Characterisation of viscosity, colour, 5-hydroxymethylfurfural content and diastase activity in raw rape honey (*Brassica napus*) at different temperatures

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Abstract The effect of heating at various temperatures (30, 40, 50, 60, 70 and 80 °C) on dynamic viscosity, colour, 5-hydroxymethylfurfural (5-HMF) concentration and diastase activity of raw rape honey were assessed. In fresh honey, moisture, ash, free acidity, pH and electrical conductivity averaged 185.3 g kg⁻¹, 1.2 g kg⁻¹, 18.71 mEq kg⁻¹, 4.2 and 0.25 mS cm⁻¹, respectively. Heating significantly ($p \leq 0.05$) increased lightness (L*), yellowness (b*), chroma (C*), hue (h°) values, but decreased redness (a*). The viscosity at 20 °C (33.6 Pa s) differed significantly ($p \leq 0.01$) with those at 30, 40 and 50 °C (8.2, 2.5, and 1.6 Pa s, respectively). Diastase activity decreased concomitant with heating at higher temperatures. Honey heated at 80 °C for 15 min showed the maximum increase of 5-HMF content, with an average of 1.9 mg kg⁻¹ (62 %), compared to unheated samples. Heating for 15 min between 50 °C and 80 °C did not significantly degrade the quality of the honey, but, slightly enhanced formation of 5-HMF and reduced the diastase activity.

Keywords Rape honey · Heat treatment · Viscosity · Colour · Diastase activity · Hydroxymethylfurfural

Introduction

Honey is a naturally sweet substance produced by honey bees (*Apis mellifera*) from the nectar of blossoms or from secretions of living parts of plants or excretions of plant-sucking insects (EU 2013). In 2012, the total number of beehives in Poland was 1.47 million with a production capacity of 12.2 t of honey (FAO 2014). Rape (*Brassica napus* L. and other species, hybrids and varieties) is largely cultivated in Europe for the seed, which is used for oil production. It is very attractive to bees both for nectar and pollen and represents one of the most important spring sources in Central and Eastern European countries, giving rise to large amounts of very pure unifloral honey (Persano Oddo et al. 2004).

The rape honey is characterised by quick granulation (often in crystallized form with very small crystals), due to the high glucose content (40.5 % on average). For this property, it is frequently used as a “crystallisation starter”, added to other honeys to obtain a finer granulation (Persano Oddo et al. 2004). The crystallisation is natural process that occurs spontaneously and does not reduce the quality of the product. Though, Polish consumers consistently prefer fresh liquid honey (‘patoka’). Improper treatment, especially melting may result in unfavourable chemical processes (reduction of α -amylase activity and increase of 5-hydroxymethylfurfural (5-HMF) concentration), leading to a decrease of the commercial quality of honey. According to Turhan et al. (2008) overheating honey is not needed for filling and packaging process. Thus, beekeepers or factories do not have to heat honey at high temperatures, on the contrary, mild temperatures (between 40 and 50 °C) is sufficient to reduce viscosity and

Research highlights

- heating (15 min at 50–80 °C) does not significantly reduce the quality of the raw rape honey,
- heating of raw rape honey enhanced the process of 5-HMF formation and reduced the diastase activity,
- heating of raw rape honey significantly increased lightness, yellowness, chroma and hue, but decreased redness.

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prevent crystallization. According to Bakier (2006), the effective liquification of honey requires heating at 52–55 °C for at least 10 min. After that, hot honey must be filtered to remove any fibrous crystals.

The objective of the present work was to study the effect of thermal treatment on the dynamic viscosity, colour, 5-hydroxymethylfurfural concentration and diastase activity in Polish raw rape honey.

Materials and methods

Honey

The present study was carried out on raw (unprocessed) rape honey collected from apiaries located in Lublin region. Honey samples were collected in May/June 2014. Fresh honey samples were poured into glass jars with a volume of one litre, sealed, and stored at 20 °C in the dark. After a few (3–5) days of crystallisation in such conditions, honey showed the state of aggregation close to that of a solid substance of plastic consistency. All analyses were performed until 15th July, 2014.

Heat treatment

The samples of each raw honey (50 g) were poured into containers and then submerged in a water bath (LaboPlay, W615, Poland), heated to a specified temperature (50 °C, 60 °C, 70 °C or 80 °C) for 15 min, and then cooled at room temperature and analysed for 5-HMF, diastase activity and colour measurements. All tests were performed in duplicate. The rheological properties were determined in duplicate on honey samples (150 g) heated in a water bath until the required temperature (30 °C, 40 °C or 50 °C) was achieved. Raw, untreated samples of honey (in both treatments) were measured at 20 °C.

Measurements and analysis

The moisture content was determined based on refractometric method using an Abbe Carl Zeiss refractometer (Jena, Germany). The refractive indices of honey samples were measured at ambient temperature, and the readings were corrected for a standard temperature of 20 °C (Bogdanov et al. 2009).

The ash content, pH and electrical conductivity were determined as described earlier (Bogdanov et al. 2009).

The colour of the honey samples was measured by a Minolta CR-310 Chroma Meter (Minolta Camera Co. Ltd., Osaka, Japan) using D65 as the standard light source. Honey samples were poured into small disposable petri dishes (60 mm in diameter, height layer of honey 10 mm), and then put onto a white standard plate. The measuring head (50 mm diameter of aperture) was inserted directly into the sample,

and the reflectance of honey surface was measured. The CIE colour parameters were L* (lightness), a* (redness/greenness), b* (yellowness/blueness), C* (chroma), and h° (hue angle) (CIE 2004).

The honey samples for colour intensity were diluted to 50 % (w/v) with warm (45–50 °C) deionised water and the solution was filtered through a 0.45 µm filter before measuring the optical density. There was a complete absence of coarse particles in the honey solutions. The absorbance was measured using a Varian Cary 300 Bio spectrophotometer (Varian Australia PTY, Ltd.) at 450 and 720 nm and the difference in absorbance was expressed as mAU (Beretta et al. 2005).

Viscosity was measured using a Zwick/Roell universal testing machine Proline BDO-FB0.5TS (Zwick GmbH and Co, Ulm, Germany) with the back extrusion rig. The measuring system consists of a back extrusion cell (a diameter 50 mm, a length of 60 mm), and a plunger (45 mm diameter). The dynamic viscosity η (Pa s) was evaluated from the measured force difference and the flow rate of the honey sample in the annular gap between the piston and back extrusion cell. The mean value was calculated based on 4 cycles (50, 100, 200 and 400 mm/min) using the testXpert II program especially developed for viscosity testing.

Free acidity was determined by potentiometric titration. Ten grams of honey sample were dissolved in 75 ml of distilled water, an alcoholic solution of phenolphthalein was added, and then the sample was titrated with 0.1 N sodium hydroxide solution (NaOH). The milliequivalents of acid per kg of honey were determined as 10 times the volume of NaOH used in titration (Bogdanov et al. 2009).

The 5-HMF (expressed as mg per kg of honey) was determined using method of White (1979) with Varian Cary 300 Bio spectrophotometer (Varian Australia PTY, Ltd.) following the equation:

$$5\text{-HMF}(\text{mg kg}^{-1}) = (\text{Abs}_{284} - \text{Abs}_{336}) \times 149.7 \times 5 \times (\text{D/W}) \quad (1)$$

where: Abs₂₈₄ - absorbance of the solutions at $\lambda = 284$ nm; Abs₃₃₆ - absorbance of the solutions at $\lambda = 336$ nm; $149.7 - 126 \times 1000 \times 1000 / 16,830 \times 10 \times 5 = \text{constant}$ (126 - molecular weight of HMF, 16,830 - M absorptivity ϵ of HMF at $\lambda = 284$ nm, 1000 - conversion g into mg, 10 - conversion 5 into 50 ml, 1000 - conversion g of honey into kg); 5 - theoretical nominal sample weight; D - dilution factor, in case dilution is necessary; W - weight in g of the honey sample.

The diastase activity, expressed as the diastase number (DN) in Schade units, was measured with the Phadebas tablets (Honey Diastase Test, Magle AB, Lund, Sweden) according to Bogdanov et al. (2009) using a Varian Cary 300 Bio spectrophotometer (Varian Australia PTY, Ltd.) at 620 nm.

Statistical analysis

The analyses were performed using the SAS Enterprise Guide 6.1 software (SAS 2013). One-way analysis of variance (ANOVA) followed by Tukey's (HSD) test was used to compare means of different parameters. The data were expressed as means $\pm$ standard deviations (s.d.). Differences between means at the 95 % and 99 % ($p \leq 0.05$ and $p \leq 0.01$, respectively) confidence levels were considered statistically significant.

Results and discussion

The results of the physicochemical characteristics of rape honey are presented in Table 1. The moisture content was in an agreement with the results of other authors (Kuś et al. 2014; Szczesna et al. 2011), however, the rape honey from Poland usually contains a wide range of moisture, i.e. from 154 g kg⁻¹ to 199 g kg⁻¹.

The legislative provisions do not specify the precise ash content in honey. The concentration of ash was closely related to the electrical conductivity of honey (Feás et al. 2010). In comparison to the electrical conductivity results obtained in the present study, Szczesna et al. (2011) showed slightly lower conductivity for rape honey, with an average of 0.20 mS cm⁻¹. However, the range in the earlier study varied to a greater extent (from 0.12 to 0.34 mS cm⁻¹) than reported in this investigation.

All of the raw rape honeys analysed were found to be acidic (Table 1) and in close agreement with values reported earlier by Wilczyńska (2012) and Szczesna et al. (2011). Furthermore, the pH values of European rape honey have been found on average to be 4.1, irrespective of the variable geographical origin (Persano Oddo et al. 2004). Semkiw et al. (2010) reported mean value of acidity for rape honey was 16.83 mEq kg⁻¹ and range of acidity – 12.7–20.0 mEq kg⁻¹, which was comparable to present results. According to Regulation (MARD 2004), the acceptable water content, free acids and conductivity must not exceed 200 g kg⁻¹, 50 mEq kg⁻¹ and 0.8 mS cm⁻¹, respectively.

Table 1 Descriptive characteristics of physicochemical properties of raw rape honey

Specification	Mean	Range
Moisture (g kg ⁻¹)	185.3 $\pm$ 7.4	174.0–194.2
Ash (g kg ⁻¹)	1.2 $\pm$ 0.4	0.7–1.9
Free acidity (mEq kg ⁻¹)	18.71 $\pm$ 4.16	11.50–24.00
pH	4.20 $\pm$ 0.16	4.04–4.51
Electrical conductivity (mS cm ⁻¹)	0.25 $\pm$ 0.06	0.17–0.32

The results of the analysis on colour of Polish rape honey samples at different temperatures are reported in Table 2. The considerably high L*, and lower b*, C* and h° values were found for fresh honey as compared to heated samples. Kuś et al. (2014) reported L* value of 81.8, and b* of 28.4 for rapeseed honey collected in Poland. Contrary to the findings, the value of a* was slightly below zero (green component). In present study both the fresh and heated samples of honey can be classified as light honeys (with L* > 50) (González-Miret et al. 2005). Ajlouni and Sujirapinyokul (2010) studied Australian honeys and reported that a rainforest honey exhibited the largest degree of brightness (L* value = 101.27 $\pm$ 0.34), while the Grey box sample showed the least (L* = 80.81 $\pm$ 0.06).

Heating significantly influenced all CIE colour parameters of rape honey (Table 2). In general, values of L*, b*, C*, h° increased concomitantly with increasing temperature. The opposite trend was observed for a*.

It should be also noted that perception of colour by human is influenced by all three coordinates (L*, a* and b*) at the same time. The higher lightness of honey between 50 and 80 °C could be also connected with significantly ($p \leq 0.01$) higher yellowness (from 23.37 for unheated up to 39.05 at 80 °C) and concomitant reduced redness (from 7.52 for unheated and 8.48 at 50 °C, $p > 0.05$, up to 5.86 at 80 °C).

One of the effects of thermal treatments of honey is the acceleration of Maillard reactions incorporating the sugars and free amino acids or fructose caramelisation. These reactions would be associated non-enzymatic chemical changes of browning, leading to the formation of a variety of brown pigments, and simultaneously the formation of intermediate products as HMF. Such significant ($p \leq 0.01$) changes consisting in the lightness reduction (L*), but increase of yellowness (b*) and yellowish hue (h°), as well as insignificant rise of redness (a*) and chroma (C*) values were observed in present study between honey samples heated at 20 and at 50 °C. Moreover, it should be pointed out that there was no significant change in colour intensity (ABS₄₅₀) of honey as a result of the heat effect (Table 2). However, the values of net absorbance increased concomitantly with higher temperature levels from 416.88 to 467.90 mAU.

Darkening of honey between 20 and 50 °C could be related to Maillard reaction or fructose caramelisation. The difference in browning rate of honey could be explained by differences in its amino acid and reducing sugar contents. Other factors that would influence the kinetics of Maillard browning could be the type and thermal stability of amino acids and reducing sugars which participate in the reaction (Turkmen et al. 2006). According to Abu-Jdayil et al. (2002), the stability of colour upon heating is an indication of no protein precipitation, but only protein denaturation in solution.

Additionally, decrease of a* ($p \leq 0.05$), and increase of L* and b* values ($p \leq 0.01$) values in samples heated between 50

Table 2 CIE colour parameters of rape honey at different temperatures (mean $\pm$ s.d.)

Temperature	L*	a*	b*	C*	h°	ABS ₄₅₀ (mAU)
20 °C (unheated)	84.32 ^D $\pm$ 5.55	7.52 ^{ab} $\pm$ 0.85	23.37 ^A $\pm$ 2.44	24.56 ^A $\pm$ 2.51	72.2 ^A $\pm$ 1.33	416.88 $\pm$ 93.37
50 °C	67.39 ^A $\pm$ 4.11	8.48 ^b $\pm$ 2.14	33.44 ^B $\pm$ 6.70	34.56 ^{AB} $\pm$ 6.65	75.6 ^B $\pm$ 3.94	413.07 $\pm$ 104.52
60 °C	73.24 ^{AB} $\pm$ 8.02	6.48 ^{ab} $\pm$ 2.90	32.19 ^B $\pm$ 3.59	32.93 ^B $\pm$ 3.78	78.8 ^{BC} $\pm$ 4.55	433.24 $\pm$ 97.47
70 °C	77.33 ^{BC} $\pm$ 7.00	6.17 ^{ab} $\pm$ 2.52	36.74 ^{BC} $\pm$ 4.96	37.30 ^{BC} $\pm$ 5.17	80.74 ^C $\pm$ 3.11	445.86 $\pm$ 91.07
80 °C	80.35 ^{CD} $\pm$ 4.44	5.86 ^a $\pm$ 2.12	39.05 ^C $\pm$ 6.07	39.51 ^C $\pm$ 6.28	81.74 ^C $\pm$ 1.99	467.90 $\pm$ 83.68

Mean with different letters in the same column are significantly different: a-b – $p \leq 0.05$; A-D – $p \leq 0.01$

and 80 °C, could be linked to changes of the morphology of the crystalline structure of the analysed honeys. Bakier (2008) revealed the highest mass fraction of the crystalline phase for the rape honey, characterized by the highest glucose content, compared with multifloral or buckwheat honeys. Furthermore, heating of rape honey between 25 and 45 °C increased fraction of the smallest crystals (<10 μ m) from 65.1 % to 72.2 %, but decreased share of large ones (40–50 μ m) from 2.1 to 0.6 % (Bakier 2008). Such changes of the morphology of the crystalline structure of honey (more finer crystals) heated between 60 and 80 °C, might have modified the optical properties, and consequently caused the lightening of honey.

de la Paz Moliné et al. (2015) evaluated microwave effect (800 watts during 45 and 90 s) on honey colour (mm Pfund). The initial L* value of untreated samples averaged 63.4, after 45 s of heating at 83 °C, increased to 88.9 after 90 s at 108 °C, and then dropped to 83.4 mm Pfund. Similar tendency related to lightening of honey heated at higher temperatures (between 50 and 80 °C) was observed in present research.

Figure 1 provides the temperature-dependent dynamic viscosity for rape honey samples evaluated in this study. Dynamic viscosity (called ‘viscosity’ or ‘absolute viscosity’), is the internal friction of a liquid or its tendency to resist flow. It is calculated as a quotient of the shear stress and the shear rate. According to Bourne (2002) the procedure of back extrusion viscometry, although mathematically complex, has

Fig. 1 Dynamic viscosity of rape honey samples at 20, 30, 40 and 50 °C

great potential for the food industry because the experimental procedure is simple, easy to perform, rapid, and uses robust attachments for a universal testing machine. As expected, the dynamic viscosity decreases substantially with an increase in temperature for all individual samples. The effect of temperature was more pronounced for temperatures up to 30 °C, and was not significant above this level. The mean value of viscosity at 20 °C (33.6 Pa s) differed significantly ($p \leq 0.01$) with averages at 30, 40 and 50 °C (8.2, 2.5, and 1.6 Pa s, respectively). Juszczak and Fortuna (2006) using a rotational rheometer determined a viscosity of rape honey (at moisture content of 180 g kg⁻¹) ranging between 20.9, 5.5 and 1.8 Pa s at 20, 30 and 40 °C, respectively. In addition, our results of viscosity are similar to those reported earlier by Dobre et al. (2012) at 10–40 °C, Yanniotis et al. (2006) at 25–45 °C, and Oroian (2013) at 20–50 °C for different types of honeys. Usually, Polish honeys are reported as a Newtonian liquid (Juszczak and Fortuna 2006). However, Dobre et al. (2012) reported clearly non-Newtonian flow behaviour of rape honey, which was also related to a high amount of carbohydrates (78.3 %).

The results of diastase activity in raw honey samples and after heating treatment are reported in Table 3. In comparison with previous research carried out by Polish authors (Szczęsna et al. 2011; Wilczyńska 2012), the values obtained in the present study for diastase activity in raw rape honeys were higher. Szczęsna et al. (2011) indicated for fresh rape honey collected in 2007–2010 had greater fluctuations (from 7.7 to 35.6) in the value of diastase activity, and the average value (14.0) was two times lower than mean value (28.37) which was reported for unheated samples in the current investigation. However, Wilczyńska (2012) revealed three times (9.8) lower diastase activity. In contrast, Semkiw et al. (2010) obtained the highest diastase activity (averaged 42.44) in rape honeys harvested traditionally, and this value is similar to the highest value determined in the present study (sample no. 5).

Changes in diastase activity may be due to the modification of the enzymatic activity, brought about by the structural changes in enzyme molecules, promoted by heating. Most proteins are heat labile substances. According to the theory of Eyring, during transient heating all molecules whose free energy exceeds the energy barrier undergo a complete and

Table 3 Diastase activity in rape honey at different temperatures

Temperature	Sample no.							Mean value
	1	2	3	4	5	6	7	
20 °C (unheated)	20.41	33.48	25.02	21.60	43.50	37.24	30.60	28.37 ^a ± 9.53
50 °C	17.10	29.33	24.65	22.66	43.78	33.39	26.09	26.55 ^a ± 9.13
60 °C	19.33	31.65	25.04	22.40	43.00	32.22	30.89	27.39 ^a ± 8.91
70 °C	16.25	29.80	21.92	24.05	39.64	34.41	33.39	26.60 ^a ± 9.24
80 °C	14.44	20.75	17.55	16.96	34.35	24.79	21.03	20.19 ^a ± 7.03

Mean with different letters in the same column are significantly different: a-b – $p \leq 0.05$

irreversible denaturation (Tosi et al. 2008). Thus, protein denaturation involves conformational change from the native, folded state to the denaturated, unfolded state accompanied by an endothermic heat effect (Al-Malah et al. 1995). Nevertheless, Tosi et al. (2008) revealed that during the isothermal heating, in samples maintained at 60, 70, 80 and 90 °C, the number of activated molecules which could exceed the energetic barrier of the transition stage was low. As a consequence, the reaction towards complete denaturation did not occur to any large extent. When heating stopped, all activated molecules that did not surpass the energy barrier returned to a native-like state, and the enzymatic activity was recovered. Those transitory changes may be explained by a reversible loss of protein structure.

In the present study, the diastase content decreased concomitant with heating at higher temperatures (Table 3). However, the stated differences were not confirmed statistically due to large variability. It is noteworthy that the lowest diastase content obtained for sample no.1 heated at 80 °C was almost two times higher than the regulatory value (not less than 8) set in Polish regulations (MARD 2004). Probably, this honey sample showed the natural poor level of amylase.

The results of the diastase content analysis indicated a high quality of all honey samples. Particularly, sample 5 showed the highest diastase activity and concurrent the lowest 5-HMF content (Table 4), which confirmed high quality of analysed honeys. Both HMF and diastase activity are the international parameters used to control the limit for thermal treatment of honey (Chua and Adnan 2014). The correlation between HMF content and diastase activity showed strong negative

relationship ($r = -0.605$). Therefore, those two parameters are inversely proportionate to each other.

The content of 5-HMF in the investigated raw rape honeys was much lower (about ten times) than the acceptable limit (not exceed 40 mg kg⁻¹) permitted by the Polish regulation (MARD 2004) (Table 4). However, such those results were in agreement with results of Wilczyńska (2012) which reported similar range of 5-HMF content (between 0.6 and 4 mg kg⁻¹). Obtained results indicated unequivocally, that honey samples were fresh and unheated earlier. According to Tosi et al. (2004) in fresh honeys there was practically no HMF, but it increases upon storage, depending on honey pH and on storage temperature. However, Chakir et al. (2011) indicated that honey samples collected from beekeepers in different regions of Morocco obtained values of HMF ranging from 0.1 to 53.4 mg kg⁻¹. Moreover, floral origin, chemical properties, such as pH, mineral content or total acidity also affects HMF content (Ajlouni and Sujirapinyokul 2010; Singh and Bath 1998).

Samples of honey heated at temperatures from 50 to 80 °C for 15 min contained higher amounts of 5-HMF in comparison with the control samples (unheated). However, those differences were insignificant ($p > 0.05$). The present study showed that heating of rape honey at 80 °C for 15 min resulted in the maximum increase of 5-HMF content, with an average of 1.9 mg kg⁻¹ (62 %), compared to unheated samples. An increase of 5-HMF content and a decrease of α -amylase activity during heating at higher temperatures (liquefying) were confirmed in a previous investigation conducted by Skowronek et al. (1994). The honey heated for 15 min at temperatures

Table 4 The 5-HMF content in rape honey at different temperatures (mg kg⁻¹)

Temperature	Sample no.							Mean value
	1	2	3	4	5	6	7	
20 °C (unheated)	2.16	2.29	6.51	2.87	0.9	2.84	3.93	3.07 ^a ± 1.77
50 °C	2.19	2.71	7.18	3.53	1.5	3.92	4.67	3.67 ^a ± 1.88
60 °C	2.58	3.53	8.73	3.34	1.08	4.03	3.98	3.90 ^a ± 2.36
70 °C	3.38	3.47	8.86	4.07	1.79	5.81	5.42	4.69 ^a ± 2.28
80 °C	3.80	3.64	9.15	5.42	1.78	5.01	6.02	4.97 ^a ± 2.31

Mean with different letters in the same column are significantly different: a-b – $p \leq 0.05$

from 60 to 80 °C showed higher 5-HMF content than control samples. Differences in the cited research reports were not greater than 10 mg kg⁻¹, excepting samples with a high initial content of this compound. On the other hand, the diastase activity decreased, but did not exceed the recommended level (not less than 8; MARD 2004). In contrast, Wilczyńska (2011) noticed that a heat treatment at 50, 60, 70 and 80° for 72 h in temperature-controlled oven resulted in a significant increase in 5-HMF content. The concentration of this compound in honey heated at 50 °C ranged from 1.1 to 70 mg kg⁻¹, from 105 to 551 mg kg⁻¹ at 70 °C, and from 226 to 960 mg kg⁻¹ at 80 °C. Bath and Singh (2001) studied the effect of microwave heating on HMF formation in two types of Indian honeys. These authors reported that the formation of HMF gradually increased with the increase in microwave power levels (70–280 W) and duration of heating (1–4 min). However, Tosi et al. (2004) revealed that the temperature beyond 80 °C until 140 °C, at very short times seemed not to cause deleterious effects on honey if measured by HMF and diastase activity modification.

Conclusion

The rape honey from the Lublin region showed low initial content of 5-HMF and the high diastase activity. Heating for 15 min at temperature range 50 °C to 80 °C did not significantly degrade the quality of the honey, but it slightly enhanced the process of 5-HMF formation and reduced the diastase activity. Moreover, heating of raw rape honey significantly increased lightness (L*), yellowness (b*), chroma (C*), hue (h°) values, but decreased redness (a*) and dynamic viscosity.

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