lipase

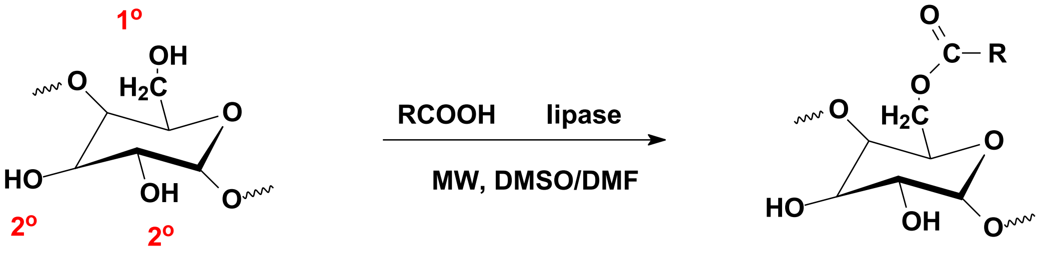
3. Introduction

Enzymes of different origins are going to play a significant role in modern organic synthesis on both laboratory and industrial scale [1]. Most of them work effectively in water reach environment only [2]. On the opposite site of biocatalysis some enzymes may be found to work in low-water or anhydrous reaction media. Lipases are one of the examples. It is known that lipase may be employed as effective catalyst of esterification, transesterification or ester hydrolysis in organic solvents [3-6, 9]. From the other hand polysaccharides that may be treated as polyalcohols dissolve mainly in water [4-5]. In the case of starch only limited number of solvent is known. The esterification of starch by means of biocatalysis may allow obtaining the product with higher degree of substitution [7-8]. Nowadays, such kind of material that possesses thermoplastic properties looks very promising as an eco-friendly and biodegradable.

Presented works focuses on some preliminary studies on starch (maize) esterification using carboxylic acids with different aliphatic chains (acetic, caprylic and stearic acid respectively).

4. Experimental

The reactions were carried out according to scheme 1, at different substrate: enzymes ratio. For all the reaction two different solvents were used i.e. DMSO and DMF.



$R = -CH_3, -C_{10}H_{21}, -C_{17}H_{35}$

Scheme 1

Obtained esters were analysed according to the degree of substitution (saponification of the ester and titration of the free acid), degradation of polymer chains (GPC method) and changes in topography of modified starch granules (SEM).

In a typical experiment the appropriate amount of maize starch – 4g (25mmol of AGU) was mixed up with 60ml of the solvent. The mixture was stirred magnetically until starch granules disappear (dissolved). Than carboxylic acid was added (25mmol) when stirring. Afterwards the pancreas lipase was added (2g) and the reaction mixture was stirred for 6 hours at 25 and 50^{o}C respectively. When the reaction was finished the mixture was poured into the acetone in order to deactivate the enzyme as well as starch ester to precipitate. The obtained sediment was separated by centrifugation (4500rpm, 15min), washed with water and dried.

5. Results and Discussion

All investigated processes testify that pancreas lipase may be treated as effective biocatalyst for polysaccharide esterification in conventional conditions. The reaction seems to be substrate independent.

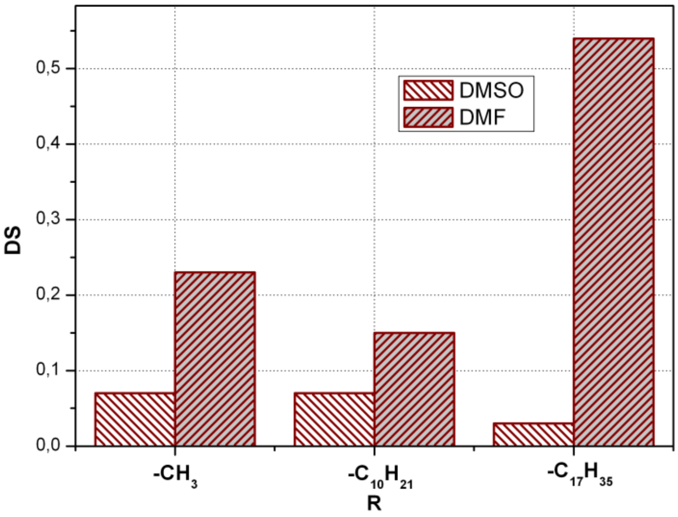


Figure 1. Solvent effect in maize starch esterification

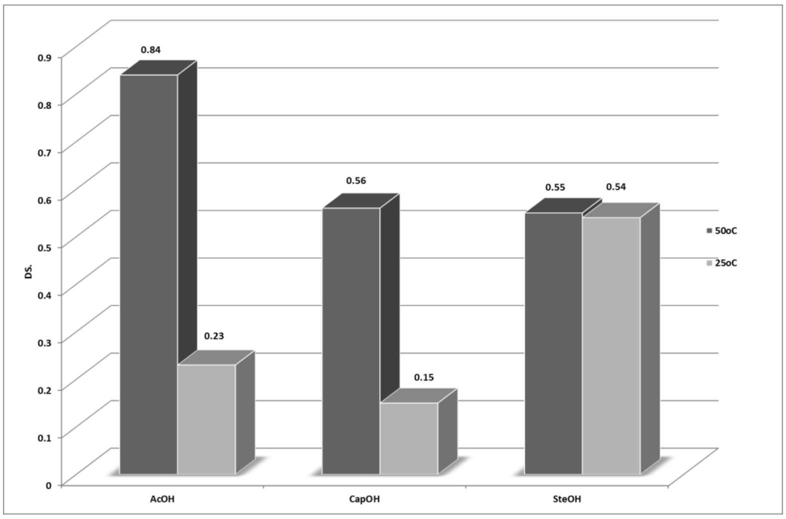
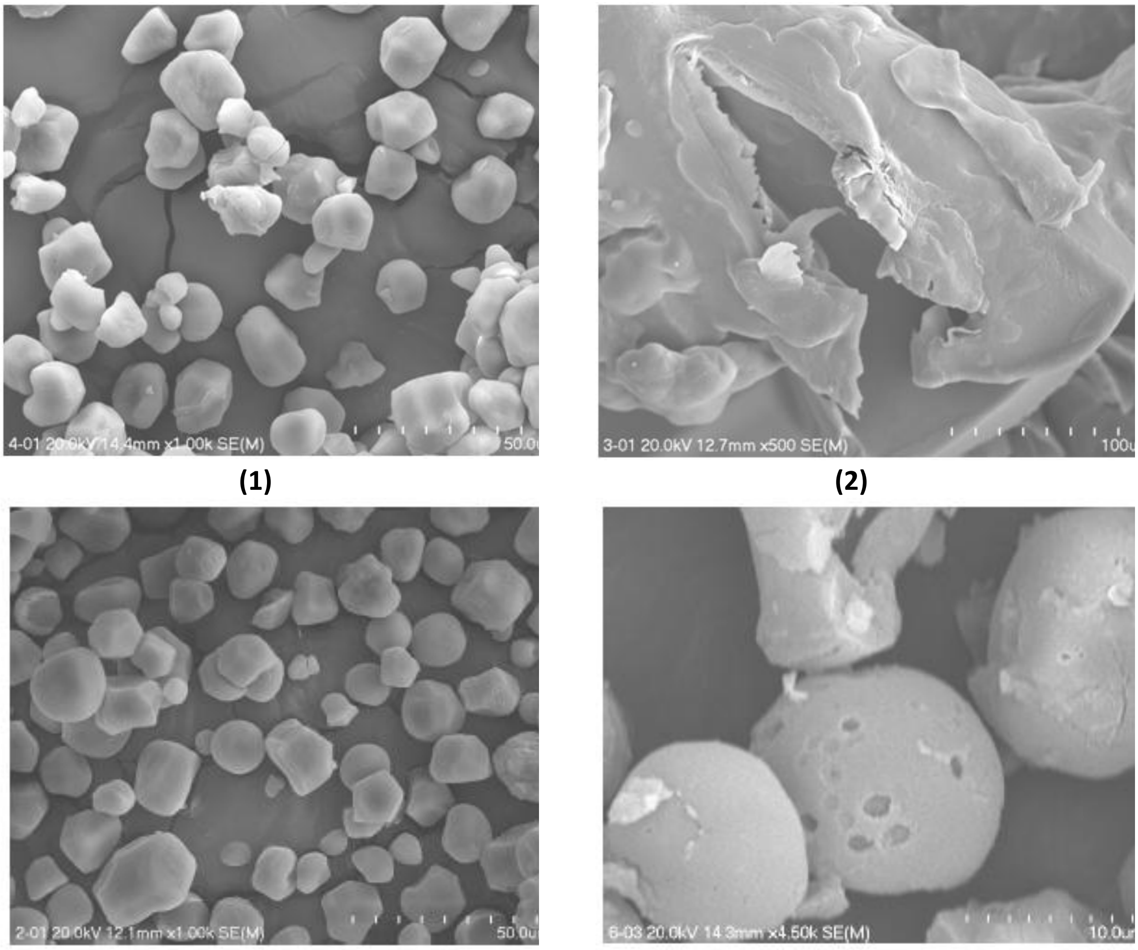


Figure 2. Temperature effect in lipase catalyzed starch esterification

Carboxylic acids may be introduced into the carbohydrate backbone even in case of very short (acetic) or very long (stearic) alkyl chain - Figure 1 and 2. It may be also noted that very common starch solvent i.e. DMSO gives worse result when compared to DMF. In all cases DMF used as a solvent gives better reaction effectiveness - Figure 1. Interesting results was also obtained when the influence of reaction temperature was investigated – Figure 2. In case of acetic and caprylic acid the yield of the ester is temperature dependent. From the other hand no differences was observed when stearic acid was used as a donor at 25 and 50°C respectively. The phenomenon looks interesting and needs further research.

Additionally some microphotographs were taken to check the structure of starch granule after modification – Figure 3. As it may be seen on SEM microphotographs the inner and outer starch structure in this case changed dramatically.



(1) **(2)**

(3) **(4)**

Figure 3. Microphotographs os obtained starch esters: (1) – CapOH, (2) – StOH, (3) – native starch, (4)- AcOH

In some cases (stearic acid esterification) the obtained material might be treated as a thermoplastic starch - Figure 3(2). Even though the granular structure is destroyed in most cases the molecular mass distribution does not change – Figure 4. For all modification products the similar monomodal peak is observed with no shifts to higher or lower fractions. Such results have powerful application consequences. As it is known almost all polysaccharide modification causes the shortening of carbohydrate chain by means of hydrolytic or oxidative pathway. The commercial starch esters obtained in conventional or industrial processes have far lower molecular weight especially when compared to native non-modified starch.

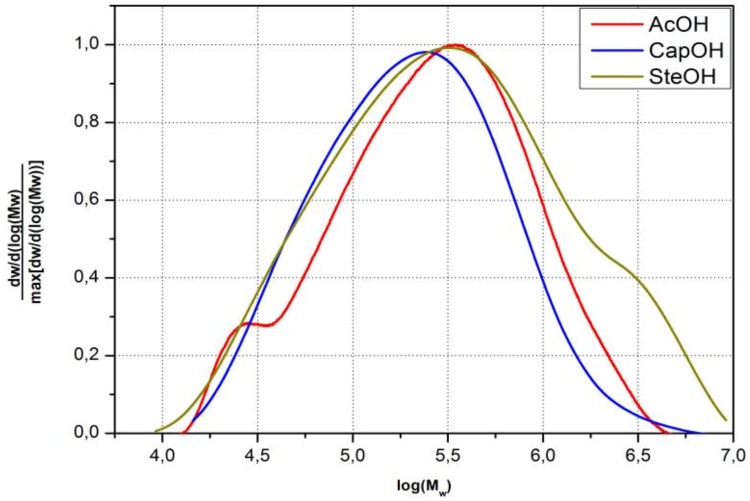


Figure 4. Molecular mass distribution in obtained starch esters

6. Conclusions

Obtained results shows that investigated method may be used for obtaining high substituted starches; however the DS strongly depends on the aliphatic chain of the acid. The basic properties of the product testify that they may be use at different fields of application as a thermoplastic starch. Obtained preliminary studies allow to hope in application usefulness of described protocols but further studies needs to be done.

7. References

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