Non-starch contents affect the susceptibility of banana starch and flour to ozonation

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Abstract The properties of native flour and starch were compared and the changes in their properties were evaluated following ozonation at 100 and 200 ppm. X-ray diffraction analysis indicated that crystallinity index of both ozonated banana flour and starch decreased by 1.6%, B-type pattern of native banana flour and starch did not change following ozonation. The presence of higher amounts of non-starch components decreased the sensitivity of flour to the oxidation, as indicated by the lower carboxyl content compared to that of starch. The flour also required higher ozone concentration than starch to alter its properties, particularly pasting properties. Ozonation tended to increase peak, hold and final viscosity of both. A prominent change in the freeze thaw stability of both flour and starch following ozonation was the most encouraging result. Ozonation also improved the solubility of flour which was important to reduce cooking loss when applied in a range of food products. The solubility improvement in the flour might be linked to the formation of new binding

following ozonation presumably involving protein present in the granule surface.

Keywords Ozone · Banana · Flour · Starch · XRD · Pasting properties

Introduction

Starch has been long regarded as the main source of energy and carbohydrate in the human diet. A number of studies on starch have been carried out using different starch sources (Manthey 2016; Zhang et al. 2017). Cassava, maize, wheat and potato have been commonly used across the world as the main sources of starch (Clifton and Keogh 2016; Hoover 2010). Another starch source such as banana has been rarely studied despite the fact it is an interesting alternative product and worth investigating due to its abundance, particularly in tropical countries.

Native starch exhibits some poor properties when used in industrial applications, however it may be possible to overcome these by modifying the starch. Ozonation is a chemical method of starch modification considered as an environmentally friendly technology due to the absence of any residues left in food or the environment following the treatment (Çatal and İbanoğlu 2014). Additionally, ozone has a powerful oxidant capacity since it has an extra oxygen atom (Kaur et al. 2012).

Study on starch ozonation has prompted research and it was reported that ozonation could modify significantly starch properties (Sandhu et al. 2012). Ozone can be applied as a gaseous phase or aqueous solution. Several studies have reported the effectiveness of ozone gas in oxidizing starches from different sources such as corn, sago and tapioca (Chan et al. 2009), yam and cocoyam

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(Oladebeye et al. 2013) and rice (An and King 2009). The authors found out that ozonation affected pasting properties, swelling volume and freeze thaw stability with different extents of starch oxidation depending on types of starch.

As an oxidizing agent, ozone can oxidize hydroxyl groups present at C2, C3, and C6 positions on glucose molecule to carbonyl and carboxyl groups. The presence of these groups could then increase swelling power and alter pasting properties of starch (Chan et al. 2009). The repulsion between negative charges of carboxyl groups results in increased swelling of starch granules during heating in water.

Ozonation of starch form has been more commonly investigated than its flour form despite the fact that flour form is also required for certain food applications. Different chemical compositions particularly in non-starch content may, however impart different responses to the ozonation. The objectives of this study were (1) to carry out the ozonation on starch and flour form of unripe green banana, (2) compare the effectiveness of ozonation on banana starch to the flour form and consider the difference in non-starch contents. In order to compare the sensitivity to ozonation, functional and pasting properties, crystallinity and carboxyl content of both starch and flour form were examined following ozonation. In the present study ozone in gas phase was applied to starch and flour of banana var kapas.

Materials and methods

Materials

The flour and starch used in this study were extracted from unripe green banana (*Musa acuminata colla*, AAA group, Cavendish subgroup), local name known as pisang kapas, purchased in market Gede Bage (Bandung, Indonesia). This banana is the most abundant in the country. For the present study, green banana samples with a diameter of around 3 cm, length of 15–20 cm were chosen. They were harvested after around 3 months of flower emergence. Flour and starch production was carried out immediately after the obtainment of the fresh samples. Reagents used in this study such as HCl, NaOH, HgO, K₂SO₄, H₂SO₄, Na₂S₂O₃, C₆H₁₄, I₂, KI, CH₃COOH, C₂H₅OH were of analytical grade and used directly without further purification.

Preparation of banana starch and flour

The method of banana flour preparation referred to a previous study (Yangilar 2015) with a slight modification. The peel of banana fruit was stripped off after previously

dipped in water. The pulp was cut into transverse slices of around 2 mm thick, and then the slices were dipped in water for 10 min, drained and dried at 50 °C overnight. The pulp slices were then milled by grinder and passed through 80 mesh screens. Banana flour was stored in impermeable plastic bags and stored at 25 °C for later analysis. Meanwhile the starch was extracted from homogenate following peeling, washing and disintegrating banana pulp in water (1:3 ratio) using waring blender. The homogenate was passed through a mesh sieves. The starch suspension was decanted off for 24 h. During decantation, water in the suspension was replaced with the fresh one every 6 h. The white starch sediments were dried at 50 °C overnight. The starch was then subjected to the treatment as applied for banana flour as explained above.

Ozonation

One hundred grams of banana starch or flour was placed in a 1 L cylindrical stainless container and gas containing 100 or 200 ppm of ozone was flowed into it for 15 min. The container was then immediately sealed, left at room temperature for 60 min during which every 5 min it was vertically rotated to allow homogenous exposure of starch or flour to ozone gas.

X ray diffraction (XRD)

X-ray diffraction analysis of the starches and flours was performed on powder using X-ray diffractometer (PANalytical X'Pert PRO seri PW3040/x0) with Cu K α value of 1.54 radiation with a 2θ range of 3°–50° using a voltage of 40 kV and filament current 30 mA. The relative crystallinity was calculated according to the method reported in literatures (Cheetham and Tao 1998; Nara and Komiya 1983) using Sigma plot programme.

Swelling volume and solubility

Swelling volume and solubility measurement was carried out by suspending 0.35 g (db) banana flour or starch in 12.5 mL of water in centrifuge tube. The suspension was then mixed using vortex for 30 s, and then placed in a 92.5 °C water bath for 30 min. Samples were then cooled in ice water for 1 min, followed by centrifugation at 3500 rpm for 30 min. Supernatant volume was measured and the solubility was measured from supernatant previously dried in a hot air oven (Collado and Corke 1999).

$$\text{Swelling volume (mL/g)} = \frac{\text{Total volume} - \text{supernatant volume}}{\text{Weight sample (db)}} \quad (1)$$

$$\text{Solubility (\%)} = \frac{\text{Weight dried supernatant}}{\text{Weight sample (db)}} \times 100\% \quad (2)$$

Freeze-thaw stability

The freeze-thaw stability was determined according to the method of Wattanachant et al. (2003). Five percent of flour or starch (w/v dry basis) was first of all suspended in water and then heated at 95 °C for 30 min under constant mild agitation. It was then cooled to room temperature in an iced shaking water bath. The paste of 20 g was taken and placed in a centrifuge tube and subjected to freeze thaw cycle by storing at 4 °C for 24 h, then freezing at – 15 °C for 48 h, followed by thawing at 25 °C for 3 h and then centrifugation at 3500 rpm for 15 min. The supernatant removed from the gel was then weighed. The extent of syneresis was expressed as the percentage of separated liquid per total weight of sample in the centrifuge tube.

Gel strength

Gel strength was determined by suspending 1% of starch or flour (w/v dry basis) in distilled water and then heated at 95 °C for 30 min with constant stirring. The resulting paste was then poured into a pipe container (2.5 cm diameter, 3 cm high) at room temperature for 1 h to form gel. It was covered with aluminium foils and then stored in refrigerator (4 °C) for 24 h. Texture Analyzer (TA-XT2) was used to measure gel strength.

Water absorption capacity

Water absorption capacity of the flour or starch was determined as follows; suspension of flour or starch was first of all prepared by suspending 1 g of sample in 10 mL of water in a centrifuge tube. The suspension was then left at room temperature (26 ± 2 °C) for 1 h, and then subjected to centrifugation at 200 g for 30 min. The volume of supernatant was then measured. Water absorption capacity was calculated as the volume of water absorbed per weight of flour (mL/g).

Rapid visco analyser (RVA)

Pasting properties of flour or starch samples were determined using a Rapid Visco Analyzer (RVA-SM2), Warriewood Australia. Approximately 3.5 g sample were added into 25 mL distilled water in the RVA canister. Viscosity was recorded with temperature profile as follows; holding at 50 °C for 1 min, heating from 50 to 95 °C in 3.7 min, holding at 95 °C for 2.5 min, and then cooling down to 50 °C in 3.8 min. The resulting gel was

maintained at 50 °C for 2 min with constant paddle rotating speed at 160 rpm throughout the entire analysis except for rapid stirring at 960 rpm for the first 10 s to disperse the starch sample. Total run time for each sample was 13 min. Parameters including peak viscosity (PV), hold viscosity (HV), final viscosity (FV), breakdown, setback, and pasting point were recorded. All measurements were reported in cp.

Determination of carboxyl content

The carboxyl content was determined according to the method reported in literature (Chattopadhyay et al. 1997) with a slight modification. Approximately 2 g of sample (banana flour or starch) was mixed with 25 mL of 0.1 N HCl, and the slurry was stirred occasionally for 30 min with a magnetic stirrer. The slurry was then filtered through a 150 mL medium-porosity fritted glass funnel at vacuum and washed with 400 mL of distilled water. The starch cake was then carefully transferred into a 500 mL beaker, and the volume was adjusted to 300 mL with distilled water. The starch slurry was heated in a boiling water bath with continuous stirring for 15 min to ensure complete gelatinisation. The hot starch dispersion was then adjusted to 450 mL with distilled water and titrated to pH 8.3 with standardized 0.01 N NaOH. A blank test was performed with unmodified banana starch or flour. Carboxyl content was calculated as follows:

$$\begin{aligned} &\text{Milliequivalents of acidity/100 g banana starch or flour} \\ &= \frac{(\text{Sample} - \text{blank}) \text{mL} \times \text{Normality of NaOH} \times 100}{\text{Sample weight (db) in gram}} \end{aligned} \quad (3)$$

$$\begin{aligned} &\text{Percentage of carboxyl content} \\ &= \frac{\text{Milliequivalents of acidity}}{100 \text{ g banana starch or flour}} \times 0.045 \end{aligned} \quad (4)$$

Statistical analysis

Data were collected in triplicate and analysed by the statistical package software (SPSS), using Anova followed with post hoc test of Duncan.

Results and discussion

X-ray diffraction patterns and relative crystallinity

X-ray diffraction patterns of native or ozonated starch and flour were depicted in Fig. 1. Banana flour and starch ozonated at 200 ppm (referred to as OBF200 and OBS200 respectively) were used as representative samples

Fig. 1 XRD diffractograms of native starch and flour along with their corresponding ozonated forms

characterised by XRD. This concentration was the highest in the present study, therefore it was expected that there was a distinctive change in starch structure at this ozonation level. NBF and NBS showed similar XRD patterns characterised with the presence of a small peak at 5.51° , strong peaks at 15° and 16.95° with a shoulder around 17.8° and a broad peak at 22.98° 2θ , a feature characteristic of a B-type pattern (Pukkahuta and Varavinit 2007). Banana starch has been reported to exhibit B type crystallites (Agama-Acevedo et al. 2015). It was however found that A (Bello-Pérez et al. 2000) and C type also existed (Bi et al. 2017; Jane et al. 1997).

The relative crystallinity of NBF and NBS was 36.6 and 35.8% respectively. Literatures documented the crystallinity index ranging from 28 to 30% for different variety of banana grown in Mexico (Agama-Acevedo et al. 2015) and 30–35.5% for those studied in China (Bi et al. 2017). Variety and environment where they grew could also affect crystallinity. Moreover, a slight increase in the relative crystallinity in the flour compared to the starch form suggested that the other components present in the flour such as protein, lipid, cellulose contributed to the crystallinity. This was also observed in another study (Bi et al. 2017).

When the starch and flour were subjected to ozonation (OBF200 and OBS200), the diffractogram remained unchanged demonstrating that the ozonation did not affect the XRD pattern. Relative crystallinity however reduced slightly by 1.8% in both flour and starch forms. The absence of the change in the crystalline type and the reduction in crystallinity following ozonation was in agreement with previous studies on cassava starch suspension oxidized by ozone and also on ozonated rice flour using nonthermal plasma technology (Klein et al. 2014; Pal et al. 2016). The reduction in crystallinity following ozonation might result from the oxidation taking place in the semi-crystalline region of starch i.e. depolymerisation

of amylopectin chains through the cleavage of α -(1,4)-glucosidic linkages (Klein et al. 2014).

Functional properties

Comparison of native flour to the native starch properties

Characterisation of chemical composition showed that the contents of ash, protein, fat and carbohydrate of native banana flour (NBF) used in this study were around 1.87, 2.9, 0.45 and 87.1% respectively. Starch and amylose content of the flour were approximately 79.2 and 38.6% respectively. The chemical composition was in similar range to that reported in another study on banana flour (Bi et al. 2017). Meanwhile native banana starch (NBS) contained 0.13% ash, 6.93% moisture, 0.01% fat, 1.45% protein and 83.3% starch.

Comparing the properties of native flour to those of the native starch (Table 1), it was obvious that swelling volume and gel strength of NBF were similar to those of NBS, suggesting that the non-starch content which was higher in the flour than that in its counterpart did not affect the properties. Solubility, freeze thaw stability, and water absorption capacity of the flour form, were however different from those of its counterpart. NBF solubility was around 11.93%, which was almost three times higher than that for the starch one. This high value of flour solubility might be linked to the higher non-starch content in the flour form. Following the solubility test carried out at around 92°C , the non-starch content in the native flour might be excluded from the gel structure and solubilised in water particularly ash and protein content, hence higher solid content in water. Consequently, the high solubility of the flour did not reflect solely the amount of amylose leaching from the starch structure into water.

The syneresis extent of the native flour which was around 33% was slightly higher than that of the native starch which was 26.9%. It suggested that the starch form was more stable to retrogradation than its counterpart form. Water absorption capacity of the flour compared to its counterpart was nearly double, suggesting the influence of non-starch content on the increased property.

Ozonation effect on functional properties

For certain properties such as gel strength and water absorption capacity, neither flour nor starch properties was significantly altered by ozonation. On the other hand, swelling volume, solubility and freeze thaw stability of the flour significantly changed following ozonation.

Ozonation increased the swelling volume of both flour and starch. Starch form required lower ozon concentration (100 ppm) to alter the property than the flour form

Table 1 Functional properties of banana flour and starch following ozonation at different concentration

Parameter	Flour			Starch		
	NBF	OBF100	OBF200	NBS	OBS100	OBS200
Swelling volume (mL/g)	10.14 ± 0.77 ^{aA}	11.15 ± 0.59 ^{abA}	12.34 ± 0.74 ^{bA}	9.60 ± 0.58 ^{aA}	11.09 ± 0.77 ^{bA}	11.92 ± 0.76 ^{bA}
Solubility (%)	11.93 ± 1.03 ^{aA}	6.43 ± 0.43 ^{bA}	7.03 ± 1.46 ^{bA}	4.63 ± 0.38 ^{aB}	5.42 ± 1.16 ^{aA}	6.78 ± 1.09 ^{aA}
Freeze thaw stability (% syneresis)	33.23 ± 6.50 ^{aA}	8.01 ± 4.58 ^{bA}	2.01 ± 1.08 ^{bA}	26.927 ± 3.48 ^{aB}	5.742 ± 3.24 ^{bA}	0.004 ± 0.002 ^{cB}
Gel strength (gF)	3.15 ± 0.58 ^{aA}	3.45 ± 0.17 ^{aA}	3.46 ± 0.28 ^{aA}	2.80 ± 0.35 ^{aA}	3.03 ± 0.51 ^{aA}	3.21 ± 0.16 ^{aA}
Water absorption capacity (g/g)	2.43 ± 0.18 ^{aA}	2.46 ± 0.09 ^{aA}	2.20 ± 0.06 ^{aA}	1.37 ± 0.03 ^{aB}	1.98 ± 0.32 ^{aA}	2.13 ± 0.76 ^{aA}

Upper case letters indicate a comparison of different form (starch or flour form) in the same treatment (native or ozonation). Lower case for comparison of different treatment in the same form. Different letters in the compared values denote significant difference at $p < 0.05$

(200 ppm). Seemingly the higher non-starch content in the flour form hindered the ozone gas from diffusing into starch granule of the flour or simply it was reactive to oxidation, thereby less effective in flour ozonation. Interestingly, solubility was much lowered in the flour form when ozonation was applied, while there was no difference in the ozonated starch solubility. This finding suggested that solubility was affected by the change in the properties of non starch compound present in the flour, demonstrating that the oxidation also occurred to the compounds located on the granule surface. Protein has been believed to be reactive to the ozonation (Gozé et al. 2016) as also demonstrated by another study which found that deproteinisation pretreatment significantly altered starch properties following ozonation (Chan et al. 2012). A study on ozonated wheat flour using atmospheric cold plasma also revealed that the secondary structure of gluten proteins changed following the treatment (Misra et al. 2015).

In the present study, it appeared that ozonation triggered the formation of new binding i.e. covalent binding or cross linking presumably involving protein present slightly higher in the flour and located on the starch granule surface. This new formation was effective in preventing solid leaching from the flour when heating process was applied, thereby lower solubility. Formation of disulfide bond due to protein oxidation following ozonation has been indicated in previous studies (Segat et al. 2015; Wan and Yu 2012). The formation however was not observed in the study of ozonated short and long grain rice flour (Pal et al. 2016). It is thus interesting to further examine the effect of ozonation on protein oxidation from different sources including the protein present in banana flour, taking into account the amino acid compositions. Certain amino acids have been reported to be more reactive to ozonation than the others (Kotiahio et al. 2000).

To underpin the role of non-starch contents in the starch oxidation, further test on carboxyl content was then performed. It was found that flour ozonated at 100 and

200 ppm contained respectively 0.0007 and 0.0038% carboxyl. The content increased incredibly to 0.0114 and 0.0235% carboxyl when ozonation was employed to the starch form at 100 and 200 ppm respectively. This finding suggested that non-starch content in the flour prevented the effective oxidation. It thus explained why the flour form required higher ozone concentration than the starch form to result in pronounced change in its properties.

Furthermore, freeze-thaw stability of both flour and starch was ameliorated when ozonation was applied, indicated by the remarkably reduced syneresis extent. Amongst the properties studied, freeze thaw stability was seemingly the more sensitive property to the ozonation indicated by the change magnitude of the value compared to the native one.

Pasting properties

Comparison of native flour to native starch properties

The effect of ozonation on pasting properties of banana flour and starch was presented in Fig. 2 and Table 2. Pasting properties of native flour were different from those of the starch. The flour pasting point was around 79.9 °C which was slightly higher than the starch one of around 77.8 °C. Furthermore, peak, hold and final viscosity of flour were also higher than those of starch. Regarding the pasting point, the presence of non starch components apparently hindered effective heat transfer to the starch granule, hence a higher energy requirement for starch granule swelling and gelatinisation, and consequently higher pasting point. The presence of non-starch components also seemed to contribute to the more viscous characteristics as shown in the higher peak, hold and final viscosity of flour compared to those of starch. Pectin may contribute to the increase in the viscosity as reported in another study (Bai et al. 2017; Sasaki and Kohyama 2012).

Fig. 2 Pasting properties of banana flour and starch before and after ozonation

Pectin content in banana flour reportedly ranged from 3.3 to 5.6% (Bi et al. 2017).

In terms of stability, native flour breakdown value of 2863 cp was lower than the starch one (3388 cp) which means that the flour form was more stable to heat and shear than the counterpart form. Flour setback value however was higher than the starch one suggesting that the flour form was relatively unstable to the cooling process.

Ozonation effect on pasting properties

Graph of pasting profile of NBF and OBF100 (Fig. 2) superimposed one over another suggesting that ozonation at 100 ppm was not effective to change the pasting properties. It required a higher concentration at 200 ppm to result in a change in the properties. The starch ozonated at 100 ppm (OBS100) however exhibited already effective

treatment to change the properties, while at 200 ppm (OBS200), the graph superimposed over that of OBS100. This observation on pasting properties was in agreement with that on functional properties that is to say the effectiveness of ozonation on starch compared to flour form.

Ozonation in general affected peak and hold viscosity of both flour and starch form. This finding was in line with previous study on ozonated short and long grain rice flour (Pal et al. 2016). Ozon concentration requirement in the starch form to change peak and hold viscosity was lower than in the counterpart one. At 100 ppm peak and hold viscosity of the starch form were significantly altered from the initial values of respectively 5592 and 3069 to 6531 and 3455 cp, while it was required the concentration at 200 ppm to give significant effect in the flour form. This result was in agreement with previous observation on functional properties that flour required higher ozone concentration than the starch form due to the higher content of non starch components. It was also in line with carboxyl content in flour compared to the starch.

No change occurred in the other pasting properties of the starch following ozonation. In the flour form the increase in the breakdown and final viscosity was observed while setback did not change following the ozonation. The increased breakdown and final viscosity was also reported in another study on ozonated long grain rice flour (Pal et al. 2016). Augmented breakdown in the ozonated flour indicated that ozone treatment lowered the stability of the flour to heating and shearing process. Lower breakdown value of native flour and that ozonated at 100 ppm indicated that it was relatively more stable to heat and shear during pasting

Table 2 Pasting properties of flour and starch of banana following ozonation

Parameter	Flour			Starch		
	NBF	OBF100	OBF200	NBS	OBS100	OBS200
Pasting point (°C)	79.91 ± 0.07 ^{aA}	78.1 ± 1.04 ^{bA}	79.7 ± 0.12 ^{aA}	77.6 ± 0.11 ^{aB}	77.8 ± 0.33 ^{aA}	77.5 ± 0.06 ^{aB}
Peak viscosity (cp)	6439.0 ± 17.35 ^{aA}	6516.2 ± 133.54 ^{aA}	6912.2 ± 175.97 ^{bA}	5592.2 ± 78.62 ^{aB}	6531.8 ± 63.94 ^{bA}	6536.3 ± 24.70 ^{bA}
Hold viscosity (cp)	3580.5 ± 7.78 ^{aA}	3654.8 ± 79.54 ^{aA}	3814.7 ± 87.99 ^{bA}	3069.7 ± 43.02 ^{aB}	3455.7 ± 68.24 ^{bA}	3499.8 ± 62.22 ^{bA}
Breakdown (cp)	2863 ± 14.14 ^{aA}	2861.3 ± 75.65 ^{a,b,A}	3097.5 ± 101.75 ^{bA}	3388.3 ± 1403.62 ^{aB}	3076.2 ± 82.189 ^{aB}	3036.5 ± 82.98 ^{aA}
final viscosity (cp)	5899 ± 57.98 ^{aA}	5872.8 ± 52.82 ^{aA}	6012.5 ± 40.06 ^{bA}	4173.5 ± 1423.78 ^{aB}	5281 ± 47.01 ^{aB}	5315.2 ± 29.34 ^{aB}
Setback (cp)	2329 ± 50.20 ^{aA}	2218 ± 32.98 ^{aA}	2197.8 ± 92.95 ^{aA}	1930.3 ± 31.26 ^{aB}	1825.3 ± 115.25 ^{aB}	1815.3 ± 47.27 ^{aB}

Upper case letters indicate a comparison of different form (starch or flour form) in the same treatment (native or ozonation). Lower case for comparison of different treatment in the same form. Different letters in the compared values denote significant difference at $p < 0.05$

compared to the starch ozonated at corresponding concentration. Setback values of ozonated flour were virtually higher than those of starch at corresponding ozone concentration, indicating that flour in both native and ozonated form was relatively unstable to cooling process following pasting time.

Conclusion

Flour and starch form of banana exhibited different functional and pasting properties as well as different relative crystallinity. Although B-type patterns in native flour and starch remained unchanged following ozonation, slight decrease in relative crystallinity was observed. The presence of higher content of non-starch component in the flour affected the properties of native and ozonated form. Flour required higher ozone concentration to significantly change the properties compared to the starch form. It was linked to the presence of non-starch components in the flour which might serve as a barrier to the ozonation as indicated by lower carboxyl content in the flour form following oxidation by ozone. Amongst the functional properties studied freeze-thaw stability was remarkably ameliorated following the ozonation in both flour and starch. Solubility of flour could be also significantly reduced by ozonation, which was probably as a result of new binding formation on granule surface supposedly involving protein following oxidation. In terms of pasting properties, the presence of non-starch components in the flour increased the pasting point and viscosity during pasting compared to the starch form. More viscous suspension observed in the flour form was seemingly attributed to pectin component. Ozonation augmented the peak and hold viscosity of both flour and starch.

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Compliance with ethical standards

Conflict of interest The authors declare no competing financial interest.

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