Chemical Interesterification with Regioselectivity for Edible Oils[1]

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Chemical interesterification reaction conditions that provide regioselectivity regarding fatty acid positions in triacylglycerol have been investigated. Sodium methoxide-catalyzed ester interchange between soybean oil and methyl stearate was performed in hexane at low reaction temperature, *i.e.*, 30 to 60°C. The results showed regioselectivity was obtained at 30°C. The ester interchange at 1,3-carbons progressed 1.7 times faster than at 2-carbon of the glycerol moiety of triacylglycerol at 24 h. Preheating of the mixture of reactant and catalyst at 60°C for 15 min promoted catalyst activation to accelerate the interesterification while maintaining regioselectivity. This method is believed to be feasible for modification of edible fats and oils.

KEY WORDS: Interesterification, methyl stearate, regioselectivity, sodium methoxide, soybean oil.

Interesterification of edible oils is an important process for the modification of physical and functional properties, as are hydrogenation and fractionation (1). Interesterification alone and in combination with other processes extends the utility of edible oils, and the modified oils are extensively used in a wide variety of foods (2–4). There have been a number of reports published concerning interesterification, which discussed random and directed ester interchange reactions, the reaction mechanism and the characteristics of several catalysts such as sodium potassium alloy, metallic sodium, sodium methoxide and sodium hydroxide (5–7). Others have reported the effect of fatty acid rearrangement by random and directed interesterification on physical characteristics such as melting point, dropping point, solid fat content (or index) and crystalline form (2,7–13) and on functional characteristics such as oxidative stability (14,15). There is a consensus that, contrary to lipase-directed reaction, chemical interesterification progresses randomly with no regioselectivity (positional specificity) on the carbons of the glycerol moiety of the triacylglycerol (TAG) (1,6,16). Much research on ester interchange by lipases has been conducted to produce useful TAG mixtures, for example cocoa butter substitutes, which are considered impossible to obtain by chemical interesterification (2,17–19). However, these researchers found that interesterification by a chemical catalyst still has some advantages such as the ease of processing due to the reactants being in one phase, easier separation of TAG from products mixture and lower catalyst cost.

Interesterification of edible oil usually involves at least two oils that have different fatty acid compositions. Soybean oil and methyl stearate were employed as reactants to simplify the interpretation of the experimental results in this study. Reaction conditions that provide regioselectivity regarding the fatty acid positions in the TAG were studied for chemical interesterification of soybean and methyl stearate, catalyzed by sodium methoxide in an organic solvent.

EXPERIMENTAL PROCEDURES

Materials. Soybean oil was obtained from a commercial source. Methyl stearate and sodium methoxide catalyst were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). The solid-phase extraction columns (6.5 mL vol, loaded with 2000 mg silica) used for isolation of TAG from reaction products were purchased from Baxter Health Care (Muskegon, MI). The solid-phase extraction columns ("Bond Elut", 3 mL vol, loaded with 200 mg silica) used for resolution of lipolysis mixtures were purchased from Varian Co. (Harbor City, CA). Pancreatic lipase (EC 3.1.1.3, type II, crude from porcine pancrease) was obtained from Sigma Chemical Co. (St. Louis, MO). Potassium hydroxide used as catalyst for transmethylation of fatty acids of TAG or 2-monoacylglycerol was purchased from Aldrich Chemical Co., Inc. All organic solvents used in this research were high-performance liquid chromatography-grade.

Interesterification. Sodium methoxide-catalyzed ester interchange was carried out in 500 mL hexane contained in a temperature-controlled bench-scale batch reactor (1 L, Parr Instrument Co., Moline, IL). The reactants, 20 g of soybean oil and 80 g of methyl stearate, were dried in the reactor at 60°C under vacuum for 30 min. After temperature of the reactants had reached the reaction temperature, sodium methoxide dispersed in 500 mL hexane was added to the reactants, and then the reactor was purged with nitrogen gas at 80 psi. Samples (10 mL) were withdrawn at timed intervals after the reaction temperature was obtained. The samples were diluted with 4 mL diethyl ether, washed with distilled water three times and dried by anhydrous sodium sulfate. Interesterified TAG was isolated from each sample by solid-phase extraction with solvent elution, as follows: 25 mL diethyl ether/hexane (2:98, vol/vol), fraction 1, fatty acid methyl ester (FAME); 20 mL, diethyl ether/hexane (10:90, vol/vol), fraction 2, TAG; 15 mL, methanol, fraction 3, mono- and diacylglycerol. Isolation of each fraction was confirmed by thin-layer chromatography.

Analysis. The progress of the reaction was monitored by following the alteration of fatty acid composition of TAG (fraction 2) by 1,3-specific lipolysis followed by gas-liquid chromatography (GLC), and FAME analysis (fraction 1) by GLC. GLC analysis was performed with a Hewlett-Packard model 5710 A gas chromatograph equipped with a flame ionization detector (Palo Alto, CA). The glass column (2.5 × 183 cm) packed with 10% SP 2330 on 100/120 mesh Chromosorb W, AW (Supelco Inc., Bellefonte, PA), was operated at 160°C with a helium carrier gas flow rate of 20 mL/min. Sample injector and detector were at 250°C. Methyl ester identification and quantitation were calibrated with a Nu-Chek-Prep soybean

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methyl ester standard 15-A (Nu-Chek-Prep Inc., Elysian, MN). Lipolysis of TAG (fraction 2) to obtain 2-monoacylglycerol to determine the fatty acid composition at the 2-position of the TAG was by the method of Neff *et al.* (20,21). 2-Monoacylglycerol, obtained by lipolysis of TAG, was isolated by solid-phase extraction, then transmethylated by 0.5 M potassium hydroxide in methanol at 50°C for 15 min. FAMEs were extracted from the transmethylation products with petroleum ether/diethyl ether, then dried, and dissolved in hexane for GLC analysis.

RESULTS AND DISCUSSION

Stearic acid concentrations in TAG interesterified with methyl stearate at 40°C for 90 min with various amounts of sodium methoxide are presented in Table 1. The stearic acid concentration in the interesterified TAG was not increased significantly in the presence of 4.0 wt% (wt:wt of reactants) or less of sodium methoxide. Sodium methoxide at 10 wt% improved ester interchange to increase stearic acid concentration in TAG. Although sodium methoxide is usually used at low levels such as 0.2–2 wt%, 10 wt% of the catalyst was required under these reaction conditions. The reactants were dissolved, and the catalyst was dispersed in hexane in the reactor, so the contact between the reactants and the catalyst was less in this reaction compared to reaction without hexane.

The dependency of reaction rate on temperature was studied at 30, 40, 50 and 60°C for 6 h for the reactants in the presence of 10 wt% of sodium methoxide. The further the ester interchange progressed, the more methyl stearate is consumed and the more stearic acid is incorporated into TAG. The methyl stearate concentration decreased with increasing reaction time at each reaction temperature, as shown in Figure 1. The higher the reaction temperature, the greater the rate of methyl stearate consumption. After 2 h at 60°C, methyl stearate concentration did not decrease further, indicating that the reaction was complete.

The yield of TAG recovered from the products at 6 h is given in Table 2. The TAG yield was determined by weighing fractions from solid-phase extraction. The higher the reaction temperature in the presence of 10 wt% sodium methoxide, the lower the TAG yield due to the formation of di- and monoacylglycerols, which was observed as an increased weight percentage of fraction 3 from solid-phase extraction. The lower reaction temperature improved TAG yield.

The intermolecular ester interchange between TAG and methyl stearate took place so rapidly at 60°C that the introduction of stearic acid at both 1,3- and 2-positions

TABLE 1

Stearic Acid Concentration of Interesterified Oil at 40°C for 90 min

$NaOCH_3$ (wt%)	Stearic acid (%)
Unreacted	3.1
0.1	4.4
0.4	4.2
2.0	5.8
4.0	5.5
10.0	10.1

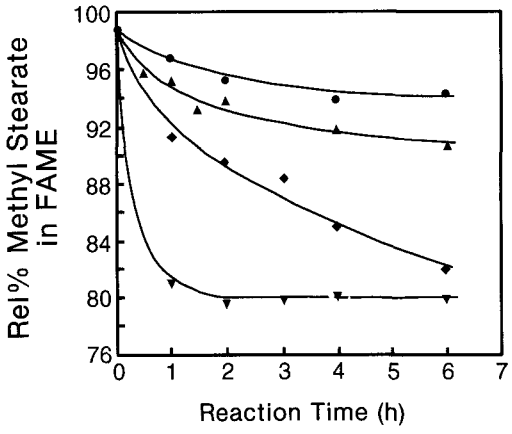

FIG. 1. Reduction of stearic acid from methyl stearate during the reactions at 30–60°C. Inverted triangle, 60°C; closed diamond, 50°C; triangle, 40°C; closed circle, 30°C. FAME, fatty acid methyl esters.

TABLE 2

Triacylglycerol (TAG) Yield of Interesterified Oil at 30–60°C for 6 h

Reaction temperature (°C)	TAG yield (wt%)
30	88.7
40	83.4
50	76.1
60	58.6

reached approximately 65% at *ca.* 2 h, as shown in Figure 2. The stearic acid at 1,3-positions decreased gradually from 2 h to 4 h but increased at the 2-position. This indicates that the stearic acid in all positions was randomized to reach equilibrium at 4 h by intramolecular reaction. Stearic acid concentrations at 50°C increased at the same rate at both 1,3- and 2-positions, and the rate was slower than those at 60°C (Fig. 2). The reaction was accelerated after 3 h. At 40°C (Fig. 3), stearic acid concentration at both 1,3-and 2-positions increased linearly at relatively slow rates compared with those at 50 and 60°C. At 30°C (Fig. 3), slow parallel increases of the

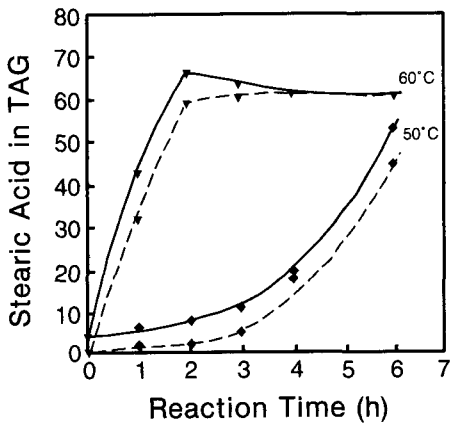

FIG. 2. Stearic acid in triacylglycerol (TAG) at 60 and 50°C. Symbols are the same as in Figure 1; —, 1,3-positions; – –, 2-position.

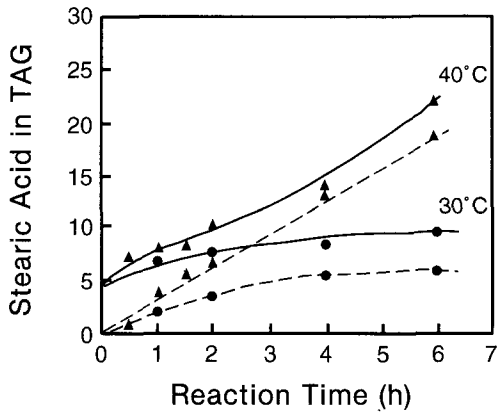

FIG. 3. Stearic acid in triacylglycerol (TAG) at 40 and 30°C. Symbols are the same as in Figure 1; —, 1,3-positions; – –, 2-position.

stearic acid concentration at 1,3- and 2-positions were observed.

The difference of palmitic acid concentrations between 1,3- and 2-positions with reaction time is presented in Figure 4. Palmitic acid concentration at 1,3-positions decreased as the concentration at the 2-position increased, which indicated intramolecular ester interchange, because no palmitic acid was supplied to the reaction as methyl ester. Intramolecular reactions were so rapid at 60°C that palmitic acid was randomized in the TAG at *ca.* 3 h. Reactions at 50 and 40°C had similar profiles of palmitic acid randomization, such that the positional difference of palmitic acid concentration disappeared by 6 h. The 30°C reaction was so slow that palmitic acid was not randomized within 6 h.

These results indicate that the higher the reaction temperature, the faster the ester interchange progressed. However, the intramolecular reaction was also promoted more at the higher temperature. The 30°C reaction was suitable to depress the intramolecular ester interchange to obtain regioselectivity, although the ester interchange progresses slowly.

The regioselectivity of the 30°C reaction was confirmed by interesterification of soybean oil with methyl stearate for 24 h. No marked alteration in palmitic acid concentrations occurred after 6 h, as shown in Figure 5. The intramolecular ester interchange remained depressed for 24 h. The stearic acid concentrations at 1,3- and 2-positions increased slowly until 12 h, as shown in Figure 5. After 12 h, the increase of stearic acid concentration at 1,3-positions was accelerated, while the concentration at 2-position stabilized. The intermolecular ester interchange was apparently dominant after 12 h. Regioselectivity regarding the fatty acid position in triacylglycerol was evaluated by the ratio defined as follows,

$$\Delta S_{1,3}/\Delta S_2 = \frac{S_{1,3}{}^t - S_{1,3}{}^0}{S_2{}^t - S_2{}^0} \quad [1]$$

where S is the concentration of stearic acid, 1,3 and 2 are the fatty acid positions in the TAG, t and 0 mean reaction time. The ratio, $\Delta S_{1,3}/\Delta S_2$, is theoretically 1.0 with a nonregioselective reaction, however, the experimentally determined ratio ranged from 0.7 to 1, which results from the randomization in which the stearic acid at 1,3-positions migrated to the 2-position. Plots of $\Delta S_{1,3}/\Delta S_2$ *vs.* ΔS_T for reactions at 30, 40 and 50°C are given in Figure 6. S_T is total stearic acid concentration so that ΔS_T showed the progress of the reaction. The values of $\Delta S_{1,3}/\Delta S_2$ at 60°C were 0.9 to 1.0 (not included). In the initial stage of the reaction at 50 and 40°C, $\Delta S_{1,3}/\Delta S_2$ were as high as 1.7 to 2, which indicated regioselectivity. But as ΔS_T increased, the regioselectivity disappeared. At 30°C, the minimum value of $\Delta S_{1,3}/\Delta S_2$ occurred at approximately $\Delta S_T = 4$, followed by an increase of $\Delta S_{1,3}/\Delta S_2$ to 1.7 when ΔS_T was 12.4 at 24 h. Thus, the interesterification progressed with regioselectivity at 30°C for 24 h.

Several organic solvents, in addition to hexane, were investigated to enhance the progress of the regioselective reaction at 30°C. Ten vol% ethanol or acetone added to

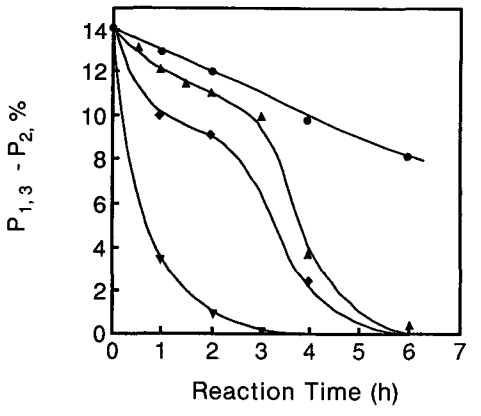

FIG. 4. The difference of palmitic acid at 1,3- and 2-positions. Symbols are the same as in Figure 1. $P_{1,3}$, palmitic acid concentration at 1,3-positions; P_2, palmitic acid concentration at 2-position.

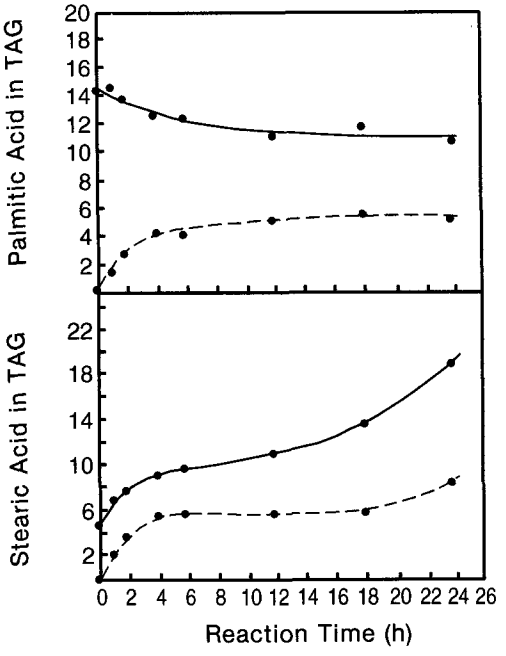

FIG. 5. Alteration of palmitic and stearic acid in triacylglycerol (TAG) at 30°C for 24 h. —, 1,3-positions; – –, 2-position.

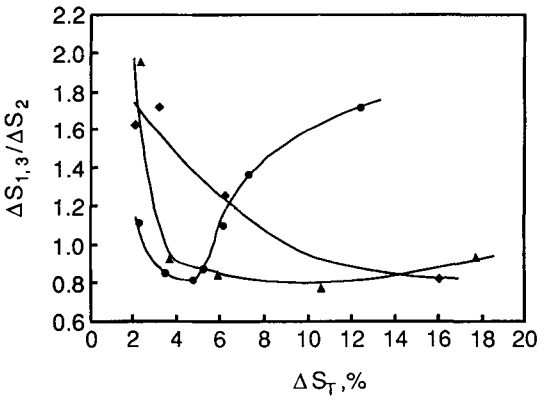

FIG. 6. $\Delta S_{1,3}/\Delta S_2$ *vs.* ΔS_T at 30–50°C. Symbols are the same as in Figure 1.

hexane converted TAG to fatty acid alcohol ester within 1 h. Thus ethanol and acetone promoted an undesirable reaction. In diethyl ether and hexane (10:90, vol/vol), a larger amount of methyl stearate was consumed than without diethyl ether. The intramolecular ester interchange was not influenced by 10 vol% diethyl ether. However, diethyl ether promoted the increase of stearic acid concentration at the 2-position, and the reaction in diethyl ether and hexane had less regioselectivity. Of the solvents investigated, only hexane enhanced the regioselective reaction.

The catalytic activity of sodium methoxide itself is low and intermediates, like sodium glycerate formed during the reaction, may possibly work as the active catalyst (6,7). The strong dependency of reaction rate on reaction temperature was discovered in this study. The higher temperature gave higher catalytic activity. It was considered that the activation of the catalyst depended on the temperature and the catalyst was activated sooner at the higher temperature, since the acceleration of the reaction rate was observed more at 50 and 60°C than at 30°C (Figs. 2 and 5). The thermal dependency of catalyst activation was evaluated by preheating before the reaction at 30°C. The catalyst was added to the reactant, the mixture was heated rapidly up to 60°C, maintained for 15 min and then cooled to 30°C. The effects of preheating on the rate of methyl stearate reduction and the percentages of palmitic and stearic acids in TAG were determined (Fig. 7). The dashed line before 0 h of reaction time indicates the alteration of concentration during preheating. The greater amount of methyl stearate was consumed in the reaction at 30°C with preheating than without preheating (Fig. 7a). The difference of palmitic acid concentrations between 1,3- and 2-positions decreased by approximately 5% during the preheating process, while the change of concentrations was not significant during the reaction at 30°C (Fig. 7b). The intramolecular ester interchange was still depressed at 30°C. Preheating did not influence the stearic acid concentrations until 6 h (Fig. 7c), after which concentrations at 1,3- and 2-positions increased remarkably and consequently $\Delta S_{1,3}/\Delta S_2$ was 1.7 at 33.6 of ΔS_T (Fig. 8). Preheating at 60°C for 15 min was so effective that the reaction rate was accelerated while maintaining regioselectivity.

In this study, ester interchange between soybean oil and methyl stearate was conducted to investigate the reaction conditions that provide regioselectivity regarding fatty

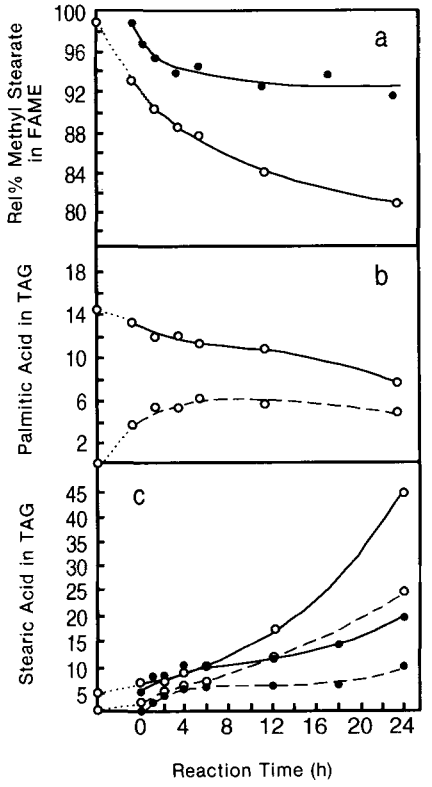

FIG. 7. Effect of preheating on interesterification reaction. a, reduction of stearic acid from methyl stearate; b, alteration of palmitic acid in triacylglycerol; c, alteration of stearic acid; ●, without preheating; ○, with preheating; —, 1,3-positions; – –, 2-position. FAME, fatty acid methyl esters; TAG, triacylglycerols.

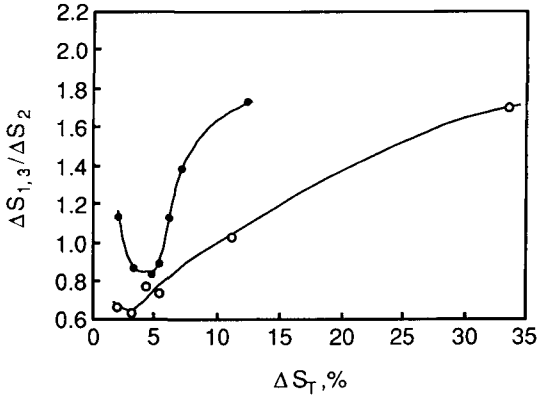

FIG. 8. Effect of preheating on $\Delta S_{1,3}/\Delta S_2$ *vs.* ΔS_T. Symbols are the same as in Figure 7.

acid positions in TAG. When two oils are interesterified in this manner, instead of a oil and a FAME, the conditions (*e.g.*, reaction temperature, catalyst concentration or organic solvent to be used) to obtain regioselectivity may depend on characteristics of those two oils used as reactants, such as melting points, solubilities in hexane.

The regioselective interesterification reaction reported here, including the catalyst activation process, appears to be feasible for modification of vegetable oils for enhanced utilization as edible fats and oils.

REFERENCES

1. Hustedt, H.H., *J. Am. Oil Chem. Soc. 53*:390 (1976).
2. Young, F.V.K., *Ibid. 62*:378 (1985).
3. Wiedermann, L.H., *Ibid. 55*:823 (1978).
4. Sonntag, N.O.V., in *Bailey's Industrial Oil and Fat Products*, Vol.II, 4th edn., edited by D. Swern, John Wiley and Sons, New York, 1982, p. 147.
5. Sonntag, N.O.V., *Ibid.*, p. 153.
6. Going, L.H., *J. Am. Oil Chem. Soc. 44*:414A (1967).
7. Sreenivasan, B., *Ibid. 55*:798 (1978).
8. Laning, S.J., *Ibid. 62*:400 (1985).
9. List, G.R., E.A. Emken, W.F. Kwolek, T.D. Simpson and H.J. Dutton, *Ibid. 54*:408 (1977).
10. Eckey, E.W., *Industrial and Engineering Chemistry 40*:1184 (1948).
11. Chobanov, D.G., and M.R. Topalova, *J. Am. Oil Chem. Soc. 56*:581 (1979).
12. Sreenivasan, B., U.S. Patent 3,748,348 (1973).
13. Dominick, W.E., U.S. Patent 2,625,485 (1953).
14. Tautorus, C.L., and A.R. McCurdy, *J. Am. Oil Chem. Soc. 67*:525 (1990).
15. Wada, S., and C. Koizumi, *Yukagaku 35*:549 (1986).
16. Chobanov, D., and R. Chobanova, *J. Am. Oil Chem. Soc. 54*:47 (1977).
17. Chang, M.K., *Ibid. 67*:832 (1990).
18. Macrae, A.R., *Ibid. 60*:243A (1983).
19. Bloomer, S., P. Adlercreutz and B. Mattiasson, *Ibid. 67*:519 (1990).
20. Neff, W.E., M.A.M. Zeitoun and D. Weisleder, *J. Chromatogr. 589*:353 (1992).
21. Zeitoun, M.A.M., W.E. Neff, E. Selke and T.L. Mounts, *J. Liquid Chromatogr. 14*:2685 (1991).

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