

Physicochemical characterization of honey from different regions in Rio Grande do Sul State labeled with different inspection service stamps

Caracterização físico-química de méis de diferentes localidades do Rio Grande do Sul com diferentes selos de serviços de inspeção

Ludmila Noskoski Salazar¹, Ana Beatriz Benevides de Freitas², Mariela Valério da Luz³, Patrícia Bersch⁴, Rodrigo Fernando dos Santos Salazar⁵

¹Profª. Adjunta I. Medicina Veterinária. Centro de Ciências da Saúde e Agrárias (CCSA) - UNICRUZ, Cruz Alta - RS, Brasil
lnoskoski@unicruz.edu.br

²Residente em Medicina Preventiva, Análises Micotoxicológicas e Patologia Aviária. Programa de Residência Multiprofissional Integrada em Sistema Público de Saúde - UFSM, Santa Maria - RS, Brasil
ana.beatriz.benevides@hotmail.com

³Acadêmica do Curso de Medicina Veterinária. Centro de Ciências da Saúde e Agrárias (CCSA) - UNICRUZ, Cruz Alta - RS, Brasil
marylelaluz@hotmail.com

⁴Profª Assistente I. Engenharia Civil, Centro de Ciências Humanas e Sociais (CCHS) - UNICRUZ, Cruz Alta - RS, Brasil
pbersch@unicruz.edu.br

⁵Prof. Titular I. Programa de Pós-Graduação *Stricto Sensu* em Atenção Integral à Saúde (PPGAIS) - UNICRUZ/UNIJUI, Cruz Alta - RS, Brasil
rsalazar@unicruz.edu.br

Resumo

O mel é um produto com elevado valor nutritivo e propriedades terapêuticas. Entretanto, pode apresentar diferentes defeitos decorrentes do mal processamento ou fraudes, necessitando de rigoroso controle de qualidade e fiscalização em toda cadeia produtiva. Neste sentido, avaliou-se a qualidade físico-química de méis de diferentes localidades do Rio Grande do Sul. A amostragem consistiu de 12 lotes de méis de 12 fabricantes distintos entre cooperativas e industriais. Foram determinados a acidez (mEq kg^{-1}); cinzas ($\%\text{m/m}$); corantes (qualitativo); pH; reação de Fiehe (qualitativo); reação de Lugol (qualitativo); reação de Lund (mL) e umidade ($\%\text{m/s}$). Empregou-se os protocolos padronizados da Association of Official Analytical Chemists (AOAC) para as determinações analíticas. As amostras apresentaram teores de umidade, cinzas, pH, acidez e a reação de Lund em torno de $18,88 \pm 5,5 \%\text{m/s}$, $0,040 \pm 0,014 \%\text{m/m}$, $4,23 \pm 0,19$; $25,28 \pm 8,95 \text{ mEq kg}^{-1}$ e $1,92 \pm 0,60 \text{ mL}$, respectivamente. As amostras deram negativo para o teste de detecção de corantes, para reação de Lugol e Reação de Fiehe. Por fim, Verificou-se a boa procedência dos méis, remetendo às boas práticas de fabricação que tangem os apicultores até as cooperativas e industriais envolvidas no acabamento final dos méis comercializados, independentemente do selo de inspeção.

Palavras-chave: Controle de qualidade. Inspeção. Segurança Alimentar.

Abstract

Honey is a food of high nutritional value and therapeutic properties; however, it may present different non-conformities due to poor processing or fraud. Therefore, the honey production chain requires strict quality control and inspection. Accordingly, the physicochemical quality of honey from different agribusinesses in Rio Grande do Sul State was herein assessed. The sample consisted of 12 honey types from 12 different cooperatives and manufacturers. Honey acidity (mEq kg^{-1}), Ash ($\%\text{m/m}$), Dyes (qualitative), pH, Fiehe reaction (qualitative), Lugol reaction (qualitative), Lund reaction (mL) and humidity ($\%\text{m/s}$) were found. The standard protocols set by the Association of Official Analytical Chemists (AOAC) were used for analytical purposes. The samples presented moisture content, ash, pH, acidity and Lund's reaction of approximately $18.88 \pm 5.5 \%\text{m/s}$, $0.040 \pm 0.014 \%\text{m/m}$, 4.23 ± 0.19 ; $25.28 \pm 8.95 \text{ mEq kg}^{-1}$ and $1.92 \pm 0.60 \text{ mL}$, respectively. They presented negative results in the dye detection test, and in the Lugol and Fiehe reactions. Furthermore, the food safety of the assessed honey types was checked and the results showed that good manufacturing practices affect the beekeepers in the cooperatives and production plants involved in the final commercial honey production, regardless of the inspection stamp.

Keywords: Food inspection. Quality control. Food safety.

1 Introduction

Honey is a food of animal origin widely appreciated in different cultures due to its high nutritional value, characteristic flavor and therapeutic properties; besides, it is used as natural sweetener and energy source because of its medicinal importance (AROUCHA, 2008; JATI, 2007; RICHTER, 2011; BULIGNOL et al, 2016). According to the Brazilian Administration of Agriculture, Livestock and Food Supply, also known as MAPA, honey is the food produced by honeybees who collect flower nectar or the secretion from plant living-parts, or even the excretion from plant-sucking insects to be their raw material. These nectars, secretions and excretions lie on plant living-parts, and the bees collect, transform, and combine them with specific substances from the bees' themselves; next, they store and let the mixture ripe in the hive combs (MAPA, 2000). Honey chemical composition comprises different sugars, mainly monosaccharides such as glucose and fructose. In addition, it is possible finding levels of proteins, amino acids, enzymes, organic acids, mineral substances, pollen and other substances in it (RICHTER, 2011; BULIGNOL et al, 2016). Some polysaccharides such as sucrose, maltose, malaise, and dextrin are components commonly found in honey, regardless of the flowering or bee species (JATI, 2007). Other honey components found in small concentrations are fungi, algae, yeasts and other solid particles resulting from its production process (CODEX ALIMENTARIUS, 2001).

Different honey types usually come from different flowering plants; however, eucalyptus and citrus flowers, as well as wildflowers are the most used in large-scale honey production and trade (AIDAR, 1997). It is common finding different physical and chemical compositions depending on the honey type, because several factors, such as climatic conditions, maturation phase, bee species and flowering type, (PÉREZ et al., 2007), as well as product processing and storage (AROUCHA et al, 2008) influence its quality. However, according to the current legislation, honey cannot comprise sugars and/or other substances because they change its composition (MAPA, 2000).

The Brazilian Administration of Agriculture, Livestock and Food Supply (MAPA) implemented the "Technical Regulation on Honey Identity and Quality" through Normative Instruction N. 11 from October 20th, which sets the quality standards and the identity of the honey in order to assure the quality of the product sold throughout the country. The Normative Instruction also establishes the acceptable incidence levels of certain physicochemical components, the sensorial attributes and the microbiological parameters that may be present or absent in honey. These levels are set in order to allow honey processing and trading without any food-safety or health damage risk (MAPA, 2000).

Among the main adulterations made in honey, it is possible mentioning the addition of commercial sugars, glucose, molasses and inverted sugar solution (RYBAK-CHMIELEWSKA, 2003); these changes are often practiced during processing (filtration, centrifugation and decantation) (AROUCHA et al, 2008). Besides these changes, natural modifications resulting from excessive moisture, heat or aging may also occur (RICHTER, 2011). Accordingly, the quality control achieved through the physical and chemical tests applied to honey contributes to the inspection of the commercialized product and to the quality control of internally produced honey throughout the extraction, processing and commercialization stages (CRANE, 1985; CAMARGO et al, 2003; NUNES, 2005; WELKE et al, 2008). The test results are compared to the standards set by the official international bodies or to the national standards as a way to protect consumers from acquiring an adulterated product (MARCHINI, 2004; RICHTER, 2011).

According to MAPA (2000), the physicochemical analyses indicated for pure *Apis* honey quality control are divided in four subgroups: i) maturity (reducing sugars, moisture, apparent sucrose); ii) purity (solids insoluble in water, minerals or ash, pollen); iii) and degree of deterioration (free acidity, diastase activity and hydroxymethylfurfural - HMF) (MAPA, 2000). Therefore, we sought to assess the physicochemical attributes, purity and degree of deterioration

of different honey brands sold in Rio Grande do Sul State, Southern Brazil, in order to check these honey types quality and adequacy to the Brazilian legislation standards.

2 Material and methods

Sampling

The honey sample comprised different brands from different rural districts and colonial fairs from nine locations in Rio Grande do Sul State. Producers and manufacturers' names were kept secret and codified (Table 1). All the purchased samples were kept in their selling packages and sent to the laboratory of General Chemistry at Cruz Alta University.

Table 1 – Batch description based on city of origin, location (latitude and longitude), inspection stamp (SIM, CISPOA or SIF), flowering type

Sample	City	Geographical coordinates		Flowering	Inspection Stamp *
		Latitude	Longitude		
1	Cambará do Sul - RS	29°02'52" S	50°08'42" W	Sylvan	SIM
2	Cambará do Sul - RS	29°02'52" S	50°08'42" W	Eucalyptus	SIF
3	Içara - SC	28°42'48" S	49°18'00" W	Sylvan (Organic)	SIF
4	Prudentópolis - PR	25°12'46" S	50°58'40" W	Sylvan	SIF
5	Guarapuava - PR	25°23'42" S	51°27'28" W	Citrus	SIF
6	Xaxim - SC	26°57'42" S	52°32'05" W	Sylvan	SIF
7	Porto Alegre - RS	30°01'58" S	51°13'48" W	Citrus	SIF
8	Caçapava do Sul - RS	30°30'43" S	53°29'27" W	Sylvan	Cispoa
9	Barão de Cotegipe - RS	27°37'15" S	52°22'48" W	Eucalyptus	SIM

* Cispoa: Coordination of Animal Origin Product Sanitary Inspection; SIF: Federal Inspection Service; SIM: Municipal Inspection Service

In addition to the samples from different cities in Rio Grande do Sul State, the choice was made to assess up to two honey types from Paraná and Santa Catarina states in order to compare them to data of the honey produced in Rio Grande do Sul State apiaries. Furthermore, the present study also had the aim of investigating whether the nature of the flowering and the inspection service stamps are possible parameters to be used in honey physicochemical quality differentiation.

The procedures suggested by Nunes (2005) were followed for sample conservation until the moment to perform the analyses. The room temperature faced approximately 25 °C oscillation throughout the analyses. The physicochemical tests were performed according to the methodological procedures proposed by the National Animal Reference Laboratory (LANARA, 1981) and by Zenebon et al. (2008). The determination in each parameter was conducted in quintuplicate per sample (n = 5).

Reagents and solutions

The reagents used in the present study were of analytical grade. Distilled and deionized water were used to clean and prepare the solutions, respectively. The flasks, glassware and polypropylene materials were washed and soaked in 10%_{v/v} HNO₃, and thoroughly washed in deionized water to avoid contamination.

Analytical procedures

Moisture, ash content, pH determination, acidity, Lugol reaction, Fiehe reaction, and Lund reaction analyses, and dye research were performed. The results were compared to the recommendations of the Brazilian Administration of Agriculture, Livestock and Food Supply (MAPA, 2000).

The moisture analysis was performed according to the techniques described by the National Animal Reference Laboratory (LANARA, 1981). Refractometry was the method of choice, and its interpretation was based on the Chataway table using the Quimis® refractometer. One drop of honey was placed on a specific position in the refractometer for sample reading. The reading based on the Brix scale was adapted according to the Chataway table.

The ash content analysis was performed according to the techniques described by LANARA (1981). Two grams of sample were weighed in a porcelain crucible, preheated in a muffle oven for 30 minutes, cooled in a desiccator using silica gel and weighed again. The sample was charred in a Bunssen's beaker and, then, transferred to a muffle furnace at 600 °C. White ash calcination was performed by leaving the sample for 4 hours inside the muffle oven. Next, the sample was cooled in a desiccator and weighed again. The procedure was repeated for one more hour, until the weight became constant. Calculations were made by applying equation 1, wherein P is the ash mass (g) and P' is the total mass of the sample (g) before calcination, and Ash (%_{m/m}) is the percentage of ash content in the honey sample.

$$Ash (\%_{m/m}) = \left(\frac{P}{P'} \right) \cdot 100 \quad (1)$$

The pH determination was performed according to the techniques described by LANARA (1981). Ten grams (10.0 g) of honey were weighed in an analytical scale and diluted in 75.0 mL of distilled water; the sample was then read using a pHmeter.

The acidity analysis was performed according to the techniques described by LANARA (ANIMAL, 1981). The honey solution prepared for pH determination and titrated with 0.1 N sodium hydroxide solution was used to reach the turning point of the indicator and to record the spent volume. Calculations were made by means of equation 2, wherein V is the consumed NaOH volume (mL) and f is the analytical-standard correction factor; acidity was reported through mEq/kg of honey:

$$Acidity (mEq/kg) = V \cdot f \cdot 10 \quad (2)$$

The reaction using the Lugol's solution was adopted to investigate starch and dextrin presence in the honey. The investigation was performed according to the techniques described by Zenebon et al. (2008), ten grams (10.0 g) of sample were weighed in a 50 mL beaker; next, 20 mL of water were added to it and the sample was homogenized. The

sample was kept in boiling water for 1 hour and, subsequently, left to cool down to room temperature; 0.5 mL of the Lugol solution was added to the sample after heating. The solution turned from reddish-brown to blue in the presence of commercial glucose or sugar syrups. The color intensity depends on the quality and quantity of dextrin or of the starch found in the adulterated sample.

The qualitative evidence of hydroxymethylfurfural (HMF), or Fiehe reaction, was performed according to the techniques described by LANARA (1981). In order to proceed with the method, 10 mL of 50%_{v/v} honey solution were stirred in a test tube containing 5 mL of ethyl ether. The sample was allowed to rest until the ethereal layer became clear. Then, 2 mL of the ether layer was transferred to another test tube containing 5 drops of fresh 1% resorcinol solution in concentrated HCl. The sample was stirred again and the staining that acquired the resorcinol drops at the bottom of the test tube was observed. If the immediate cherry-red or salmon color showed up, it would indicate the presence of invert sugar caused by the acid treatment, as well as by the intense heating or prolonged storage at high room temperature (CRANE, 1985; CAMARGO et al., 2003; NUNES, 2005).

The Lund reaction is applicable to honey samples and it indicates the presence of albuminous cells; however, its absence indicates fraud. This reaction was performed according to the techniques described by Zenebon and collaborators (2008). Two grams (2.0 g) of the sample were weighed, transferred to a 50.0-mL beaker and added with 20.0 mL of water and 5 mL of 0.5%_{v/v} tannic acid solution. Thereafter, water was added until reaching the total volume 40.0 mL, and stirred for complete mixing. The sample was once more allowed to rest for 24 hours. A precipitate (0.6 to 3.0 mL) forms at the bottom of the beaker in the presence of pure honey. The precipitate shall not form or exceed the aforementioned maximum volume range in the presence of adulterated honey (CAMARGO et al, 2003; ZENEBO et al, 2008).

The dye research was performed according to the techniques described by the Brazilian Society of Pharmacognosy (SBF, 2016). One gram (1.0 g) of honey was weighed and dissolved in 10.0 mL of distilled water. Approximately 2.0 mL of 5% w / v sulfuric acid solution was added to the diluted-honey sample. The honey should remain unchanged according to the analysis protocol (SBF, 2016). When coloring substances are added to the honey, the color gradually changes from violet to pink (CRANE, 1985; CAMARGO et al, 2003; NUNES, 2005).

3 Results and discussion

Initially, the physicochemical quality of the assessed honey types showed no differences resulting from state of origin (Paraná, Rio Grande do Sul or Santa Catarina), inspection stamp (Municipal Inspection Service - SIM; Coordination of Industrial and Sanitary Inspection of Products of Animal Origin - Cispoa; Federal Inspection Service - SIF) or flowering (citrus, eucalyptus or sylvan). The only difference between samples concerned the physicochemical properties. The moisture, ash content, pH, acidity, Lugol reaction, Fiehe reaction, and Lund reaction analysis results, as well as the dye research of the different honey samples are shown in Table 2.

Table 2 - Physicochemical parameters determined to honey samples. Values are represented by the mean and the standard deviation (n = 5)

Sample	Moisture (% _{m/v})	Ash (% _{m/m})	pH	Acidity (mEq/kg)	Lugol reaction*	Fiehe reaction*	Lund reaction(mL)	Dyestuff*
1	18.27 ± 0.91	0.055 ± 0.014	4.16 ± 0.21	23.04 ± 1.04	N	N	2.0 ± 0.1	N
2	16.80 ± 0.75	0.046 ± 0.002	4.14 ± 0.20	21.12 ± 0.74	N	N	2.5 ± 0.2	N
3	21.18 ± 1.36	0.029 ± 0.003	4.20 ± 0.14	40.32 ± 2.42	N	N	1.5 ± 0.1	N
4	6.62 ± 0.13	0.003 ± 0.001	4.55 ± 0.13	21.12 ± 1.48	N	N	1.5 ± 0.1	N
5	24.6 ± 1.46	0.002 ± 0.001	4.39 ± 0.25	27.84 ± 1.50	N	N	2.5 ± 0.3	N
6	19.13 ± 0.48	0.005 ± 0.002	4.4 ± 0.23	11.52 ± 0.58	N	N	1.0 ± 0.2	N
7	19.72 ± 1.06	0.008 ± 0.002	3.96 ± 0.15	30.72 ± 1.98	N	P	2.0 ± 0.1	N
8	24.01 ± 1.44	0.170 ± 0.032	4.0 ± 0.12	34.56 ± 1.56	N	N	1.5 ± 0.3	N
9	19.63 ± 0.69	0.012 ± 0.004	4.27 ± 0.23	17.28 ± 1.38	N	N	2.8 ± 0.2	N

* N: negative; P: positive

We chose to present each parameter in independent sections in order to better present and discuss the results.

Moisture

The honey moisture content is an analysis of great importance due to this variables' influence on parameters such as taste, viscosity, and fluidity (BULIGNOL et al, 2016). In addition, the water excess may compromise the honey shelf-life, since the moisture content directly influences microorganism's water activity; besides, honey may undergo fermentation inside the package (VILHENA; ALMEIDA-MURADIAN, 1999; ARAÚJO et al., 2006). The fermentation process may occur due to yeast activity and, consequently, it makes the product unsuitable for consumption and trade (RIBEIRO et al, 2009). The moisture content in the samples used in the present study ranged from 6.62 to 24.6%_{m/v}.

According to the current legislation, the maximum moisture content allowed in honey is 20%_{m/v} (MAPA, 2000) and 3 samples presented water content higher than that recommended in the legislation. However, as the acidity values were within the parameter set forth in the legislation, it is possible concluding that there were no indications of fermentative processes. High moisture levels could result from the lack of good manufacturing practices at some harvest stages, or it could indicate early harvesting, which might have happened when the honey could be "green" or unripe (RICHTER et al, 2011).

Ashes

It is possible determining some irregularities in honey processing such as lack of hygiene and absence of decantation and / or filtration at the end of the honey removal process performed by beekeepers (EVANGELISTA-RODRIGUES et al., 2005). The ashes express the mineral content in the food, as for honey, the differences may be related to floral source, environment, and to production and processing conditions (FELSNER, 2007; BULIGNOL et al, 2016). The

maximum ash content allowed is 0.6g for each 100g of honey, but in case of molasse honey and of its mixtures with floral honey, up to 1.2g of ash for each 100g of honey can be tolerated (MAPA, 2000). Regarding the ashes determinations in the current study, it was verified that all the analyzed samples were within the recommendations in the legislation in force (MAPA, 2000).

pH

Although the pH value does not appear as a mandatory analytical procedure for Brazilian honey quality control, this parameter is a useful auxiliary variable in quality evaluations (SOUZA; BAZLEN, 1998; SILVA, 2004). The honey pH value can be influenced by the nectar and soil pHs or by the association between vegetables contributing to honey composition (CRANE, 1985; MARCHINI et al, 2004). Honey pH is important because it influences the hydroxymethylfurfural formation rate (HMF) (SOUZA; BAZLEN, 1998). Accordingly, pH determination becomes an interesting quantification parameter due to the low analytical complexity it presents at the time to collect data about the acid content in the medium. In parallel it also contributes with complementary information when the idea is to find out whether the honey was subjected to high thermal amplitude or just to aging (CRANE, 1985; MARCHINI et al., 2004; BULIGNOL et al, 2016).

The mandibular substances found in bees are added to the nectar when they transport it to the hive, and this mixture may alter honey pH (EVANGELISTA-RODRIGUES et al., 2005). The pH influences honey texture, stability and shelf-life, because its values may indicate honey fermentation or adulteration caused by the bees themselves (TERRAB et al., 2004). All the samples in the current study presented acidic pH values ranging from 3.96 to 4.55.

Acidity

Honey acidity may also derive from the presence of gluconic, succinic, malic, acetic, citric, formic, lactic, folic and butyric acids (PEREIRA, et al., 2003; RIBEIRO, 2009). The acidity origin in the honey results from organic acid variations, which are caused by different nectar sources, by the action of the enzyme glucose oxidase on the glucose giving origin to gluconic acid (TERRAB et al., 2004; EVANGELISTA-RODRIGUES et al., 2005). This enzyme keeps its action during storage, since it remains in activity even after honey processing (NOGUEIRA–NETO, 1997). In addition to nectar source, other factors, such as the production of acids by bacteria during maturation and the amount of minerals in the honey, influence its acidity (WHITE, 1989).

The organic acids in the honey represent less than 0.5% of the solids found in it, but they have a pronounced effect on flavor, because they are partially responsible for the high microbiological stability (PEREIRA, et al., 2003). The legislation allows maximum acidity 50 mEq/kg in honey (BRASIL, 2000); thus Table 2 shows that all the herein analyzed samples have recorded values within the legally recommended rates.

Assessed Adulterants

Lugol reaction

According to Wiese (2000), the application of iodine and potassium iodide (Lugol reactant) adulterates honey, which shows a characteristic color reaction due to the presence of starch and dextrin, because these components are not naturally found in pure honey. Starch addition is a fraud easy to be observed when producers intend to increase the

content of solids in the honey (CAMARGO et al., 2003; BULIGNOL et al., 2016). Table 2 shows that the reaction to the Lugol agent was negative and that the analyzed batches were not adulterated by starch addition.

Fiehe reaction – a qualitative analysis applied to HMF

Hydroxymethylfurfural is used as quality indicator, since it results from the degradation of enzymes found in different honey types, although only small amounts of enzymes are found in mature honey. Small HMF amounts are found in freshly harvested honeys, but more significant values may indicate significant changes caused by longer storage at high room temperature and / or under overheating (VILHENA; ALMEIDA-MURADIAN, 1999), or by adulterations resulting from inverted sugar addition (SILVA et al., 2004; BULIGNOL et al., 2016). According to Bertoldi et al. (2004), honey adulterations can be carried out by using corn syrup, beet and also inverted syrup, because the inverted syrup is generated by the acid hydrolysis of corn syrup, which contains high hydroxymethylfurfural levels. In addition, the HMF content in honey can also be affected by acidity, pH, water content and minerals (WHITE JÚNIOR, 1979).

The Fiehe reaction is a qualitative test that assesses HMF presence. The persistent red color indicates positivity for or strong HMF presence (possibly more than 200 mg / kg) (LEAL et al., 2001). Just one of the herein analyzed samples presented positive result in this test.

Lund reaction

The Lund reaction indicates the presence of albuminoid substances, which are normal honey components precipitated by the tannic acid added to the sample. This precipitate forms a 0.6 to 3.0 mL deposit at the bottom of the test tube in presence of natural honey. However, the reaction does not occur in artificial honey; therefore, in the case of adulterated honey, the precipitate volume will be lower (BERTOLDI et al., 2004). All samples presented precipitate volume within the expected.

Dyestuff research

According to the current legislation (MAPA, 2000), honey color should not be changed due to the presence of sulfuric acid (5% m / v) when the dye test is applied to the samples. The color change from honey to violet is an adulteration indicator (CODEX ALIMENTARIUS, 2001; ZENEBON et al, 2008). No color change was observed in any of the herein analyzed samples.

4 Conclusion

Based on the physicochemical parameters used to assess the quality of the honey from different locations in Rio Grande do Sul State, it is possible stating that they all complied with the legislation regarding the quality and food safety of the marketed products. In addition, it was not possible distinguishing the quality of the honey according to the inspection stamp chosen for each product since there was no difference between the quality of the honey using the municipal inspection stamp (SIM) and that of honey using the Coordination of Sanitary Inspection of Products of Animal Origin (Cispoa) inspection stamp or the Federal Inspection Service (SIF) stamp. Therefore, these results showed that according to the honey samples from the Southern Brazil production region, this agribusiness sector works with products of good applicability that meet the normative issues to assure the quality and safety of the produced and marketed honey.

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