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Purification of Isoflavone from Soybean Hypocotyl Using Different Solvents

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Abstract - Composition of isoflavone in cotyledon and hypocotyl of soybean were detected using HPLC. Optimum conditions for extracting isoflavone from hypocotyl were studied as well. Contents of isoflavone in soybean cotyledon and hypocotyl were 482.5 mg 100 g^{-1} and 3453.3 mg 100 g^{-1}, respectively. Hypocotyl contained 7~8 times more isoflavone than corresponding cotyledon of the soybean. Malonyl glycoside accounted for more than 70% of the total isoflavone, followed by glycoside, acetyl glycoside, and aglycone. Aqueous ethanol of 60~80% was the most suitable solvent for extracting isoflavone from the hypocotyl. Optimum temperature and time was 90°C, 1 hr. Acetic acid, NaCl, and NaOH added to 80% ethanol suppressed extraction yield of the phytochemical.

Key words : ethanol, extraction, isoflavone, organic solvents, soybean

INTRODUCTION

During the last two decades, isoflavone from soybean was found to have activities against cancer (Adlercreutz *et al.* 1992; Adlercreutz 1995), cardiovascular diseases (Potter 1995), and osteoporosis in women (Kurzer and Xu 1997). Anticarcinogenic activity of genistein has been predominantly reported after the discovery that genistein is a potent and a specific inhibitor of protein tyrosine kinases (Akiyama *et al.* 1987). While the biological activities of isoflavone have been studied intensively, the research on the large-scale production of isoflavone from soybean and its by-products produced during manufacturing of soybean foods are rather limited.

Isoflavone was first isolated from soybean meal in 1931 (Walz) and later confirmed in 1941 by Walter. Due to the presence of hydrophobic ring structure and hydrophilic hydroxides, isoflavone was reported to be best solubilized in aqueous organic solvent such as 80% methanol or 80% ethanol, and the solubility was increased at high temperature. In the extracts, pigments, soybean saponin and oligosaccharide such as raffinose, stachyose and sucrose were also contained as well as isoflavone. Murphy (1981) reported that extraction of isoflavone with acetonitrile and 0.1 N HCl maximizes recovery and minimizes the coextractives. Coward *et al.* (1993) extracted isoflavone from soybean using 80% aqueous methanol. Wang *et al.* (1990) also used similar solvents to extract isoflavone. Chromatography has been adopted by many researchers as a purification process in the production of isoflavone from soybean. Kitada *et al.* (1996) reported that isoflavone could be isolated from the soybean cooking water, which was produced as a byproduct in the miso manufacture.

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Because the production of isoflavone from whole soybean is expensive and makes large amounts of wastes, alternative sources of raw materials are necessary. Soy whey, a byproduct of soy protein manufacture is obtained after acid or ethanol precipitation of protein (Waggle and Bryan 2001). Concentrated soy whey is a good source of isoflavone production (Kitada *et al.* 1996). Defatted soy flake, a byproduct of soy oil manufacture, is also used as a raw material for the production of isoflavone. Soy hypocotyl, byproduct in the soymilk and tofu manufacture could be a useful source for isoflavone production. In this paper, we report feasibility of using soy hypocotyl for industrial production of isoflavone and optimum conditions for the purification process.

MATERIALS AND METHODS

1. Materials

Soybean, imported from USA, and soy hypocotyl were obtained from Dr. Chung's Food Co., (Cheongju, Korea), a local soymilk manufacturer.

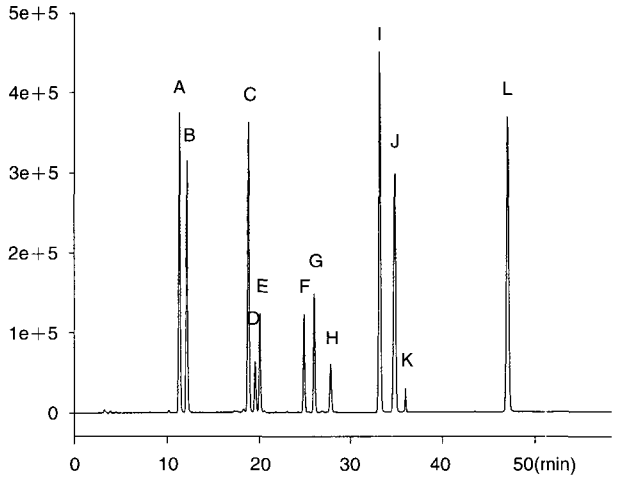
2. Isoflavone detection

To identify content of isoflavone in soybean samples, cotyledon and hypocotyl were hand separated and powdered using a hammer mill and dried in a vacuum drier at 60°C for 12 hr. One tenth gram of each powdered samples were dispersed in 0.5 mL of 80% aqueous ethanol and stirred for 24 hr at room temperature. The solutions were centrifuged at 12,500 rpm for 5 min and the supernatants were filtered with 0.45 μm membrane (Whatman, Germany) and analyzed with HPLC with conditions as below (Table 1). JASCO (Japan) HPLC system was used with ODS A303 (4.6 × 250 mm, YMC, U.S.A) column. Detector used was UV detector at 254 nm, and the flow rate of the solvent was 1.0 mL min^{-1}. HPLC chromatograms of 12 isoflavone isomers are as in Fig. 1.

Table 1. HPLC solvent system for determination of isoflavone of soybean

Time (min)	Solvent composition (%)	
	Solvent A	Solvent B
0	15	85
50	35	65
55	35	65
60	100	0
75	15	85

Solvent A : 0.1% acetic acid in acetonitrile
Solvent B : 0.1% acetic acid in water



A: Daidzin, B: Glycintin, C: Genistin, D: Malonyl-daidzin, E: Malonyl-glycitin, F: Acetyl-Daidzin, G: Acetyl-glycitin, H: Malonyl-genistin, I: Daidzein, J: Glycitein, K: Acetyl-genistin, L: Genistein.

Fig. 1. HPLC chromatograms of isoflavone isomers.

3. Extraction of isoflavone

To determine the optimum solvents for extracting isoflavone from powered hypocotyl, 70 g of the dried sample was dispersed in different solvents and isoflavone extracted was quantified with HPLC. Effects of time, temperature, pH, and salts on extraction were identified.

RESULTS AND DISCUSSION

1. Isoflavone composition of soybeans

Isoflavone content of soybean is known to differ depending on cultivar, growth conditions, and harvest time (Eldrige and Kwolek 1983). In Korea, known to be the place of origin for soybean, various varieties of soybeans with different shapes, colors, and sizes are consumed and used in food preparation and processing.

Isoflavone contents of soybean cotyledon and hypo-

Table 2. Isoflavone contents of hypocotyl and cotyledon of soybean

Part	Isoflavone content (mg 100 g^{-1})												
	Glucoside			Malonyl			Acetyl			Aglycon			Total
	Din	Glin	Gin	Din	Glin	Gin	Din	Glin	Gin	Dein	Glein	Gein	
Hypocotyl	425.5	441.4	111.9	1351.1	532.1	377.8	25.1	28.9	tr	74.8	57.7	26.2	3453.3
Cotyledon	42.4	nd	67.8	115.8	4.9	211.6	16.3	nd	nd	9.2	2.3	12.2	482.5

Abbreviations : Din: daidzin, Glin: glycitin, Gin: genistin, Dein: daidzein, Glein: glycitein, Gein: genistein, tr: trace, nd: not detected.

cotyl were 482.5 mg 100 g^{-1} and 3453.3 mg 100 g^{-1}, respectively. Hypocotyl contained 7~8 times more isoflavone than corresponding cotyledon of the soybean (Table 2). This result coincided well with reports by other researchers (Walter 1941; Wang *et al.* 1990). The major types of isoflavone are 6″-O-malonyl genistin, 6″-O-malonyl daidzin and 6″-O-malonyl glycitin which accounted for more than 70% of the total phytochemical, though malonyl forms are known to be labile to heat hydrolysis. Acetylglycosides were present in trace amounts. Content and composition of isoflavone differed between cotyledon and hypocotyl of the same bean. For example, glycitin, acetyl glycitin, and genistin were not detected in cotyledon, while glycitein and malonyl-glycitin were present in traceable amount in cotyledon. However, substantial amounts of these compounds were present in hypocotyl. This result support the speculation that isoflavone might be synthesized through separate pathways in cotyledon and hypocotyl (Eldrige and Kwolek 1983).

2. Isolation of isoflavone from hypocotyl

Since the chemical structure of isoflavone contains both hydrophobic ring and hydrophilic hydroxides, the chemical is reported to be best solubilized in aqueous alcohols (Walter 1941; Waggle and Bryan 2001). Fig. 2 shows the effects of different volumes of 80% aqueous ethanol added to 70 g of dried hypocotyl powder on extraction of isoflavone. Extraction was conducted at 70°C for one hour and the supernatant was analyzed for the phytochemical solubilized. Below solvent-to-hypocotyl ratio of 8 (v/wt), extraction percentage of isoflavone increased with increase in solvent volume; above the ratio of 8, the extraction percentage remained almost constant at 85%. Effect of solvent temperature on extraction of isoflavone is shown in Fig. 3, at the solvent hypocotyl^{-1} (v/wt) of 10 : 1 with 80% aqueous ethanol as the solubilizing solvent. Heating mantle was used to maintain the temperature from 40°C to 90°C. At all the temperatures tested, solubilizing process of isoflavone was found to complete within 60 min, and the extraction percentage of the chemical remained relatively constant thereafter. Extraction percentage of isoflavone was 50%, 72%, and 91% at 40°C, 70°C, and 90°C, respectively. Temperature was important factor in isoflavone extraction: higher the temperature, higher the efficiency of extraction. Since isoflavone extraction at 90°C showed the best result, further experiments were conducted at 90°C. Fig. 4 shows the effects of ethanol with different percentage of water on extraction of isoflavone at 90°C. Pure ethanol and water were not efficient extraction media for the phytochemical. 60~80% aqueous ethanol resulted in maximum yield with 85% of isoflavone being solubilized

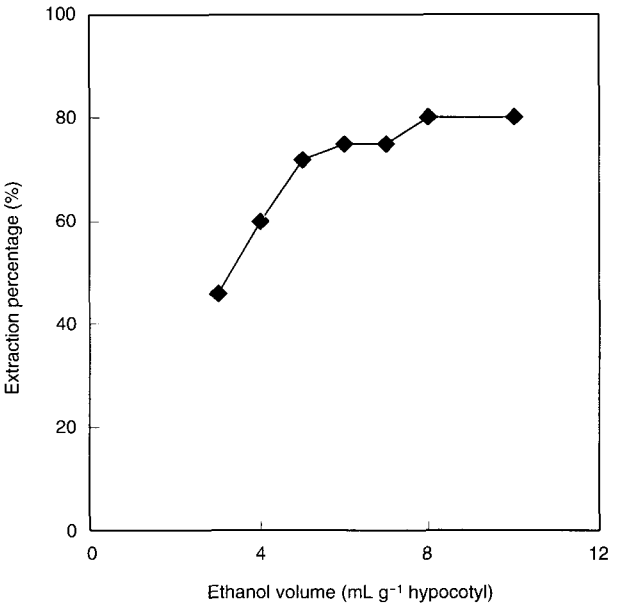


Fig. 2. Effects of 80% ethanol volume on percentage of isoflavone extracted.

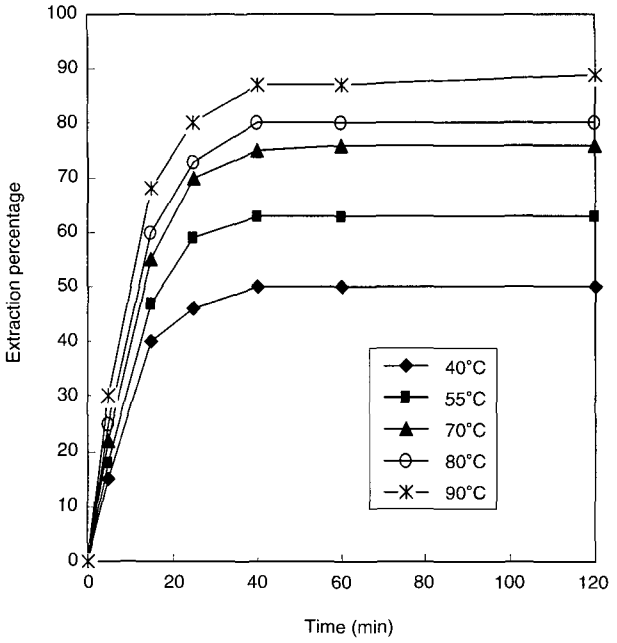


Fig. 3. Effects of temperature on extractability of isoflavone using 80% aqueous ethanol.

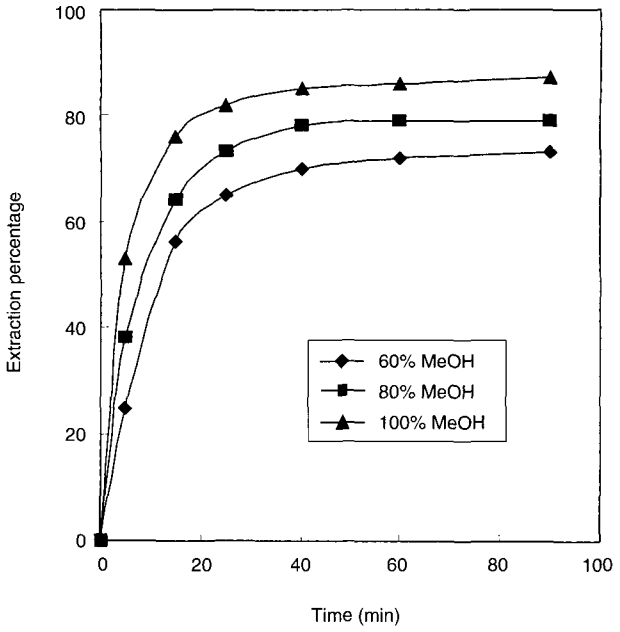


Fig. 5. Extraction yield of isoflavone with aqueous methanol at 90°C.

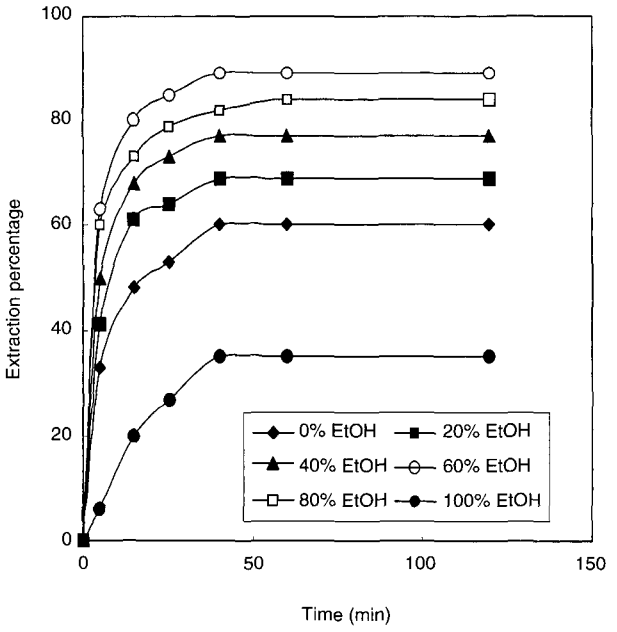


Fig. 4. Effect of different concentration of aqueous ethanol on extraction of isoflavone at 90°C.

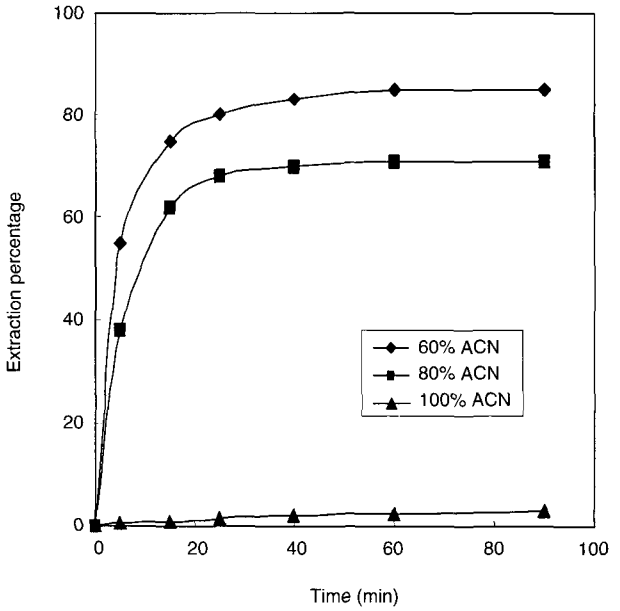


Fig. 6. Extraction of isoflavone with aqueous acetonitrile at 90°C.

into the solvent. Aqueous methanol showed similar results, with 60~80% aqueous solvents showing the maximum extraction percentage. Pure methanol was more efficient than 100% ethanol as solubilizing solvent for isoflavone (Fig. 5). Fig. 6 shows the effect of acetonitrile with different percentage of water on extraction of isoflavone. Extraction pattern of aqueous acetonitrile was similar to that of aqueous alcohols, though 100% acetonitrile showed extremely low solubility for the phy-

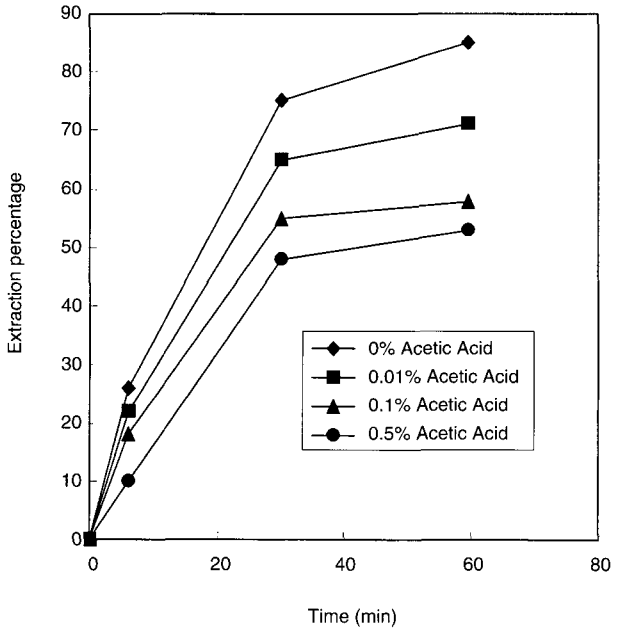


Fig. 7. Effect of acetic acid on extraction yield of isoflavone when using 80% aqueous ethanol as the solubilizing solvent.

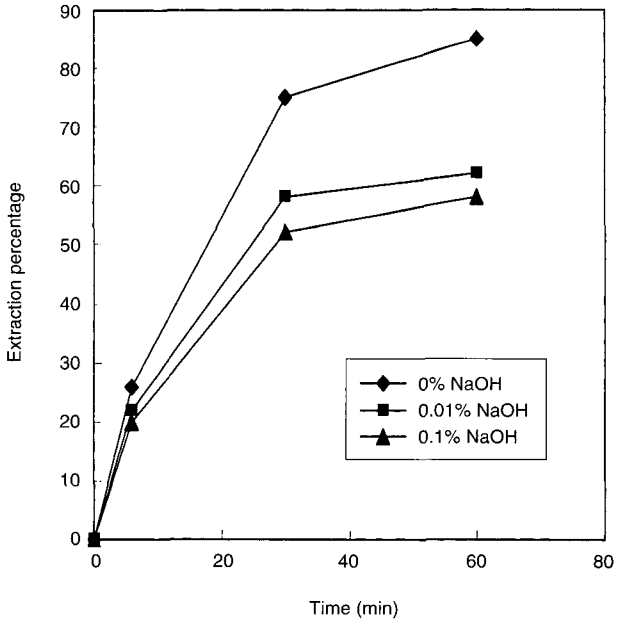


Fig. 8. Effect of NaOH on extraction yield of isoflavone when using 80% aqueous ethanol as the solubilizing solvent.

tochemical. This is probably due to higher hydrophobic property of the solvent.

To elucidate the effects of pH and polarity on extraction of isoflavone, acid, alkali, and salt were added to the 80% aqueous ethanol and the change in concentration of isoflavone in solvent was checked. All of acetic acid (Fig. 7), NaOH (Fig. 8), NaCl (Fig. 9) added to the extracting solution of 80% ethanol lowered extraction yield of isoflavone, suggesting the importance of optimum hydrophobic hydrophilic^{-1} ratio of the solution for solubilizing the chemical.

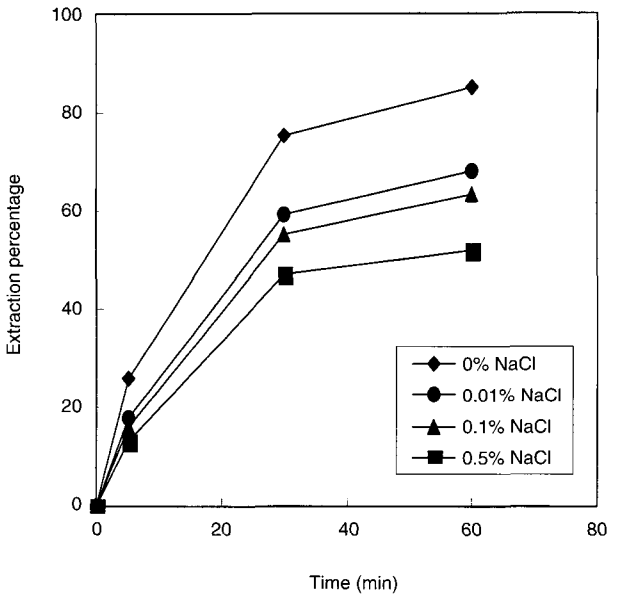


Fig. 9. Effect of NaCl on extraction yield of isoflavone when using 80% aqueous ethanol as the solubilizing solvent.

This research revealed that soy hypocotyl could be an excellent source for mass purification of isoflavone and that 60~80% aqueous ethanol at 90°C was the solvent of choice for solubilizing the phytochemical. However, for industrial applications, still more works are needed.

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REFERENCES

Adlercreutz H, Y Mousavi, J Clark, K Hockerstedt, E Hamalainen, K Wahala, T Makela and T Hase. 1992. Die-

tary phytoestrogens and cancer: *in vitro* and *in vivo* studies. J. Steroid Biochem. Mol. Biol. 41:331-337.

Adlercreutz H. 1995. Phytoestrogens: Epidermiology and a possible role in cancer protection. Environ. Health Perspect. 103:103-112.

Akiyama T, J Ishida, S Nakagawa, H Ogawara, S Watanabe, NM Itoh, M Shibuya and Y Fukami. 1987. Genistein a specific inhibitor of tyrosine specific protein kinases. J. Biol. Chem. 262:5592-5595.

Coward L, NC Barnes, KDR Setchell and S Barnes. 1993. Genistein, daidzein, and their β-glycoside conjugates: antitumor isoflavones in soybean foods from American and Asian diets. J. Agric. Food Chem. 41:1961-1967.

Eldrige AC and WF Kwolek. 1983. Soybean isoflavones: Effect of environment and variety on composition. J. Agric. Food Chem. 31:394-396.

Kitada Y, M Mizibuchi, Y Ueda, N Yamamoto, M Ishikawa and S Kawanishi. 1996. Recovery of isoflavone from soybean cooking drain of a Miso factory. Nippon Shokuhin Kogyo Gakkaishi 33:821-824.

Kurzer MS and X Xu. 1997. Dietary phytoestrogen. Annu. Rev. Nutr. 17:353-381.

Murphy PA. 1981. Separation of genistin, daidzin and their aglucones and coumesterol by gradient high-performance liquid chromatography. J. Chromatogr. 211:166-169.

Potter SM. 1995. Overview of proposed mechanisms for the hypocholesterolemic effect of soy. J. Nutr. 125:606-610.

Waggle DH and BA Bryan. 2001. Recovery of isoflavones from soy molasses. U.S. Patent 6,323,018.

Walter ED. 1941. Genistin (an isoflavone glucoside) and its aglucone, genistein, from soybeans. J. Am. Chem. Soc. 63:3273-3276.

Walz E. 1931. Isoflavone and saponin glucosides in soya hispida, Justus Liebigs Ann. Chem. 489:118-155.

Wang G, SS Kuan, OJ Francis, GM Ware and AS Carman. 1990. A simplified HPLC method for the determination of phytoestrogens in soybean and its processed products. J. Agric. Food Chem. 38:185-190.

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