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PROPERTIES OF MAIZE HYBRID GRAIN FOR UTILIZATION IN WET MILLING

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Abstract

A grain of eight selected hybrids from the Maize Research Institute Zemun Polje was used as the subject of this study. The kernel structure, physical properties and chemical composition of the grain and starch (amylose content) were analyzed before the laboratory wet milling process for starch extraction. The *in vitro* enzymatic digestibility of the isolated starch was also determined. The wet milling properties were later correlated to the physicochemical characteristics of the grain. The results showed that hybrid ZP 677 had the highest starch yield, followed by hybrid ZP 704wx, while the highest starch recovery was observed for hybrid ZP 341. The highest bran yield (10.52%) was obtained by hybrid ZP 434, and the highest germ yield (8.86%) by hybrid ZP 172/8. The correlation analysis showed that starch yield was positively correlated to 1000-kernel weight and crude fiber content, while oil content negatively influenced starch extraction and yield. The starch digestibility was negatively correlated to the amylose content. These findings can be useful for the starch processing industry as well as in the production of ethanol and alcoholic drinks.

Keywords: maize, starch, wet milling, technological quality.

Introduction

At present, maize (*Zea mays* L.), also referred to as corn, is the most important cereal crop in the world, and Serbia is currently taking the 20th place by producing about 6.2 million metric tons yearly. According to the predictions of the United States Department of Agriculture, the estimated world maize production for the 2021/2022 marketing year will be 1,210.45 million metric tons, which could represent an increase of 84.57 million tons or 7.51% in global maize production (World Agricultural Production.com, 2022).

Maize is a cereal crop with high kernel starch content (around 70%) that provides over 85% of this valuable carbohydrate produced worldwide (Milašinović et al., 2007; Zhang et al., 2021). As a significant segment of human nutrition, starch plays a key role in providing metabolic energy. It greatly influences the overall texture, appearance, and end-use quality of cereal-based food products. Mercantile starches utilized in the food-processing industry, are mostly obtained from the grain of standard, waxy, and high-amylose maize, followed by wheat, and other cultivars such as rice, potato, sweet potato, and cassava (tapioca starch) (Milašinović-Šeremešić et al., 2012). The structure, size, and shape of the starch granules are genetically predisposed and differ among botanical species. The different amylose-amylopectin ratios, as well as the structure of starch granules, affect the starch's functional and physicochemical properties, the processing properties of flour, digestibility and the sensory properties of the finished products (Zhang et al 2013; Jane et al, 1999). The two main types of molecules found in starch are amylose and

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amylopectin, and their portion varies depending on the genotype. Maize starches can be classified into three main groups: normal (25-30% amylose and 70-75% amylopectin), waxy (mostly amylopectin and 0-8% amylose), and high-amylose starches (40-85% amylose) (Li et al., 2008). Amylose is essentially a linear polymer of (l→4)-linked α-D-glucopyranosyl units with some minor branching and a degree of polymerization (DP) in the range of 500–6000 glucose residues, whereas amylopectin is a high-molecular-weight branched polymer synthesized through α-(l→4) and α-(l→6) linkages that create branch points with a DP ranging from 3×105 to 3×106 glucose units (Obadi et al, 2020; Zhang et al 2013).

There are two basic technologies for processing maize grain that are used in the production of primarily starch, as well as ethanol and bioethanol fuel: 1) dry milling) and 2) wet milling (Semenčenko, 2013). The technology of maize grain wet milling, which is considered the most complex technology in the food industry, is an integral process of several successive operations, after which the grain is separated into its basic constituents (Drapcho et al., 2008; Milašinović, 2005). The wet milling technology was introduced in the process of maize starch production, more than 150 years ago (Drapcho et al., 2008). The technological process used in wet milling, i.e. starch production, consists of several basic phases: grain steeping (soaking), germ separation, bran separation, gluten separation, starch washing and starch drying (Johnson, 2000). It is of utter importance to note that chemically or biologically treated grain is not used in wet milling during maize starch processing (White et al., 2003), which means that the products of these processes are safe from the aspect of nutrition, for both the humans and farm animals. The wet milling technology of maize processing was developed so that high yields of starch from maize kernels could be obtained for the production of maize-based sweeteners, as well as ethanol by fermentation (Parris et al., 2006).

The primary products obtained by starch processing during wet milling are starch, gluten, bran, germ and steep liquor (Radosavljević and Milašinović, 2008). The average starch yield is 66-68% of the dry matter (Johnson, 1994). Yield, recovery and purity of starch, i.e. protein content in the isolated starch, are the most important parameters in assessing the technological value of corn hybrids in wet milling. Starch, as the primary product of wet processing of maize grain, is the starting material for numerous transformation processes in further industrial production, i.e. higher stages of starch processing. Outstanding nutritional and functional properties such as energy content, digestibility, viscosity, color, taste, water binding capacity and stickiness, swelling ability, gelatinization and fermentability, make maize starch a raw material of special importance for food and a wide range of industrial applications (Milašinović, 2005).

Some of the possibilities of using native starch are, for example: in bakery products, production of high-fructose syrups and sweeteners, sweets, chewing gum, baby food, pudding, toppings, production of glue, briquettes, as a binder component for wax paints and chalks, paper inks , in the production of antibiotics and disinfectants, powdered cosmetics, soap production and so on (White et al., 2003).

By-products make up one third of the initial mass of maize grain, are low-cost, rich in nutrients, and differ regarding the contents of basic chemical components such as protein, oil, carbohydrates, and mineral elements (Zhang et al., 2021). These by-products can be used for various purposes, for example feed production, fermentation, nutrient extraction, functional food ingredients, nutraceuticals etc. For instance, high-protein maize gluten can be repurposed as a quality animal feed, and maize germ can be used as a feedstock for the production of first-grade edible oil (Deepak et al., 2022; Zhang et al., 2021). Although the maize starch processing industry has developed in proportion to population growth in the past, it has grown rapidly in recent years due to increased demand for high-fructose syrups (substitutes for imported sugar cane sugar) used in many foods and bioethanol increasing octane unleaded fuels, replaces gasoline and does not pollute the air (Johnson, 1994). By increas-

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ing the volume of production, in addition to the primary product - starch, the amount of by-products of the process of starch processing of maize is growing.

The main goal of this study was to investigate the grain properties of eight ZP maize hybrids significant for their utilization in wet milling processing. Physical properties and chemical composition of the maize hybrids grain, were evaluated prior to wet milling in order to determine which parameters influenced the yield and recovery of starch as the main product, as well the other wet milling characteristics.

Material and methods

Eight maize hybrids of different genetic background and maturity, namely: ZP 172/8, ZP 243, ZP 341, ZP 434, ZP 505, ZP 548, ZP 677 and ZP 704wx, were selected as the subject of this study. The hybrids were grown in the same agro-technical conditions in the experimental field of the Maize Research Institute Zemun Polje, Serbia (44°52'N, 20°19'E, 81m asl) and standard cropping practices were applied. The maize hybrids were sown in a two-replicate trial by following the randomized complete block design. The sowing density amounted to approximately 66.700 plants per hectare, i.e. the length of the plots was 5 m, with intra and inter-row spacing of 0.20 m and 0.75 m, respectively, in four rows. The harvesting of maize ears from two inner rows of each replicate took place in the full physiological maturity stage.

The basic chemical composition, kernel structure and physical properties of the maize grain, i.e. grain technological quality, were determined according to standard laboratory methods (AOAC, 1990) described in detail in previously published papers (Radosavljević et al., 2001; Milašinović-Šeremešić et al., 2012; Nikolić et al., 2020). The content of starch was analyzed according to Ewers polarimetric method (ISO 10520, 1997). The wet milling properties of the examined ZP maize hybrids grain were assayed by applying a 100-g laboratory method by Eckhoff et al. (1996), modified by Singh et al. (1997). For determination of amylose content, i.e. the ratio of amylose and amylopectin in isolated maize starch, a modified colorimetric method introduced by McGrance et al. (1998) was used. A modification of this colorimetric method consisted of dissolving starch samples in dimethyl sulfoxide (DMSO) at a slightly higher temperature (90 °C) and for a longer period of time (30 min). *In vitro* dry matter digestibility of the isolated starch samples was analyzed by the enzymatic method according to Aufrere (2006).

The results represent the average values of at least three replications shown as the percentage per dry matter (d.m.). Interrelations of certain investigated parameters are shown in table 5 as Pearson's correlation coefficients.

Results and discussion

The kernel structure and physical properties of the evaluated maize hybrids are shown in Table 1.

The highest pericarp content obtained by manual dissection of the maize kernels was detected in hybrid ZP 341 (7.26%), the highest germ share in waxy hybrid ZP 704wx, while the highest endosperm percentage was in hybrid ZP 243 (82.45%). Soft endosperm fraction ranged from 51.60% (ZP 548) to 65.69% (ZP 704wx). The significant physical parameter of grain quality, 1000-kernel weight, varied between 241.19 g (ZP 172/8) and 384.45 g (ZP 434). From the wet milling perspective, a higher value of 1000-kernel weight is a preferential property because, in general, it provides higher starch and protein yields and lower fiber yields. Notwithstanding the fact that it is a less important indicator of maize quality for processing and milled products, test weight is a standard easily measurable indicator of maize grain quality that notably affects its economic value (Paulsen et al., 2003, Lee et al., 2007). The test weight of the assayed ZP maize genotypes ranged from 749.20 kg m^{-3} (ZP 243) to a maximum of 846.84 kg m^{-3} (ZP 505). These results are in accordance with the findings of Lee et al. (2007) and Milašinović et al. (2007). All the maize hybrids examined in this research had test weights higher than 650.0 kg m^{-3}, as one of the requirements for animal feed according

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Table 1. Kernel structure and physical properties of the evaluated maize hybrids
Tabela 1. Struktura i fizička svojstva zrna ispitivanih hibrida kukuruza

Genotype	Pericarp (%)	Germ (%)	E (%)	SE (%)	HE (%)	Kwt (g)	Twt (kgm^{-3})	Den (gcm^{-3})
ZP 172/8	5.44	12.41	82.15	60.27	39.73	241.19	829.32	1.27
ZP 243	5.07	12.48	82.45	57.14	42.86	292.89	749.20	1.20
ZP 341	7.26	12.30	80.44	58.46	41.54	351.98	785.89	1.24
ZP 434	7.02	12.11	80.87	56.69	43.31	384.45	778.31	1.26
ZP 505	6.20	12.76	81.04	64.45	35.55	315.82	846.84	1.30
ZP 548	5.78	13.02	81.20	51.60	48.40	342.36	808.81	1.24
ZP 677	6.20	11.80	82.00	61.09	38.91	367.77	788.52	1.27
ZP 704wx	5.76	13.40	80.84	65.69	34.31	319.20	780.72	1.25
Min	5.07	11.80	80.44	51.60	34.31	241.19	749.20	1.20
Max	7.26	13.40	82.45	65.69	48.40	384.45	846.84	1.30
Average	6.09	12.54	81.37	59.42	40.58	326.96	795.95	1.25
SD	0.75	0.51	0.73	4.52	4.52	45.57	31.06	0.03

E-endosperm; SE-soft endosperm; HE-hard endosperm Kwt-1000-kernel weight; Twt-test weight; Den-density

to Serbian regulations (Pravilnik o kvalitetu hrane za životinje, 2017), as well as 69.50 kg hl^{-1} (695.0 kg m^{-3}) as a requirement for US Grade No. 2 corn (Somavat et al., 2016). Moreover, in Serbia, there are currently no regulations for this indicator of maize grain quality for food used in human nutrition. Grain density did not vary significantly among the tested maize hybrids (Table 1).

The chemical composition of the tested maize hybrids grain is given in Table 2.

Table 2. Chemical composition of the evaluated maize hybrids grain (%)
Tabela 2. Hemijski sastav zrna ispitivanih hibrida kukuruza (%)

Genotype	Dry matter	Protein	Oil	Crude fiber	Ash
ZP 172/8	90.20	9.35	7.15	2.06	1.44
ZP 243	88.89	9.40	6.23	1.98	1.25
ZP 341	88.04	9.75	6.28	2.33	1.34
ZP 434	88.44	10.15	6.02	2.42	1.40
ZP 505	88.86	9.88	6.38	2.21	1.31
ZP 548	90.19	9.19	6.08	1.97	1.41
ZP 677	88.29	9.07	5.00	2.50	1.36
ZP 704wx	89.13	10.30	5.71	2.26	1.51
Min	88.04	9.07	5.00	1.97	1.25
Max	90.20	10.30	7.15	2.50	1.51
Average	89.01	9.64	6.11	2.22	1.38
SD	0.81	0.45	0.61	0.20	0.08

Table 3. Wet milling properties of ZP maize hybrids grain (%)
Tabela 3. Karakteristike mokrog mlevenja zrna ZP hibrida kukuruza (%)

Genotype	Starch	Germ	Bran	Gluten	Steeping water	Process water
ZP 172/8	62.41	8.86	10.30	6.53	4.48	0.22
ZP 243	61.67	8.76	9.96	7.05	4.55	0.21
ZP 341	66.34	7.65	10.15	7.04	4.22	0.20
ZP 434	65.25	8.18	10.52	6.71	4.21	0.23
ZP 505	62.32	8.21	9.18	6.43	4.25	0.19
ZP 548	64.29	8.15	8.43	6.52	4.70	0.17
ZP 677	67.43	8.05	8.32	6.54	4.28	0.20
ZP 704wx	66.99	7.85	9.84	6.15	4.56	0.18
Min	61.67	7.65	8.32	6.15	4.21	0.17
Max	67.43	8.86	10.52	7.05	4.70	0.23
Average	64.59	8.21	9.59	6.62	4.41	0.20
SD	2.26	0.41	0.85	0.30	0.19	0.02

The protein content ranged from 9.07% (ZP 677) to 10.30% (ZP 704wx), oil from 5.00% (ZP 677) to 7.15% (ZP 172/8). Crude fiber content determined in the analyzed grain samples ranged from 1.97% (ZP 548) to 2.50% (ZP 677).

The wet milling properties that represent the yields of all the grain fractions obtained by the 100-g laboratory wet milling procedure on the tested hybrid maize grain samples are shown in Table 3.

Table 3 shows that the highest bran yield, a valuable by-product of the wet milling that can be used in animal feed, was obtained by hybrid ZP 434 (10.52%). Hybrid ZP 172/8 amounted to the highest yield of germ (8.86%), a co-product used in the vegetable oil industry. Studies have shown that maize germ obtained by wet milling contains around 40 to 50% oil, while dry milling germ fraction has only 20-25% oil on a dry basis (Parris et al., 2006). Furthermore, the highest gluten yield was determined in hybrid ZP 243 (7.05%).

The most important wet-milling properties of the tested ZP maize hybrids grain were assessed based on the starch yield (ration of total starch isolated from the grain), and starch recovery (a ratio between the isolated starch and the total starch content of grain), which was shown in Figure 1.

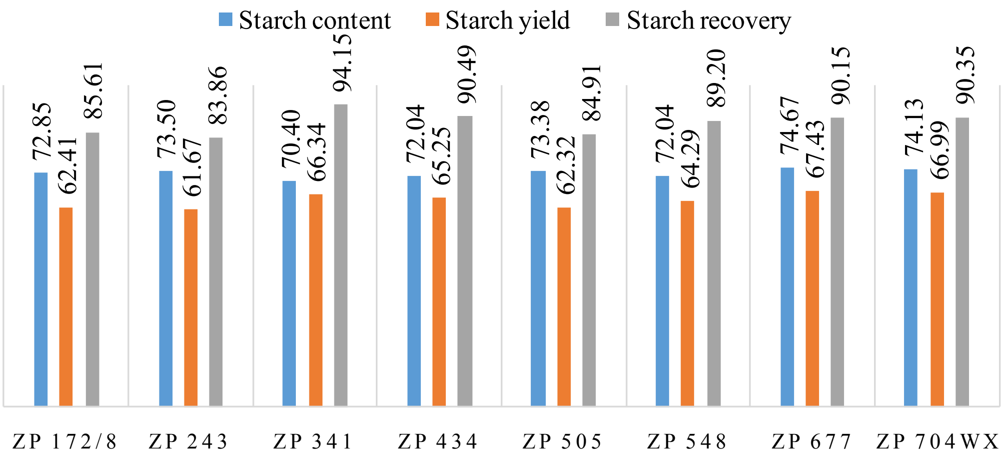

Figure 1. Content, yield and recovery of starch isolated from ZP maize hybrids grain (%)
Grafikon 1. Sadržaj, prinos i iskorišćenje skroba izolovanog iz zrna ZP hibrida kukuruza (%)

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The crucial quality indicators of a wet milling process are high starch yield, high starch recovery, and low protein content in starch (Milašinović-Šeremešić et al., 2012). Starch recovery represents the percentage of starch obtained in relation to the amount of total starch present in the grain, calculated as a percentage of dry matter. Therefore, it is necessary to determine the starch content of the whole grain in advance (Milašinović, 2005). The results presented in Figure 1 clearly show that the isolated starch yield ranged from 61.67% (ZP 243) to 67.43% (ZP 677), while the starch recovery was between 83.86% (ZP 243) and 94.15% (ZP 341). It was noticed that the recovery did not necessarily correspond to the isolated starch yields. For instance, the highest starch yielding hybrid ZP 677 had a 67.43% starch yield and recovery of 90.15%, while the highest recovery was detected in hybrid ZP 341 (94.15%) having the isolated starch yield of 66.34%. Similar results were reported earlier by Milašinović et al. (2007).

The composition of the main starch constituents: amylose and amylopectin, determined in the isolated starches, the content of protein determined in the isolated starches, as well as the *in vitro* dry matter digestibility of the obtained starch samples are given in Table 4.

The amylose content of seven out of the eight analyzed samples was in the range usual for normal maize starches (23.2 - 25.1%), while hybrid ZP 704wx had amylose content characteristic of the so-called amylopectin or waxy starches (1.0%).

The starch purity was evaluated as the protein content of the starch isolated by the wet milling procedure. The protein content in the starch samples was significantly low, ranging from 0.17% (ZP 341) to 0.27% (ZP 677), which indicates that the wet milling procedure was well conducted.

Digestibility of starch is an important factor from a nutritional point of view for humans as well as animals due to its association with the glycemic index used for the assessment of food quality (Magellanes-Cruz et al., 2017). It depends on the starch composition and changes that occur during digestion processes. Some studies have shown that high amylose starch is more prone to producing resistant starch that is unsusceptible to digestion, and that amylopectin starch may be more easily processed in the gastrointestinal system (Zhang et al., 2008). The results obtained in our study showed that the waxy hybrid ZP 704wx starch had the highest digestibility rate (92.74%), while the lowest digestibility was determined on starch isolated from hybrid ZP 243 (89.56%.).

Table 4. Composition and digestibility of starches isolated from ZP maize hybrids grain (%)

Tabela 4. Sastav i svarljivost skrobova izolovanih iz zrna ZP hibrida kukuruza (%)

Genotype	Amylose	Amylopectin	Protein in starch	Starch digestibility
ZP 172/8	23.7	76.3	0.21	90.07
ZP 243	24.4	75.6	0.20	89.56
ZP 341	23.2	76.8	0.17	90.16
ZP 434	23.6	76.4	0.26	91.24
ZP 505	25.1	74.9	0.23	89.84
ZP 548	24.1	75.9	0.26	91.40
ZP 677	23.8	76.2	0.27	89.93
ZP 704wx	1.0	99.0	0.22	92.74
Min	1.0	74.9	0.17	89.56
Max	25.1	99.0	0.27	92.74
Average	21.1	78. 9	0.23	90.62
SD	8.2	8.2	0.03	1.08

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Table 5. Correlation coefficients between the tested kernel and wet milling properties
Tabela 5. Koeficijenti korelacije između ispitivanih parametara zrna i mokrog mlevenja

	Chemical composition						Kernel structure				Physical properties		
Wet milling properties	Starch	Amylose	Protein	Oil	CF	Ash	Pericarp	Germ	E	SE	Kwt	Twt	Den
Starch yield	0.03	-0.47	0.20	-0.74*	0.75**	0.47	0.54	-0.11	-0.48	0.17	0.67*	-0.29	0.08
Starch recovery	-0.47	-0.26	0.24	-0.43	0.62*	0.37	0.77**	-0.13	-0.70**	-0.11	0.69*	-0.24	-0.03
Protein in starch	0.42	0.10	-0.19	-0.53	0.27	0.23	0.00	-0.15	0.10	-0.19	0.47	0.14	0.36
Starch digestibility	0.03	-0.80**	0.56	-0.25	0.08	0.82**	0.07	0.65	-0.52	0.08	0.24	-0.14	-0.03
Germ yield	0.27	0.38	-0.39	0.55	-0.60*	-0.24	-0.71	-0.13	0.82**	-0.16	-0.72**	0.09	-0.14
Bran yield	-0.39	-0.15	0.58	0.56	-0.01	0.09	0.17	-0.05	-0.15	0.12	-0.27	-0.25	-0.20
Gluten yield	-0.13	0.60*	-0.26	0.19	-0.09	-0.73**	0.22	-0.70	0.15	-0.05	0.10	-0.48	-0.59

CF-crude fiber; E-endosperm; SE-soft endosperm; Kwt-1000-kernel weight; Twt-test weight; Den-density

The interrelations of the assessed parameters are presented as correlation coefficients in Table 5.

The yield of starch isolated by the wet milling procedure was positively correlated to the 1000-kernel weight (r = 0.67) and crude fiber content (r = 0.75), while oil content negatively influenced yield of the isolated starch (r=-0.74). Statistical analysis has shown that the amylose content very negatively influenced the starch digestibility (r = -0.80). Pericarp content positively influenced the starch recovery (r = 0.77), while the endosperm content showed a highly negative correlation with this parameter (r = -0.70). These results are in accordance with the findings previously reported on ZP maize hybrids (Milašinović, 2007, Milašinović-Šeremešić et al., 2012).

Conclusion

The results obtained in our study showed that hybrid with the lowest protein and oil content, as well as high 1000-kernel weight (ZP 677) had the highest starch yield. However, hybrid ZP 341 showed the highest starch recovery (94.15%). Statistical analysis has shown that the amylose content negatively influenced the starch digestibility. Technological properties of all the analyzed maize hybrid grain samples may provide diverse possibilities for their industrial application. The results obtained in this study can be beneficial for the wet milling and starch processing industry, future maize breeding programmes, as well as for the various branches of industry including producers of pharmaceutical ethanol, bioethanol and alcoholic drinks.

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SVOJSTVA ZRNA HIBRIDA KUKURUZA ZA PRIMENU U MOKROM MLEVENJU

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Sažetak

Kao predmet ovog istraživanja korišćeno je zrno osam odabranih hibrida Instituta za kukuruz „Zemun Polje". Struktura zrna, fizička svojstva i hemijski sastav zrna i skroba (sadržaj amiloze) analizirani su primenom laboratorijskog procesa mokrog mlevenja za izolovanje skroba. Takođe je određena *in vitro* enzimska svarljivost izolovanog skroba. Svojstva mokrog mlevenja su potom korelisana sa fizičko-hemijskim karakteristikama zrna. Rezultati su pokazali da je najviši prinos skroba imao hibrid ZP 677, a zatim hibrid ZP 704wx, dok je najviša iskoristljivost skroba uočena kod hibrida ZP 341. Najviši prinos mekinja (10,52%) dobijen je kod hibrida ZP 434, a najviši prinos klice (8,86%) utvrđen je kod hibrida ZP 172/8. Korelaciona analiza je pokazala da je prinos skroba bio u pozitivnoj vezi sa apsolutnom masom zrna i sadržajem sirovih vlakana, dok je sadržaj ulja negativno uticao na izolaciju skroba i njegov prinos. Svarljivost skroba je bila u negativnoj korelaciji sa sadržajem amiloze. Ova saznanja mogu biti korisna za industriju prerade skroba, kao i za proizvodnju etanola i alkoholnih pića.

Ključne reči: kukuruz, skrob, mokro mlevenje, tehnološki kvalitet

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