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Rheological investigation of synergistic interactions between galactomannans and non-pectic polysaccharide fraction from water soluble yellow mustard mucilage

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ABSTRACT

Synergistic interactions between galactomannans (GMs) and non-pectic polysaccharides (NPP) from yellow mustard mucilage were investigated in the present study. Structural analysis revealed that NPP was mainly composed of β-1,4-D glucosidic linkage. Four types of GMs, namely fenugreek gum (FG), guar gum (GG), tara gum (TG) and locust bean gum (LBG) with mannose to galactose ratios (M/G) of 1.2, 1.7, 3.0 and 3.7, respectively, were blended with NPP at various ratios. The viscoelastic properties of the mixtures were measured in order to evaluate the effects of GM/NPP blending ratio, M/G ratio, total polysaccharide concentration and pH on the synergistic interactions. Results revealed that at a total polysaccharide concentration of 0.5% (w/w), the highest synergism occurred at the GM/NPP blending ratio of 3/7 for all four types of GMs. The interaction between TG and NPP showed the highest synergy, followed by LBG/NPP, FG/NPP and GG/NPP. At a higher total polysaccharide concentration (1.0% w/w), the mixture of TG and NPP still exhibited the highest synergy, however, the order of synergy between NPP and other GMs was changed as FG/NPP, LBG/NPP and GG/NPP. At the total polysaccharide concentration of 0.5% and GM/NPP blending ratio of 3/7, neutral pH (pH 6.5) showed the strongest synergy compared to that at pH 2.0 and pH 12.0. The mechanism of the synergistic effects could be explained by a combination of segregative association model and junction zone model.

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1. Introduction

Recent interest in the mucilage of yellow mustard seed is attributed to its unique rheological behaviour in solutions/dispersions and its ability to interact with galactomannans (GMs) synergistically (Cui, Eskin, Biliaderis, & Mazza, 1995; Cui, Eskin, Wu, & Ding, 2006). Synergistic interactions between polysaccharides are commercially viable because it leads to the development of new textures (Morris, 1990, chap. 8) and reduced concentration of materials, hence save costs. Synergism between yellow mustard mucilage and GMs was first reported by Weber, Taillie, and Stauffer (1974). Cui et al. (1995) further identified that the non-pectic polysaccharide (NPP) fraction from water soluble yellow mustard mucilage consisting of β-1,4-linked D-glucan backbone chain was the responsible component for the synergistic interactions with GM. NPP has a similar structure to xanthan gum, which interacts with GMs synergistically. Among the many mechanisms of polysaccharide synergistic interactions as summarized by Cui (2001) and Cui et al. (2006), they proposed the "junction zone" model (cooperative binding) for the synergistic interactions between GM and NPP assuming that the unsubstituted mannan backbone can form junction zones with the smooth region of the β-1,4 glucosyl backbone. However, due to the limited amount of this active component (Wu, Cui, Eskin, & Goff, 2009) in yellow mustard mucilage and the difficulty in obtaining it in a large quantity, more studies need to be carried out to clearly elucidate the synergistic mechanism.

There are several factors that may affect the magnitude of synergy in mixed polysaccharide systems, including molecular weight and intrinsic viscosity (Fernandes, Gonçalves, & Doublier, 1991), salt concentration (Khouryieh, Herald, Aramouni, & Alavi, 2007; Schorsch, Garnier, & Doublier, 1997), M/G ratio and fine structures (Dea & Clark, 1986; Fernandes, 1995), polysaccharide–polysaccharide blending ratio (Cui et al., 1995), total polysaccharide concentration (Bresolin et al., 1997; Mannion et al., 1992), temperature (Mannion et al., 1992; Zhan, Brownsey, Ridout, & Morris, 1993) and pH (Morris & Foster, 1994; Ross-Murphy, Morris, & Morris, 1983; Tako, 1991; Whitney, Brigham, Darke, Reid, & Gidley, 1998). In the present study, NPP was isolated as described by Wu et al. (2009), and blended with four types of GMs, namely fenugreek gum (FG), guar gum (GG), tara gum (TG) and locust bean gum (LBG). Rheological studies were carried out to investigate how the factors including GM/NPP blending ratio, M/G ratio, total

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Table 1
Charateristics of the four galactomannans.

Sample	Fenugreek gum	Guar gum	Tara gum	Locust bean gum
Moisture%	10.49 ± 0.16	10.16 ± 0.01	12.37 ± 0.05	9.77 ± 0.16
Protein%	2.62 ± 0.13	3.46 ± 0.06	0.707 ± 0.02	4.57 ± 0.37
Ash%	1.50 ± 0.03	0.72 ± 0.02	0.77 ± 0.01	1.04 ± 0.02
M/G*	1.2 ± 0.0	1.7 ± 0.0	3.0 ± 0.1	3.7 ± 0.0
Intrinsic viscosity (dl/g)	15.10 ± 0.14	15.80 ± 0.28	14.55 ± 0.07	14.20 ± 0.28
Mv (X10^6)**	3.23 ± 0.03	2.91 ± 0.05	2.23 ± 0.01	2.08 ± 0.04

$n = 2$.

* M/G, mannose to galactose ratio.

** Mv, viscosity average molecular weight.

polysaccharide concentration and pH affect synergistic interactions.

2. Materials and methods

2.1. Materials

Locust bean gum (LBG) (from *Ceratonia siliqua* seeds) and guar gum (GG) were purchased from Sigma–Aldrich, Inc. (St. Louis, USA). Tara gum (TG) was purchased from TIC Gums (Belcamp, USA). Fenugreek gum (FG) was provided by Emerald Seeds (Canafen Gum®). Characteristics of the four galactomannans were determined as described by Wu, Cui, Eskin, and Goff (submitted for publication) and are presented in Table 1. NPP was obtained as described by Wu et al. (2009). All sample solutions were prepared based on dry matter basis. Weighed samples were dispersed in distilled water with constant stirring at room temperature for 0.5 h, and dissolved in 80 °C water bath for 1h, then cooled to room temperature for 0.5 h with constant stirring. All aqueous sample solutions were prepared in the same way unless otherwise specified.

2.2. Methods

The effects of GM/NPP ratio, M/G ratio, total polysaccharide concentration and pH were investigated in the present study. At a total polysaccharide concentration of 0.5% (w/w), NPP was mixed with FG, GG, TG and LBG at the ratios of 10/0, 9/1, 7/3, 5/5, 3/7, 1/9 and 0/10, respectively. At a higher concentration (1% w/w), GMs and NPP were blended at a fixed GM/NPP ratio (3/7). The effect of pH was investigated by dissolving the polysaccharide mixtures at fixed GM/NPP ratio (3/7) at the total polymer concentration of 0.5% with pH adjusted to 2.0, 6.5 and 12.0, respectively.

Viscoelastic properties were measured on a strain controlled ARES Rheometer (TA Instruments, New Castle, DE, USA) on a parallel-plate geometry (50 mm) at gap size of 0.7 mm and strain of 1%. Storage modulus (G′), loss modulus (G″) and phase angle (δ) were determined at frequencies from 0.008 to 16 Hz at 25 °C.

3. Results and discussions

3.1. Effect of GM/NPP blending ratio

At a total polysaccharide concentration of 0.5% (w/w), four GMs were blended with NPP at 7 ratios. Fig. 1 presents frequency sweeps of all combinations except the four pure GM solutions (GM/NPP ratio of 10/0). At GM/NPP ratio of 9/1, only the mixture of LBG/NPP formed a gel. At the rest ratios all the samples showed a weak gel property, with G′ constantly higher than G″, but, both G′ and G″ are frequency dependent. Storage and loss modulus (G′ and G″) at frequency 0.8 (Hz) at the highest synergy ratio (3/7) are given in Table 2. Fig. 2 presents the results of storage modulus (G′) at frequency 0.8 (Hz). The results showed that three GM/NPP blending ratios, 5/5, 3/7 and 1/9, exhibited synergistic interaction since their G′ values were consistently higher than that of the two polymers in isolate (with GM/NPP ratios of 10/0 and 0/10). The G′ values of the GM/NPP ratios of 9/1 and 7/3 are in between those of 10/0 and 0/10, with the exception of LBG/NPP mixture, which also showed synergistic behaviour at the ratio of 7/3 with G′ higher than that of NPP solution (ratio of 0/10). The largest magnitude of synergy occurred at 3/7, followed by 5/5 and 1/9. This pattern applied to all the four GM/NPP mixtures regardless of M/G ratio. Fernandes (1995) investigated synergistic interactions between xanthan gum and different LBG fractions with various M/G ratios

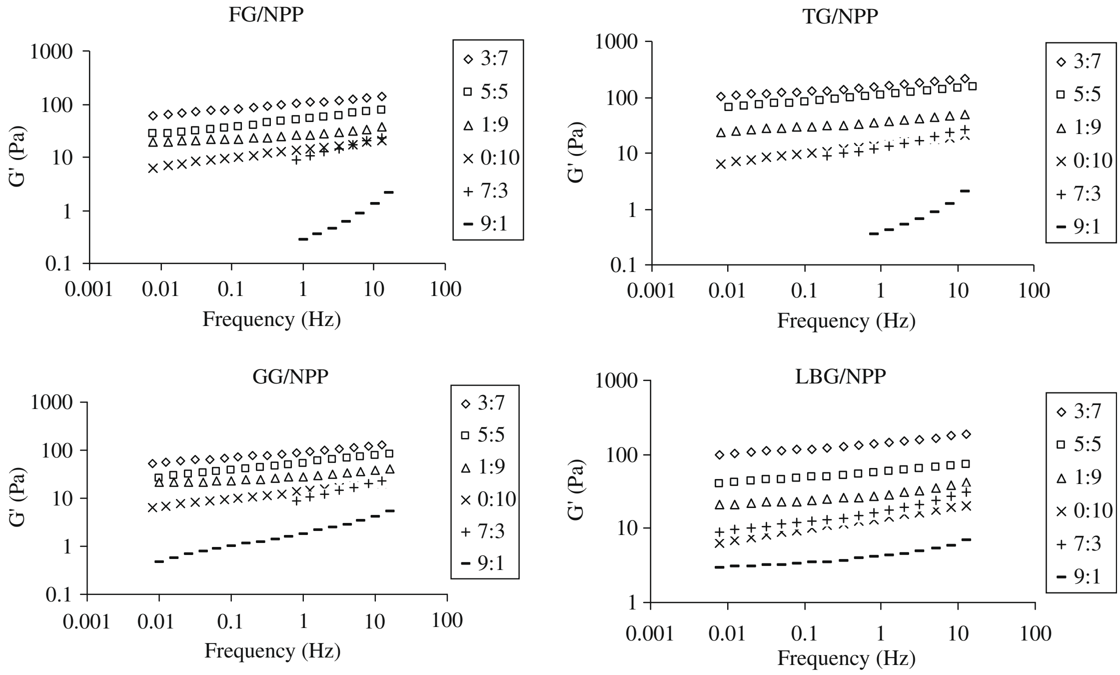

Fig. 1. Frequency sweep of GM/NPP mixtures at total polysaccharide concentration of 0.5%. GM/NPP: galactomannan to non-pectic polysaccharide blending ratio. ; FG: fenugreek gum; GG: guar gum; TG: tara gum; LBG: locust bean gum.

Table 2
Storage and loss modulus (G′ and G″) of 0.5% GM/NPP mixed gel at blending ratio of 3/7, frequency of 0.8 Hz.

Modulus (Pa)	Fenugreek gum	Guar gum	Tara gum	Locust bean gum
G′	100.63	89.29	151.62	138.62
G″	17.17	16.79	25.68	21.14

and revealed that the highest synergy occurred at 1:1 xanthan/LBG ratio at total polymer concentration of 1% for all LBG fractions regardless of M/G ratio. A similar study carried out by Mannion et al. (1992) obtained the same conclusion that the magnitude of synergy showed little dependence on the M/G ratio of the fraction.

The "junction zone" model has been generally accepted to explain synergistic interactions between GMs and cellulose-like molecules such as xanthan (Dea & Morrisson, 1975; Morris, 1990, chap. 8). Since NPP was proved possessing blocks of unsubstituted β-1,4 linked D-glucose linkages (Cui et al., 1995; Wu et al., 2009), junction zone model was adopted for the synergistic interactions between NPP and GMs (Cui et al., 1995). Nevertheless, the extension of this model to the highly substituted mannon backbone such as FG and GG was restricted due to the limited availability of unsubstituted mannon binding sites. Junction zone model is also limited to explain the phenomenon that the best synergy occurred at the GM/NPP blending ratio of 3/7 for all GMs even when the G/M ratio and the available "binding sites" varied greatly.

Another mechanism, segregative associations in single phase, as proposed by Penroj, Mitchell, Hill, and Ganjanagunchorn (2005), could be an alternative mechanism to explain the present data. The segregative association mechanism was based on a study of synergistic interactions between kappa carrageenan and konjac glucomannan: the synergistic system was a consequence of segregative association in single phase. They suggested that kappa carrageenan can promote the gelation of konjac glucomannan by reduce thermodynamically unfavourable contacts between the two polymers. In addition, association between the two polymers may also exist. Adding one non-gelling polysaccharide to promote the gelation of a second polymer was also reported by Morris and coworkers, who investigated calcium pectinate network structure induced by the addition of oxidized starch (Picout, Richardson, & Morris, 2000b), potato maltodextrin (Picout, Richardson, Rolin, Abeysekera, & Morris, 2000a) and GMs (Giannouli, Richardson, & Morris, 2004). They postulated that synergistic interactions induced by segregated association occurred regardless of the structural differences of the polymers being added into the system.

Hefford (1984) reported that the behaviour of mixed polymers in solution was clearly determined by a delicate balance of forces. After studying several pairs of polymers in aqueous systems, he found that hydrogen bonding between different polymers was the cause for the polymers being mixed rather than phase separated. The polymer interactions in the binary systems in the present study are mainly through hydrogen bonding. If NPP and GM can interact *via* hydrogen bonding, the phase separation would be less possible. The order of the associations, either intra or inter, may depend on the thermodynamic behaviours between the molecules. Tolstoguzov (2002) stated that biopolymers are limitedly co-soluble even if they are slightly different in composition, structure and /or conformation. Thermodynamically molecules favour interactions between the same molecules. There could be two kinds of associations co-exist in the current mixed gel system, self-associations and cooperative junction zones. We assume that the primary association is probably the self association, and the secondary association is the cooperative junction zone. If the cooperative junction zones are not feasible, for example, no binding sites available, phase separation might be the consequence. However, the occurrence of phase separation may depend on the mobility of the molecules in the solution. It seems difficult to happen especially in high viscous solutions. This opinion was supported by several published reports. Kohyama, Iida, and Nishinari (1993) and Kohyama, Sano, and Nishinari (1996) proposed that two crystalline regions co-exist in the mixed gel of konjac glucomannan and kappa carrageenan, one region consists of kappa karrageenan alone and the other region consists of konjac glucomannan and kappa carrageenan. The latter junction zone was weaker than the former. Garcia and Andrade (1997) studied the interaction between agarose and GG. 3-D network was observed in agarose domained area in the mixed gel while GG participated in the network formation by interacting with the surface of the agarose helix.

At present study, we propose that there are two possible mechanisms involved in the synergistic interactions: the first one is the associative interactions or formation of junction zones between the NPP backbone and the unsubstituted region of the GM backbone chains; the second mechanism is the segregative associations in single phase with NPP network dominated in the mixed system while GM distributed in the same phase with possible associations with NPP as well.

3.2. Effect of M/G ratio

At a total polymer concentration of 0.5% (Fig. 2), TG formed the strongest gel with NPP at all blending ratios tested, followed by LBG, FG and GG. This pattern applied to all GM/NPP binary solutions.

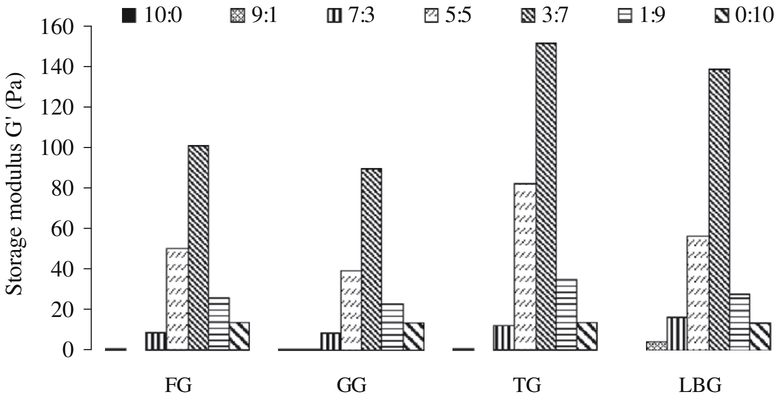

Fig. 2. Comparison of synergistic effects between galactomannan and non-pectic polysaccharides from yellow mustard mucilage with various blending ratios at total polymer concentration of 0.5%, frequency of 0.8 Hz. FG, fenugreek gum; GG, guar gum; TG, tara gum; LBG, locust bean gum.

As discussed previously, the addition of a certain amount of GMs, regardless of their M/G ratios and the fine structures, can enhance the gelling network. However, the magnitude of synergy may depend on the structural differences of the GMs. If the non-substituted GM backbone can associate with NPP by forming junction zones, the magnitude of the synergy might be increased. Therefore, as observed in the present study, TG and LBG exhibited stronger synergy with NPP, while FG and GG exhibited lower synergy (Fig. 2).

It is interesting to see that TG showed the best synergy. Table 1 indicates that intrinsic viscosity and viscosity average molecular weight (M_v) of TG was greater than LBG, while the M/G ratio of LBG was 3.7, compared to 3.0 for TG. Consequently, there should be more unsubstituted regions in LBG than in TG. Although TG is a relatively purer sample among the four with less protein content (Table 1), Bresolin, Milas, Rinaudo, Reicher, and Ganter (1999) claimed that the difference in gelling temperature and G′ of xanthan/GM mixtures cannot be attributed to a difference in protein content. Therefore, the improved synergy of TG compared to LBG should be attributed to the fine structure as indicated by Dea and Clark (1986), because the galactose distribution pattern on the mannan backbone will give a difference on the length and frequency of the unsubstituted region.

3.3. Effect of total polymer concentration

It was noticed that the order of synergy at the two polymer concentrations, 0.5% and 1.0%, switched slightly between FG and LBG (Fig. 3). Among the two GMs, FG exhibited a better synergy with NPP at the higher concentration; while at the lower concentration, LBG showed a better synergy (Fig. 3). It is obvious that with increased concentration, G′ increased accordingly for all four blends. The degree of increase varied, as shown in Fig. 3, with FG exhibited the largest increase in G′, followed by TG, LBG and GG. This can possibly be caused by two characteristics of FG: water binding capacity and conformation.

Water binding capacity may play a role in the binary system, especially with increased total polymer concentration. Sudhakar, Singhal, and Kulkarni (1995) studied the mixed polymer system with starch (5%) and GMs (0.1%, 0.2% and 0.3%) and found GG formed a superior synergy with starch compared to LBG. The authors suggested that this could be due to the greater hydration capacity of GG. In the present study, larger amount of water was imbibed within the NPP network when the polymer concentration was increased. The consequence of binding more water by FG, due to its larger water binding capacity compared to the other three GMs, is to induce a relatively higher concentration of NPP, thus strengthen the gelling network. This might explain the large increase in storage modulus of FG/NPP binary system.

Conformation of fully substituted GM in the binary system may also affect the synergy. A conformational study on GM with M/G ratio of 1/1 by using modelling approach revealed that introduction of the galactosyl residues on the mannan backbone could lower the chain dimension by the attractive interactions between the galactosyl substituents and the remaining part of the molecule (Petkowicz et al., 1999). This compact conformation may help with the synergistic interactions somehow. McCleary (1979) proposed that in GM with fully substituted galactose, the galactose might align on one side of the chain leaving the other side as the "smooth region" to interact with the cellulose-like chain. All these hypotheses should be further verified in future studies.

3.4. Effect of pH

The effect of pH conditions on synergistic interactions was investigated at a fixed GM/NPP blending ratio of 3/7 and a fixed total polysaccharide concentration of 0.5% (w/w). Three pH conditions, 2.0, 6.5 and 12.0, were investigated. Results are shown in Fig. 4. The order of magnitude of synergy followed the order of: pH 6.5 > 2.0 > 12.0. This order applied to all four GM/NPP mixtures.

Our previous study on NPP (Wu et al., 2009) showed that acidic or alkaline pHs increased the gelling property of NPP, indicating an enforced gelling network within NPP molecules. Once the NPP–NPP network is enhanced, the GM–NPP interaction is depressed, and as a consequence, synergistic interactions will decrease. When pH increases, solubility of GM molecules also increases which may reduce the GM–NPP interactions. The GM molecules could also be degraded under acidic or alkaline condition, hence led to the decreased G′ compared to the neutral condition.

4. Conclusions

The authors propose that two mechanisms of synergistic interactions co-exist in the GM/NPP binary system. One of the mechanisms is the well accepted "junction zone" model, suggesting that the unsubstituted mannan chain of GM associates with the cellulose-like backbone of NPP *via* hydrogen bonding to form junction zones. The second mechanism is that the polymers segregatively associated in single phase with the possible associations between the two different polymers. It is arguable on how the fully substituted mannan chain interacts with the cellulose-like chain of NPP, especially in the increased polymer concentration. One assumption is that the α-1,6-linked D-galactose fully or alternatively aligned on one side of the chain, leave the "smooth" side at-

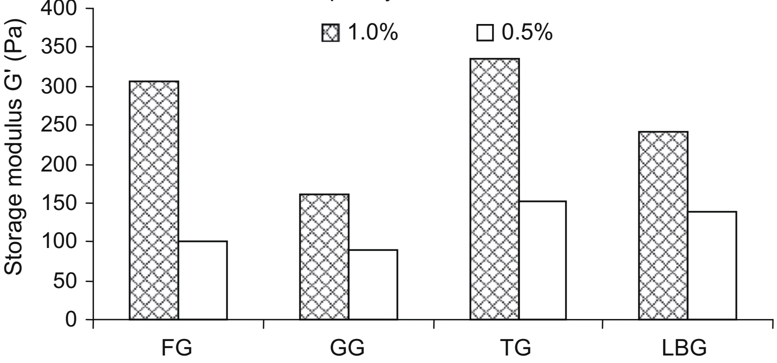

Fig. 3. Effect of total polymer concentration on synergistic interactions between galactomannan and non-pectic polysaccharides from yellow mustard mucilage at GM/NPP blending ratio of 3/7, total polymer concentration of 0.5% and 1.0%, respectively. GM/NPP: galactose to non-pectic polysaccharide blending ratio.

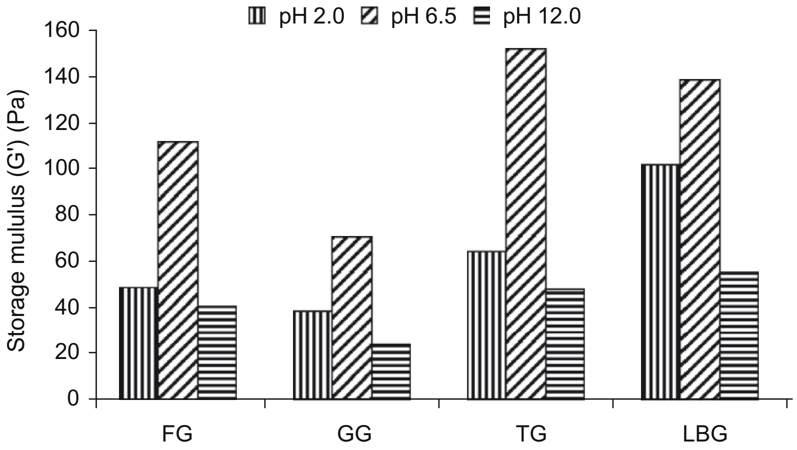

Fig. 4. pH effect on synergistic interaction between galactomannans and non-pectic polysaccharides.

tached to the cellulose-like backbone. This kind of conformation needs to be further verified using molecular scale techniques.

This study further confirmed that not only the M/G ratio of GM but also the fine structures of GM molecules determine the magnitude of synergistic interactions with NPP. Due to the difficulty in obtaining the information on fine structures, it makes the elaboration of the synergy mechanism more complicated. However, the present study is able to elucidate the synergistic effects between a certain GM and NPP. The overall magnitude of the synergistic behaviour can be controlled by adjusting GM/NPP blending ratio, total polymer concentration and pH conditions.

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