SUPPLEMENTARY MATERIAL TO

# Inovative Environmentally Friendly Method of Starch Oxidation by Hydrogen peroxide

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# 2. Materials and methods

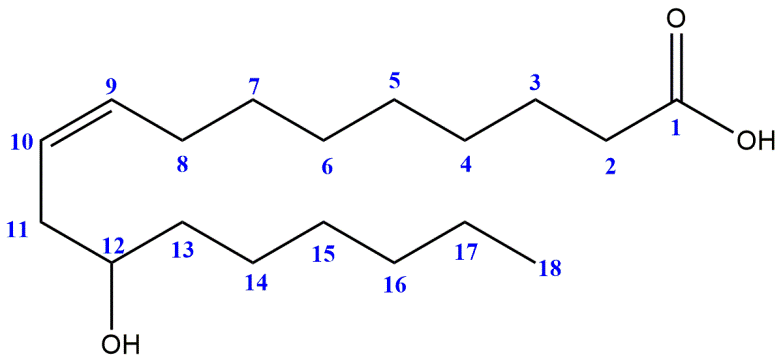
## *2.1 Materials*

Native wheat starch was purchased from local grocery store, while the chemical used for its modification are provided from following suppliers: absolute ethanol and formic acid (≥99.8% and ≥98.0%, respectively; ZORKA Pharma), potassium and sodium hydroxide (≥90.0% and ≥97.0%, respectively; HeMoss, Belgrade), concentrated HCl (Lachema, Czech Republic), diethyl ether (≥99.0%; Fisher UK), 30% solution of hydrogen peroxide (Centrohem, Stara Pazova), iron (II) sulfate heptahydrate (≥98.0%; Zorka, Šabac), copper(II) sulfate pentahydrate (99.9%; Merck KgaA, Germany), copper(II) acetate monohydrate (99.0%; Kemika, Zagreb), copper(II) chloride (98.0%; Riedel-de Haёn Germany), citric acid (≥99.5%; Sigma-Aldrich, Germany), hydroquinone (≥99.0%; Fluka, Germany), tartaric acid and isopropyl alcohol (≥99.5% and ≥98.0%, respectively; Merck KgaA, Germany).

## *Methods*

### *Laboratory isolation of ricinoleic acid*

The isolation of ricinoleic acid from castor oil was performed analogously to previous published method [1]: 233 g (0.26 mol) of castor oil and 700 mL of absolute ethanol were placed into a four-neck-reactor equipped with reflux condenser, mechanical stirrer, thermometer, dropping funnel and nitrogen inlet. After dissolving of castor oil, the 30% potassium hydroxide (0.91 mol) was slowly introduced in reactor with constant stirring. The reaction mixture was maintained at approximately 10 ºC during the addition of potassium hydroxide for 1 h. Hereafter,the reaction mixture was heated to 50 °C and temperature was kept constant for 2 h. After that, two-thirds of the solvent was removed from the mixture by distillation at atmospheric pressure. The obtained slurry was dissolved in distilled water, acidified with concentrated HCl to pH 3.0, purified with activated carbon and filtered. The obtained ricinoleic acid (RA) was extracted by diethyl ether and the obtained solution was dried with anhydrous sodium sulfate, and then in vacuum dryer at 80 oC/2000 Pa for 5 h. The FTIR spectrum was presented in the *section 3.4*. Elemental analysis calculated for C18H34O3 (Mw = 298.46 gmol-1 ): C, 71.42; H, 10.32; O, 18.26. Found: C, 70.98; H, 10.31; O, 18.71. The oxygen percent was calculated as the difference to 100%. NMR analysis (**Fig S1**): 1H-NMR (200 MHz, CDCl3-d6, δ / ppm): 0.883 (3H, s, C18H3), 1.31 (16H, m, *J* = 11.0 Hz, C4-7, C14-17H), 1.465 (2H, m, J = 11.0 Hz, C13H), 1.623 (2H, m, J = 7.0 Hz, C3H), 2.033 (2H, m, J = 7.0 Hz, C8H), 2.216 (2H, m, J = 7.0, C11H), 2.328 (2H, dd, C2H), 3.639 (1H, m, C12H), 5.396 (1H, m, C10H), 5.535 (1H, m, C9H) and 6.5 (1H, m, C12OH); 13C-NMR (50 MHz, DMSO–d6, δ / ppm): 14.0 (C18), 22.6 (C17), 24.7 (C16), 25.0 (C15), 27.3 (C14), 29.0 (C9-13), 32.0 (C8), 34.0 (C7), 35.2 (C6), 36.7 (C5), 71.7 (C4), 125 (C3), 133 (C2) and 179 (C1).



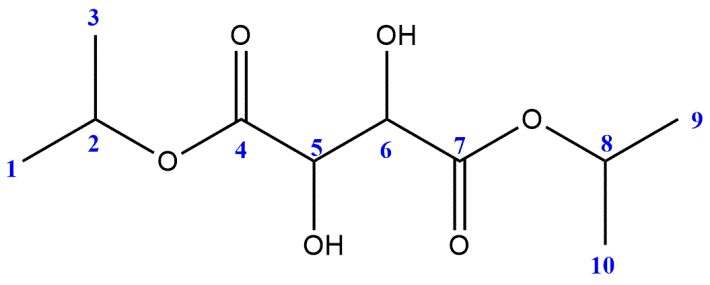
**Fig S1**. NMR analysis of ricinoleic acid

### *2.2.2 Laboratory epoxidation of soybean, linseed and sunflower oil*

Firstly, 100 g of the soybean, linseed or sunflower oil (SO, LO and SFO, respectively) and 13.9 g of formic acid were poured to glass reactor immersed into water bath and mechanically stirred at the 50 °C and 550 rpm. Afterwards, 116.9 g of 30% hydrogen peroxide was gradually charged into the mixture during the 5 h. The molar ratio of soybean oil:formic acid:hydrogen peroxide was 1:2.64:8.9, which mean the molar ratio of carbon double bonds toward hydrogen peroxide (C=C:H2O2) were 1:1.7. After the charging of H2O2 was completed, the reaction further continued for the next 5 h. Hereafter, the mixture was cooled down, dilluted with distilled water and subjected to extraction by diethyl ether to separate the oil product from the water phase. The final products, ESO, ELO and ESFO, were obtained after ether evaporation and drying at 40 °C/2000 Pa for 4 h. Epoxy number was determined using 0.4 M HCl in dioxane and titration with solution of silver nitrate in presence of the ammonium thiocyanate as indicator according to the standard method [2]. The obtained yield of epoxide is 68.9%. The FTIR spectrum of ESO was presented in the *section 3.4*.

### *2.2.3 Laboratory synthesis of diisopropil tartarate*

In a single-neck flask, 25 g of tartaric acid was dissolved in 120 ml of isopropyl alcohol. Afterwards, the solution was cooled to 0 °C in an ice bath. The esterification of tartaric acid was catalyzed by introduction gaseous HCl for 5h at 0 °C providing mixing at room temperature for 24 h. The excess ethanol was removed by distillation at 40 °C, and diisopropyl tartarate (DIPT), purified by vacum distillation, as a mildly viscous yellow liquid (b.p. 85 oC/2500 Pa; refractive index 1.437). The FTIR spectrum was presented in the *section 3.4*. Elemental analysis calculated for C10H18O6 (Mw = 234.25 gmol-1 ): C, 51.27; H, 7.75; O, 40.98. Found: C, 51.25; H, 7.70; O, 40.91. The oxygen percent was calculated as the difference to 100%. NMR analysis (**Fig S2**): 1H-NMR (200 MHz, CDCl3-d6, δ / ppm): 1.60 (12H, s, 6C1-3Hand 6C9-10H), 3.30 (2H, m, C5OH and C6OH) and 4.60-4.90 (4H, m, 4C2,5,6,8H); 13C-NMR (50 MHz, DMSO–d6, δ / ppm): 22.0 (C1, C3, C9 and C10), 70.0 (C2 and C8), 73.0 (C5 and C6) and 171 (C4 and C7).



**Fig S2**. NMR anlaysis of diisopropyl tartarate

### *2.2.4 Laboratory synthesis of copper citrate*

In a typical experiment, 4.2 g of citric acid dihydrate (0.02 mol) and 3.41 g copper chloride dihydrate (0.02 mol) were dissolved in 100 ml of distilled water. The resulting light blue solution was filtered and heated in an oven at 85 °C for 19 h in a teflon reactor. The green crystalline product was separated from the blue solution (pH 4.0) by decantation, thoroughly washed with distilled water, and air-dried (yield 3.2 g, 90% based on Cu). Cu content, determined according to atomic absorption spectroscopy, was 24.5%. The FTIR spectrum was presented in the section *3.4*.

### *2.2.5 Laboratory synthesis of copper ricinoleate*

The copper(II) acetate monohydrate in amount of 6 g (0.03 mol) was poured to flask and dissolve in ethanol with stirring at room temperature. Afterwards, 60 ml (0.210 mol) of ricinoleic acid (*synthesis 2.2.1*) is slowly added during 2h while the reaction mixture was heated to 80 °C. pH (6.5-7.0) was adjusted with sodium hydroxide. After that, the obtained mixture was cooled down and dried in vacuum dryer to evaporate the solvent. Cu content, determined according to atomic absorption spectroscopy, was 7.4% (78% conversion; the product contain ~20% of non-reacted ricinoleic acid and its content was adjusted by experimental condition to provide plasticizing effect in the course of starch oxidation). The FTIR spectrum was presented in the section *3.4*.

### *2.2.6 Laboratory preparation of oxidized starch by the SPS method*

**Tab S1**. Quantities of reactants and reaction conditions used in experiments at laboratory level

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample** | **Starch,**  **[g]** | **Plasticizer,**  **[ml]** | | **Hydrogen peroxide, [ml]** | **Catalyst,**  **[g]** | | | **T,**  **[°C]** |
| **Exp 1** | 20 | - | | 3.6 | FeSO4∙7H2O | 0.01 | | 20 |
| **Exp 2** | 20 | - | | 3.6 | FeSO4∙7H2O | 0.01 | | 80 |
| **Exp 3** | 20 | - | | 1.9 | FeSO4∙7H2O | 0.01 | | 20 |
| **Exp 4** | 20 | - | | 1.9 | FeSO4∙7H2O | 0.01 | | 80 |
| **Exp 5** | 20 | - | | 1.5 | FeSO4∙7H2O | 0.01 | | 20 |
| **Exp 6** | 20 | - | | 1.5 | FeSO4∙7H2O | 0.01 | | 80 |
| **Exp 7** | 20 | - | | 1.2 | FeSO4∙7H2O | 0.01 | | 20 |
| **Exp 8** | 20 | - | | 1.2 | FeSO4∙7H2O | 0.01 | | 80 |
| **Exp 9** | 20 | RA | 0.4 | 1.2 | FeSO4∙7H2O | 0.01 | | 20 |
| **Exp 10** | 20 | DIPT | 0.4 | 1.2 | FeSO4∙7H2O | 0.01 | | 20 |
| **Exp 11** | 20 | ESO | 0.4 | 1.2 | FeSO4∙7H2O | 0.01 | | 20 |
| **Exp 12** | 20 | ELO | 0.4 | 1.2 | FeSO4∙7H2O | 0.01 | | 20 |
| **Exp 13** | 20 | ESFO | 0.4 | 1.2 | FeSO4∙7H2O | 0.01 | | 20 |
| **Exp 14** | 20 | - | | 1.2 | - | | | 20 |
| **Exp 15** | 20 | - | | 1.2 | Cu citrate | | 0.01 | 20 |
| **Exp 16** | 20 | RA | 0.4 | 1.2 | Cu citrate | | 0.01 | 20 |
| **Exp 17** | 20 | - | | 1.2 | Cu ricinoleate | | 0.35 | 20 |

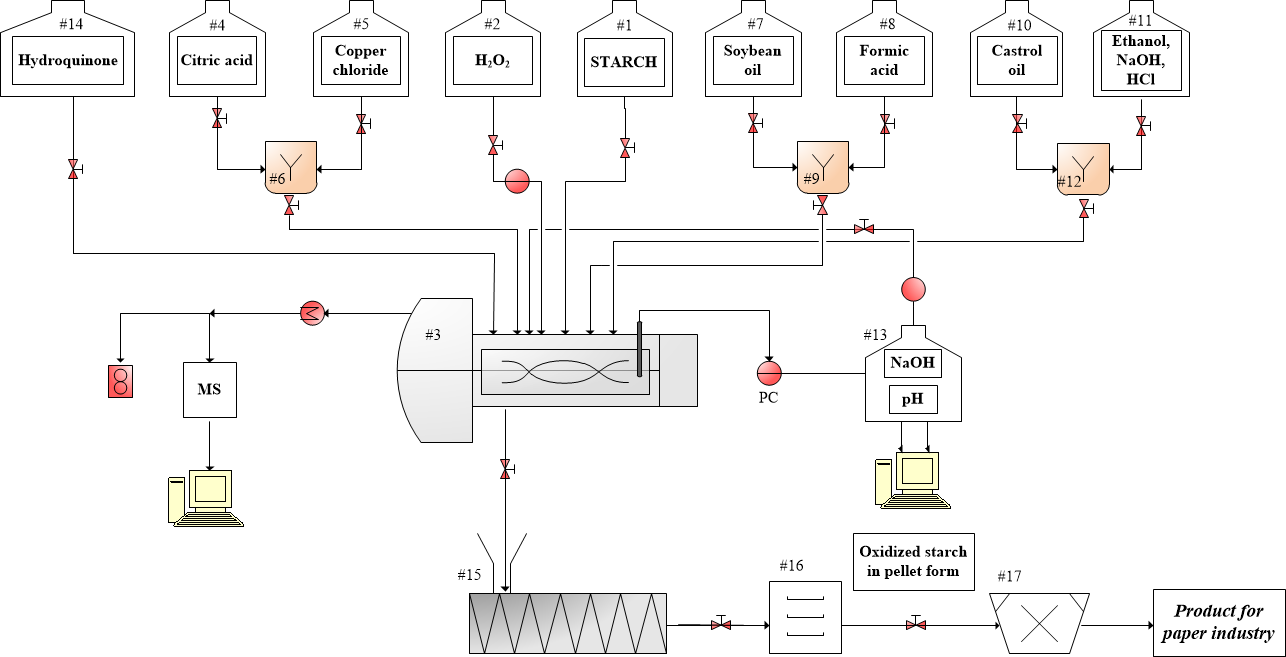


**Fig S3**. Proposed mechanism of starch oxidation with hydrogen peroxide

## *Industrial starch oxidation by the SPS method*

**Tab S2**. Quantities of reactants and reaction conditions used in experiments

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample** | **Starch, [kg]** | **Plasticizer, [l]** | | **10% H2O2, [l]** | **Catalyst, [kg]** | | **Hydroquinone, [kg]** |
| **Exp 18** | 200 | - | | 20 | Cu citrate | 0.01 | 0.4 |
| **Exp 19** | 200 | RA | 0.4 | 20 | Cu citrate | 0.01 | 0.4 |
| **Exp 20** | 200 | ESO | 0.4 | 20 | Cu citrate | 0.01 | 0.4 |



**Fig S4.** Schematic overview of the technological process for the starch oxidation

## *Characterization*

The carboxyl/carbonyl content (COOH/100 GU and CO/100 GU) and the swelling capacity (SC)/water solubility (WS) of native and oxidized/modified starch were determined as described in the previous publications [3, 4], respectively.

The viscosity of oxidized starches obtained at laboratory level was determined using capillary viscometer methods (Cannon-Fenske viscometer) (CF Method) following the method (Method 1) described in the publication [5]. The viscosity of oxidized starches obtained at industrial level was measured using Brookfield method (Brookfield DV-II + Pro Viscometer) (B Method) according to [6] with some changes: before of measuring oxidized starch was heated at 90 o for 30 min, and in the course of cooling from 60 oC viscosity was continuosly recorded.

Fourier transforms infrared (FTIR) spectra of the samples were recorded in absorbance mode using a Nicolet™ iS™ 10 FT-IR Spectrometer (Thermo Fisher SCIENTIFIC) with Smart iTR™ Attenuated Total Reflectance (ATR) Sampling accessories, within a range of 400-4000 cm–1, at a resolution of 4 cm–1 and in 20 scan mode.

Nuclear magnetic resonance (1H NMR and 13C NMR) spectra were recorded in a deuterated chloroform (CDCl3) using a Ascend 400, Bruker, USA, at 400 MHz and 25 °C.

Thermogravimetric (TG) and differential scanning calorimetry analysis (DSC) (SDT Q600 simulated TGA-DTA instrument - TA Instruments) were used for studying the thermal properties of oxidized starches. Samples were heated to 800 °C (10 °C min−1) in a flow of nitrogen (20 cm3 min−1). The morphology of native and modified starches was recorded by a scanning electron microscope (SEM) (FE-SEM, TESCAN Mira3 XMU) operated at 20 kV. Before analysis, oxidized starch products were coated with Au.

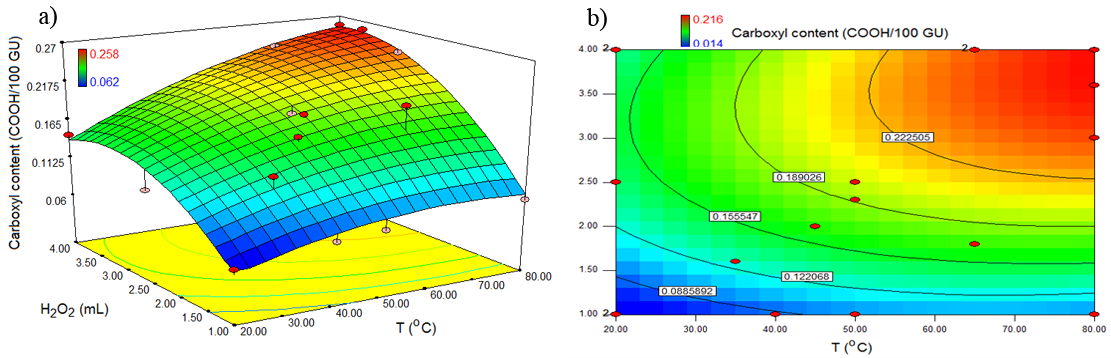
# 3. Results and discussion

## *3.1 Optimization of starch oxidation*

Optimization of starch oxidation, by applying response surface methodology (RSM) [7], is based on five-level-three-factor central rotatable composite design (blocked single factor: temperature). The coded and operational values of the selected variables are shown in **Tab S3**, together with the experimental plan, which comprised 18 experimental runs plus three replicates on the central point [8]. Each experiment (except the central point) was performed in duplicate. The output variable was the carboxyl content (COOH/100 AGU). Data obtained in these experiments were fitted with a second-order polynomial equation and the coefficients of the response function and their statistical significance were evaluated by the least squares method using commercial software Design-Expert, Software Version 9 (Stat-Ease, Inc. 2021 E. Hennepin Ave. Suite 480 Minneapolis, USA). **Fig S5** shows the effect of reaction temperature and initial concentration of H2O2 on the effectivenes of starch oxidation.

**Table S3**. D-optimal desing with two factor and three replicates used to optimize the starch oxidation

|  |  |  |  |
| --- | --- | --- | --- |
| **Run** | **Factor 1 T (oC)** | **Factor 2 H2O2 (ml)** | **Response**  **Carboxyl content (COOH/100 GU)** |
| **1** | 50 | 2.5 | 0.188 |
| **2** | 80 | 3 | 0.238 |
| **3** | 80 | 1 | 0.088 |
| **4** | 20 | 2.5 | 0.113 |
| **5** | 80 | 3.6 | 0.258 |
| **6** | 45 | 2 | 0.174 |
| **7** | 50 | 2.3 | 0.192 |
| **8** | 20 | 1 | 0.062 |
| **9** | 35 | 1.6 | 0.144 |
| **10** | 65 | 4 | 0.235 |
| **11** | 20 | 4 | 0.144 |
| **12** | 40 | 1 | 0.074 |
| **13** | 20 | 4 | 0.144 |
| **14** | 65 | 1.8 | 0.204 |
| **15** | 65 | 4 | 0.235 |
| **16** | 50 | 1 | 0.0785 |
| **17** | 80 | 4 | 0.257 |
| **18** | 20 | 1 | 0.062 |



**Fig S5**. The influence of temperature (T, oC) and concentration of H2O2 (ml) on the value of carboxyl content (COOH/100 AGU) (a) 3D plot and (b) 2D plot

An increase in carboxyl group content is observed with increasing the temperature and initial concentration of H2O2. According to the results given on **Fig S5**, as an operational parameters temperature of 80 oC (353 K) and 3.6 ml of H2O2 was selected as the most efficient for starch oxidation in presence of iron(II) sulphate catalyst. Similar results was obtained using copper(II) sulfate (differences not higher than 8%; data not presented), which means that both catalyst showed similar activity which depends on metal ion activity and the water content (starch moisture and provided by water solution containing catalyst). Obtained results also indicate significance of the temperature as reaction parameter. Higher temperature (80 oC) means better hydrogen peroxide/water diffusivity and interior material avalilability (partial starch grain gelaitinization occur). Regarding results on laboratory synthesis of oxidized starch, *i.e*. as base for scale-up, it is necessary to consider selection of optimal technology which provide product quality in accordance to customer request. It means achievement of balance of requested quality/price in relation to specific requirement from customers. Main intention of industrila level of production was focused on use lower quantity of hydrogen peroxide in combination with higher activity catlalyst, and also improved processability supported by proper selection of plasticizer.

## *3.2 Carboxyl and carbonyl contents*

**Tab S4**. Carboxyl and carbonyl contents of native and oxidized starches at laboratory level

|  |  |  |  |
| --- | --- | --- | --- |
| **Sample** | **Carboxyl content (COOH/100 GU)** | **Carbonyl content (CO/100 GU)** | **Sum (COOH+CO)** |
| **Native starch** | 0.018 | 0.026 | 0.044 |
| **Exp 1** | 0.172 | 0.250 | 0.422 |
| **Exp 2** | 0.258 | 0.292 | 0.550 |
| **Exp 3** | 0.144 | 0.271 | 0.415 |
| **Exp 4** | 0.216 | 0.279 | 0.495 |
| **Exp 5** | 0.117 | 0.172 | 0.289 |
| **Exp 6** | 0.175 | 0.213 | 0.388 |
| **Exp 7** | 0.072 | 0.355 | 0.427 |
| **Exp 8** | 0.108 | 0.318 | 0.426 |
| **Exp 9** | 0.162 | 0.229 | 0.391 |
| **Exp 10** | 0.127 | 0.285 | 0.393 |
| **Exp 11** | 0.123 | 0.219 | 0.342 |
| **Exp 12** | 0.124 | 0.227 | 0.351 |
| **Exp 13** | 0.123 | 0.221 | 0.344 |
| **Exp 14** | 0.060 | 0.368 | 0.428 |
| **Exp 15** | 0.096 | 0.367 | 0.463 |
| **Exp 16** | 0.182 | 0.248 | 0.430 |
| **Exp 17** | 0.076 | 0.350 | 0.426 |

**Tab S5**. Carboxyl and carbonyl contents of native and oxidized starches at industrial level

|  |  |  |  |
| --- | --- | --- | --- |
| **Sample** | **Carboxyl content (COOH/100 GU)** | **Carbonyl content (CO/100 GU)** | **Sum (COOH+CO)** |
| **Native starch** | 0.018 | 0.126 | 0.144 |
| **Exp 18** | 0.162 | 0.335 | 0.497 |
| **Exp 19** | 0.192 | 0.288 | 0.480 |
| **Exp 20** | 0.181 | 0.280 | 0.466 |

## *3.3 Swelling capacity (SC), water solubility (WS) and viscosity (η)*

**Tab S6**. Swelling capacity (SC), solubility (S) and viscosity (η)\* of native and oxidized starches at laboratory level

|  |  |  |  |
| --- | --- | --- | --- |
| **Sample** | **SC, [%]** | **S, [%]** | **η, [Pa·s]** |
| **Native starch** | 11.8 | 19.2 | 1.0630 |
| **Exp 1** | 22.7 | 38.6 | 0.0124 |
| **Exp 2** | 25.2 | 42.8 | 0.0121 |
| **Exp 3** | 19.7 | 33.5 | 0.0195 |
| **Exp 4** | 21.8 | 37.1 | 0.0183 |
| **Exp 5** | 18.3 | 31.2 | 0.0273 |
| **Exp 6** | 20.4 | 34.7 | 0.0252 |
| **Exp 7** | 16.3 | 27.8 | 0.0339 |
| **Exp 8** | 17.5 | 29.9 | 0.0313 |
| **Exp 9** | 23.9 | 36.2 | 0.0153 |
| **Exp 10** | 23.1 | 34.1 | 0.0175 |
| **Exp 11** | 22.7 | 32.8 | 0.0182 |
| **Exp 12** | 21.3 | 32.3 | 0.0189 |
| **Exp 13** | 21.5 | 31.5 | 0.0183 |
| **Exp 14** | 15.4 | 25.6 | 0.0323 |
| **Exp 15** | 18.6 | 29.3 | 0.0298 |
| **Exp 16** | 24.6 | 38.5 | 0.0141 |
| **Exp 17** | 16.5 | 28.2 | 0.0320 |

\*determined by CF Method

**Tab S7**. Swelling capacity (SC), solubility (S) and viscosity (η)\*\* of native and oxidized starches at industrial level

|  |  |  |  |
| --- | --- | --- | --- |
| **Sample** | **SC, [%]** | **S, [%]** | **η, [Pa·s]** |
| **Native starch** | 11.8 | 19.2 | 1.0630 |
| **Exp 18** | 16.1 | 29.8 | 0.0451 |
| **Exp 19** | 19.5 | 32.3 | 0.0440 |
| **Exp 20** | 18.3 | 31.9 | 0.0452 |

\*\*determined by B Method

## 3.4 ***Fourier transform infrared spectroscopy (***FTIR)

**Tab S8**. Area of the characteristic groups in the region of carbonyl and carboxyl vibrations

|  |  |  |
| --- | --- | --- |
| **Sample** | **Wavenumber, cm-1** | **Area** |
| **Exp 1** | 1728 | 134.621 |
| 1708 | 63.414 |
| **Exp 8** | 1726 | 136.356 |
| 1710 | 58.203 |
| **Exp 10** | 1748 | 219.932 |
| 1735 | 243.540 |
| 1719 | 71.144 |
| **Exp 16** | 1744 | 40.562 |
| 1732 | 184.328 |
| 1710 | 166.902 |

## 3.5 Thermogravimetric analysis

**Tab S9**. Thermal characteristics of native and oxidized starch

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sample** | ***T*5, [°C]** | ***T*50, [°C]** | **Residue content, [wt%]** | **DTG peaks, [°C]** | ***ΔH*, [J/g]** |
| **Native starch** | 248.3 | 312.9 | 13.72 | 314.5 | 159.86 |
| **Exp 1** | 251.4 | 308.7 | 16.59 | 307.4 | 94.85 |
| **Exp 6** | 271.9 | 307.2 | 16.89 | 305.0 | 94.40 |
| **Exp 10** | 273.4 | 305.9 | 17.48 | 308.3 | 126.5 |
| **Exp 16** | 277.8 | 308.7 | 18.16 | 305.7 | 70.18 |

## T*5 and* T*50 are temperature at 5 and 50 mass % loss of the specimen, respectively.*

## 3.7 Techno-economic analysis of industrial starch oxidation

**Tab S10**. Distribution of total monthly production, with calculated income

|  |  |  |  |
| --- | --- | --- | --- |
| **Product** | **Monthly sales, [t]** | **Selling price, [**€**/t]** | **Monthly income, [**€**]** |
| **Exp 18** | 75 | 400 | 30.000 |
| **Exp 19** | 150 | 510 | 60.000 |
| **Exp 20** | 100 | 600 | 70.500 |
| **SUM** | 325 | 512 (average) | 160.500 |

**Tab S11**. Variable costs for starch oxidation

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Variable costs (per product unit)** | | **Exp 18** | **Exp 19** | **Exp 20** |
|  | Selling price of product/service | **(€)** | **(€)** | **(€)** |
|  | Costs of raw materials | 0.33 | 0.35 | 0.38 |
|  | Costs of distribution and sale | 0.01 | 0.01 | 0.01 |
|  | Cost of packaging | 0.0042 | 0.0042 | 0.0042 |
|  | Energy costs | 0.007 | 0.007 | 0.007 |
|  | Variable labor costs | 0.0084 | 0.0084 | 0.0084 |
|  | Costs of communication with customers | 0.0004 | 0.0004 | 0.0004 |
|  | **TOTAL VARIABLE COSTS**  **BY PRODUCT UNIT (kg)** | 0.36 | 0.38 | 0.41 |

**Tab S12**. Fixed costs for starch oxidation

|  |  |  |
| --- | --- | --- |
| **Fixed monthly costs** | **€** | **Comments** |
| Fixed salaries of employees | 500 | - |
| Electrical energy | 50 | electricity consumption for the plant operation, light, heating, etc. |
| Amortization | 788 | Plant value - 165.134€, time of obsolescence - 20 years |
| Bank costs | 158 | - |
| Phone/internet | 150 | - |
| Maintenance /cleaning | 159 | - |
| Fairs/conferences | 1.000 | - |
| Research and developments | 4.000 | raw materials for further development, fine equipment, property checks, travel expenses, office materials for teamwork |
| **TOTAL FIXED COSTS** | 6.805 |  |

# References

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