Extraction of Defatted Soybean Flours and Flakes with Aqueous Alcohols: Evaluation of Flavor and Selected Properties

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One of the chief obstacles to the acceptance of soy protein products for food use is flavor. Treatment with organic solvents, primarily aqueous alcohols, has been shown by previous workers to improve color and flavor of soy proteins. In this study, three alcohols (ethanol, methanol, and isopropyl alcohol) at various aqueous concentrations were used to extract defatted soy flours and flakes at temperatures ranging from 30 to 75 °C. The odors and flavors of the extracted flours and flakes were evaluated when the products were initially prepared and also after 6 and 12 months of storage at 25 °C. After 6 months of storage at 25 °C, there were few signs of deterioration in either flavor or odor scores of the stored samples. However, after 12 months of storage at 25 °C, four samples were scored significantly lower for odor than were their unaged controls. These four samples were those (1) extracted with 70% ethanol at 30 °C, (2) extracted with 87.7% isopropyl alcohol at 30 °C, (3) extracted with 50% methanol at 45 °C, (4) extracted with 92.7% ethanol at 60 °C. Of these four samples, 1 through 3 above also scored significantly lower for flavor.

In the United States, the sale of soy proteins for human consumption shows a tenfold increase in a 5-year period (Becker and Tiernan, 1976). However, the proportion of soy for food use is small. Soy protein products are needed that are nearly bland and free of flatulence and that have good functional and nutritional properties and a long shelf life.

A sensory evaluation of commercial soy flours, concentrates, and isolates in 1971 confirmed that these products were not bland and that some beany and bitter flavors remained (Kalbrener et al., 1971). Organic solvents, primarily aqueous alcohols, have been used by Mustakas et al. (1962), Eldridge (1972), and Rackis et al. (1973) to improve color and flavor of soy proteins. Use of an azeotropic mixture of hexane–ethanol for removing the residual oil and flavor components from soybean flakes has been reported by Honig et al. (1969), Sessa et al. (1969), and Eldridge et al. (1971). In this study, three alcohols (ethanol, methanol, and isopropyl alcohol) at various aqueous concentrations were used to extract both soy flakes and flours at temperatures ranging from 30 to 75 °C. The odor and flavors of the extracted flours and flakes were evaluated organoleptically as initially prepared and after 6 and 12 months of storage at 25 °C. The present study was carried out to study the alcohol extraction of defatted soy flakes in some detail in order to characterize the products as affected by several process variables including the type of alcohol used. The study will attempt to identify those treatments which yield soy protein products having good initial odor and flavor and can retain these qualities during extended storage. Also the treatment should be effective in the removal of oligosaccharides, generally identified as being responsible for flatulence. The treatment itself must not leave significant residues of alcohol in the product. And finally, the effect of the treatment on yield, protein denaturation, and inactivation of urease will also be evaluated.

MATERIALS AND METHODS

Materials. Commercial defatted soy flakes with high nitrogen solubility index (NSI) were used for the exper-

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iments. The flakes were in the 20–50 mesh range and had an NSI of 77%. A portion of the flakes was pin milled to 100-mesh flour. The alcohols used were ACS grade.

Analytical Methods. Residual alcohols on the extracted flours and flakes were run by the method of Dupuy et al. (1975). Raffinose and stachyose were run by the method of Black and Bagley (1978). Nitrogen solubility index and protein were run by the official AOCS method (1976). Urease and trypsin inhibitor were run by official AACC methods (1962). Lipoxygenase was run by the method of Smith (1948). Urease values reported as pH units were converted to percent residual urease as described by Baker and Mustakas (1973).

EQUIPMENT AND PROCEDURES

Experimental Design. Three alcohols were used in the experiment: ethanol, methanol, and isopropyl alcohol. Ethanol or isopropyl alcohol were diluted with distilled water to make either the azeotrope or a 70% w/w solution. Methanol was diluted to either 90% or 50% w/w alcohol solutions. Both soy flakes and soy flours were extracted at temperatures ranging from 30 to 75 °C. Eleven experiments were run with soy flakes; these were then repeated with soy flours for a total of 22 experiments. The design was as follows:

alcohol	aqueous alcohol concn, wt % alcohol	extraction temp °C
ethanol	92.7 (azeotrope)	30, 60, 75
	70.0	30, 60
isopropyl alcohol	87.7 (azeotrope)	30, 60
	70.0	30, 60
methanol	90.0	30
	50.0	45

Experimental Procedure. The extraction vessel was an agitated 4-L stainless-steel beaker held in a water bath for temperature control. An experiment consisted of a 20-min batch extraction, followed by filtration and washing; the entire procedure was repeated five more times before vacuum drying overnight. The initial slurry was made at 3:1 v/w solvent-to-meal ratio and the five subsequent reslurries at a 2:1 ratio. After each extraction and filtration, the filter cake was washed with 1 volume of the appropriate alcohol at the same concentration of alcohol.

For the initial slurry, 2400 mL of aqueous alcohol was added to the beaker and heated to the design temperature.
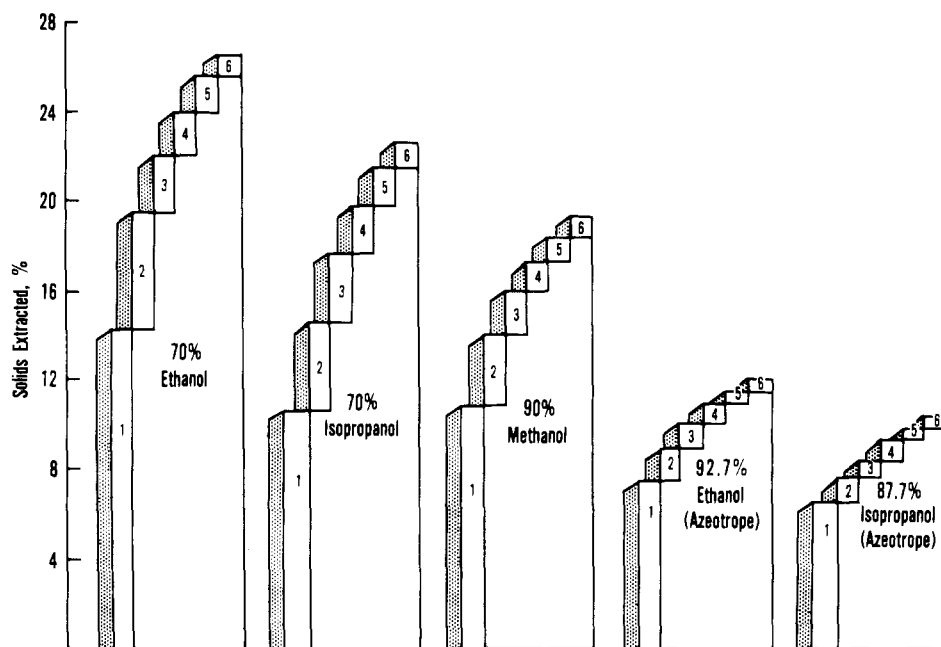
Figure 1. Relationship of aqueous and azeotropic alcohols to the amount of solids extracted from soy flakes in six batch extractions at 30 °C.

Soy flakes or flour (800 g) was added and stirred slowly for 20 min at design temperature. The slurry was then cooled and vacuum filtered over no. 2 Whatman paper on an 8-in. Buchner filter. Next the filter cake was washed with 800 mL of aqueous alcohol. For the second extraction the cake was reslurried with 1600 mL of aqueous alcohol and stirred, heated, filtered, and washed as before. The procedure was repeated four more times, and the filter cake from the sixth reslurry was vacuum dried overnight at 50 °C. Pending the completion of all experiments, the dried sample was placed in a wide-mouth capped bottle and held at 0 °F. When all experiments were completed, portions of each sample were removed for analysis, taste panel evaluation, and storage stability tests at 25 °C.

Odor and Flavor Evaluation. Eleven samples of defatted soy flakes and 11 of defatted soy flours were evaluated for odor and flavor by a trained 15-member panel experienced in testing soybean flour. Each panelist received 10 mL of a 2% dispersion in charcoal-filtered tap water and evaluated the overall intensity of odor and of flavor. They also analyzed for type and intensity of individual odors and flavors. To do this the panel used two separate scales. The individual odors and flavors that the panel member detects are rated on a scale of 1 = weak intensity, 2 = moderate intensity, and 3 = strong intensity. The intensity values of 0 to 3 that the panel gives are then averaged by a formula as follows:

OIV or FIV = [(1 × no. of weak responses) + (2 × no. of moderate responses) + (3 × no. of strong responses)]/no. of testers

where OIV = odor intensity value and FIV = flavor intensity value. The higher the OIV or FIV for a single description, the more predominant that odor or flavor is. The overall intensity for odor and for flavor is rated on a 1 to 10 scale with 10 as bland and 1 as very strong. The lower the average overall intensity score, the stronger the odor or flavor is. Because of this scoring system, those samples with low overall intensity scores will probably have high FIV's or OIV's for some individual descriptions. A balanced incomplete block (BIB) design was used. This design allowed for nine scores for each sample. Each taster

evaluated six or seven of the ten samples in the total series. Sample no. 9 and 22 were evaluated separately (not in the block design) since only ten sample blocks were feasible.

The aged samples were stored at 25 °C and tested after 6 and 12 months. Each panelist evaluated the unaged sample of each flour stored at 0 °F against the aged sample of that flour. The statistical significance of the difference in flavor or odor scores between the aged and unaged sample of each flour was determined by a two-way analysis of variance.

RESULTS AND DISCUSSION

Extraction of Solids. The most significant variations in yield of extracted solids were manifested with the type and aqueous concentration of the alcohol. The greatest amount of solids (amounting to over 30% of the starting material) was extracted with 50% methanol. A very large proportion of the solids was removed by the first three extractions, whereas the fourth through the sixth extracted successively smaller amounts (Figure 1). As might be expected, the more aqueous solvents extracted more solids. However, with both alcohols at the same 70% concentration, ethanol extracted more than isopropyl alcohol. Both azeotropes extracted the least amount of solids.

The effect of particle size on yield was most significant when extracting with the azeotropes at low temperature where the solids loss to the extracting solvent was considerably higher (47–86%) with flours than with flakes. The effect essentially disappeared at the higher temperature (60 °C) with the 70% alcohols where both flours and flakes extracted approximately the same.

The effect of temperature on yield was considerably less than the effect of either solvent type or particle size. More solids were extracted at the higher temperatures except in the extraction of soy flour with ethanol azeotrope (Table I). In general, when extracting flours the effect of temperature on yield has considerably diminished.

Nitrogen Solubility Index (NSI). Flakes were somewhat more denatured than flours in all treatments as measured by NSI (Figure 2). For a given temperature, denaturation was more complete when extractions were made with the 70% alcohols than with the azeotropes. Ethanol caused more denaturation than isopropyl alcohol.

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Table I. Yield and Analytical Data Summary of Alcohol-Extracted Soy Flours and Flakes

solvent	temp, °C	a	yield, %	NSI, %	protein, %	raffi- nose, %	stach- yose, %	residual trypsin inhibitor activity, %	residual urease activity, %
ethanol (92.7%)	30	FK	88.0	69	58.0	1.5	5.1	100	100
		FR	84.6	72		1.1	5.4	100	100
	60	FK	84.1	32	60.3	1.2	5.7	100	8
		FR	81.8	41	60.2	1.3	6.4	100	16
	75	FK	82.6	11	57.8	0.8	5.1	65	0
		FR	81.2	11	58.0	1.1	6.5	59	0
ethanol (70.0%)	30	FK	73.8	21	66.8	0.5	3.0	100	37
		FR	72.6	16		0.6	3.4	95	31
	60	FK	71.6	5	67.8	0.2	2.9	83	0
		FR	71.3	5	67.4	Tr	1.4	72	0
isopropyl alcohol (87.7%)	30	FK	89.6	67	55.9	1.4	5.8	100	100
		FR	87.1	75		1.7	5.9	100	100
	60	FK	84.6	57	55.9	1.8	5.4	100	100
		FR	83.4	63	57.1	1.6	6.8	100	100
isopropyl alcohol (70.0%)	30	FK	77.4	43	64.8	0.6	4.8	100	100
		FR	75.2	44		1.0	4.7	100	100
	60	FK	76.7	7	63.6	0.4	2.5	75	2
		FR	76.3	10	63.9	1.2	3.3	75	7
methanol (90.0%)	30	FK	80.8	39	61.1	0.8	5.3	100	2
		FR	74.4	41		0.7	1.3	100	0
methanol (50.0%)	45	FK	69.8	4	67.4	Tr	Tr	28	2
		FR	69.0	4	66.0	Tr	Tr	41	2
original flour				77	51.0	2.4	5.7	100	100

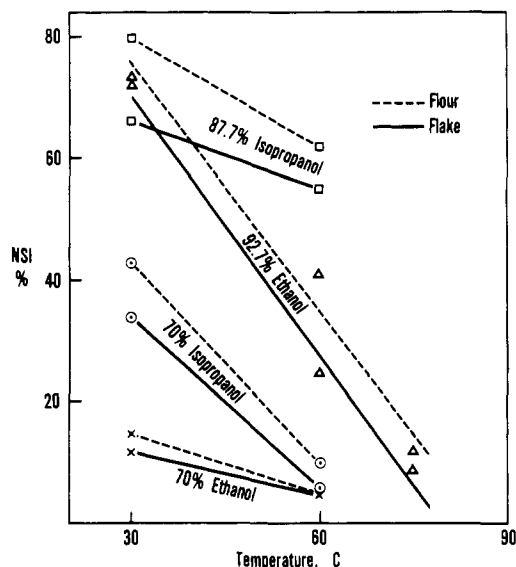*a* Flakes (FK), flour (FR).

Figure 2. Relationship of nitrogen solubility index to extraction temperature for aqueous alcohol mixtures.

Denaturation increased with temperature as expected; however, denaturation was minimal even at the elevated extraction temperature (60 °C) when extractions were made with the isopropyl alcohol azeotrope.

Enzyme Inactivation. Residual lipoxygenase in the commercial starting material was only about 7% compared to raw soy flour, whereas urease was almost 100% intact. Trypsin inhibitor was not inactivated by 13 of the 22 treatments and only partially inactivated by the other nine treatments (Table I). Urease inactivation by the various treatments ranged from none to complete inactivation. Urease inactivation was especially sensitive to the type and aqueous concentration of the alcohol used in the treatment. No inactivation occurred when extractions were made with the isopropyl alcohol azeotrope; whereas with 90% aqueous methanol, inactivation was complete at 30 °C. As expected, inactivation generally increased with temperature

Table II. Reduction of Residual Alcohol in Extracted Soy Flours by Redrying

solvent	extrac- tion temp, °C	residual alcohol, ppm		
		original vacuum drying	second vacuum drying	third vacuum drying
92.7% ethanol	30	12000	220	4
	60	5810	290	16
	75	8100	185	4
70.0% ethanol	30	2200	151	10
	60	1200	80	5
87.7% isopropyl alcohol	30	6200	148	3
	60	5300	106	2
70.0% isopropyl alcohol	30	1600	80	4
	60	3900	60	1
90.0% methanol	30	3010	135	6
50.0% methanol	45	580	48	4

except for the isopropyl alcohol azeotrope extraction, where no inactivation was observed at either 30 or 60 °C (Table I).

Oligosaccharide Extraction. Raffinose was more readily extracted than stachyose. On the average, residual raffinose content was reduced about two-thirds compared to one-third for stachyose (Table I). Extraction of either oligosaccharide was best accomplished with methanol, followed by the 70% aqueous alcohols; the azeotropic alcohols were the least effective. Temperature was not a significant factor except when extracting with 70% ethanol where the removal of oligosaccharides was much improved at the higher temperature (60 °C).

Residual Alcohol. As prepared, the vacuum-dried samples contained 580 to 12000 ppm residual alcohol (Table II). These amounts had to be reduced for taste panel evaluation. The samples were tempered to 30% moisture and redried under vacuum. This procedure removed over 90% of the residual alcohol but still left 48 to 290 ppm residual alcohol. The procedure was repeated once again, which reduced the residual alcohol to below 20 ppm, a level at which no problems were expected in the taste panel evaluation.

Table III. Summary of Sensory Data for Alcohol-Extracted Soy Flours

solvent	extraction temp, °C	zero time				aged 6 months		aged 12 months	
		flavor		odor		Δ in flavor score	Δ in odor score	Δ in flavor score	Δ in odor score
		score	grassy/beany FIV[b]	score	grassy/beany OIV[c]				
92.7% ethanol	30	6.3	1.1	7.5	0.4				
	60	7.3	0.7	8.3		-0.2	0.3	-0.5	-0.6[a]
	75	7.4	0.8	8.3	0.2	-0.1	-0.4	0.2	-0.2
70.0% ethanol	30	7.4	0.7	8.4	0.2	0.3	-0.6	-0.9[a]	-0.7[a]
	60	6.3	0.4	7.9		-0.2	0.2	-0.4	-0.6
87.7% isopropyl alcohol	30	6.8	0.8	7.7	0.2	0	0	-1.0[a]	-0.5[a]
	60	6.1	0.8	7.2		-0.1	0	-0.3	-0.3
70.0% isopropyl alcohol	30	6.6	0.7	8.0	0.2	0	0.1	-0.2	-0.4
	60	6.8	0.2	7.7	0.2	0.6	0.2	-0.1	0.3
90.0% methanol	30	6.8	0.9	7.9	0.3	-0.2	-0.3	-0.6	-0.1
50.0% methanol	45	6.6	0.4	7.2	0.5	-0.4	0.2	-0.7[a]	-0.6[a]

[a] Significant difference at 95% confidence level. [b] Flavor intensity value. [c] Odor intensity value.

Table IV. Treatments Which Failed to Meet Two or More of the Sensory Criteria

solvent	extraction temp, °C	zero time			12 months	
		flavor score	odor score	grassy beany FIV[a]	Δ in flavor score	Δ in odor score
92.7% ethanol	30	(6.3)[b]	(7.5)	(1.1)		
87.7% isopropyl alcohol	30	6.8	7.7	0.8	(-1.0)	(-0.5)
	60	(6.1)	(7.2)	0.8	-0.3	-0.3
70.0% ethanol	30	7.4	8.4	0.7	(-0.7)	(-0.9)
50.0% methanol	45	6.6	(7.2)	0.4	(-0.7)	(-0.6)

[a] Flavor intensity value. [b] Values in parentheses do not meet defined criteria.

ODOR AND FLAVOR EVALUATION

Zero Time. The odor scores for the flour samples tended to be higher than the scores for the flakes. A statistical analysis cannot be reliably done between the flour series and flakes series since direct comparisons were not made. The flour samples had lower OIV for grassy/beany than did the flake samples. An adequate statistical analysis cannot be calculated on this observation either.

Flavor scores for the flours also tended to be higher than for the flakes. The FIV for grassy/beany were generally lower for the flours. Three of the treatments for the flours gave significantly lower flavor scores than the other eight; i.e., ethanol azeotrope at 30 °C, 70% ethanol at 60 °C, and isopropyl alcohol azeotrope at 60 °C (Table III). The most significant variations in flavor scores were between solvent types used in the various treatments. For both the flour and flake series, the samples extracted by ethanol azeotrope at 30 °C were given some of the lowest scores and corresponding high FIV's for grassy/beany. This treatment was not included in the storage stability testing.

Only a few of the significant differences in flavor scores were observed when the extraction temperature was varied. At 60 °C, ethanol azeotrope received higher odor and flavor scores than the isopropyl azeotrope, although a significant difference was seen only with the flavor scores of the flours.

Storage Stability Tests. Since the differences in odor and flavor scores between flours and flakes were small, it was decided to eliminate one or the other from the storage stability tests. Flavor scores for the flours tended to be higher, so the flakes were elminated from the long-term study. After 6 months of storage at 25 °C, there were few signs of deterioration in either flavor or odor scores or types and intensities of descriptions. However, after 12 months of storage at 25 °C, four samples were scored significantly lower for odor than their unaged controls (Table III). Three of these four samples were also scored significantly lower for flavor than their unaged controls. Probable reasons for the lower scores include higher flavor intensity

values for grassy/beany, musty/stale, astringent, and "off" descriptions in the aged flours.

ARBITRARY CRITERIA FOR SELECTION OF OPTIMUM CONDITIONS

To use the accumulated data most effectively, it was necessary to set some arbitrary criteria to eliminate those treatments that produced the least satisfactory products based on sensory evaluation and selected properties.

Sensory Criteria. All flake treatments were eliminated because, for any given treatment, there were no significant differences between flour and flake odor and flavor scores, although flours tended to score somewhat higher. To

test	limit
zero time	
1. flavor score	6.5 or above
2. odor score	7.6 or above
3. grassy/beany FIV	0.8 or below
aged	
1. flavor or odor score	no significant decrease vs. unaged

qualify in this category, a sample must not have low initial flavor and odor scores or correspondingly high flavor intensity values. The aged samples must not have undergone significant decreases in odor or flavor scores as compared to the unaged controls.

Applying the above criteria, 5 of the 11 treatments were eliminated (Table IV).

Analytical Criteria. The setting of criteria for selected analyses was based on the range of results obtained over the entire series. Raffinose and stachyose are considered

test	limit
1. raffinose content	0.9% or less (40% of original)
2. stachyose content	4.1% or less (70% of original)
3. residual urease	25% or less
4. residual alcohol	50 ppm or less

responsible for flatulence often associated with soy

Separation of Wax from Sorghum Bran Extracts

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Table V. Treatments Which Exceeded Both Sensory and Analytical Criteria

solvent	°C	raffi- nose, %	stach- yose, %	residual urease activ., %	residual alcohol, ppm
70.0% ethanol	60	0.1	1.4	0	5
90.0% methanol	30	0.7	1.3	0	6
70.0% isopropyl alcohol	60	0.8	2.9	1	7

products; consequently, those treatments that are most effective in the removal of these oligosaccharides would produce the more acceptable products. The test for urease is often used as an index for adequate heat treatment in the inactivation of antinutritional factors. Since the yield and product quality appeared to be moving in opposite directions, yield was not considered in the selection of treatments, only insofar as to assess the amount of yield loss to be tolerated for a given improvement in product quality. Likewise, the degree of protein denaturation as measured by NSI was not included because this would vary with the intended end use and could not be evaluated until the use was specified.

Applying the above criteria, three of the six remaining treatments were eliminated (Table V), two because of inadequate extraction of raffinose and stachyose (92.7% ethanol at 60 and 75 °C) and one for lack of enzyme inactivation (70% ethanol at 30 °C). Of the three remaining, it is interesting to note that each of the three alcohols is represented. In the event that the emphasis was placed on a different set of objectives by the dictates of economics or other factors, then the priorities might be weighed differently and some of the eliminated treatments might be acceptable.

ACKNOWLEDGMENT

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Use of Urea as Clathrate in Separation of Wax from Sorghum Bran Extracts

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Soxhlet extraction of sorghum bran to obtain miscella was performed with eight separate solvents (absolute alcohol, acetone, ethylene dichloride, methanol, methyl ethyl ketone, *n*-butyl ether, *sec*-butyl alcohol, and Skellysolve B) and two azeotropic mixtures (ethylene dichloride–heptane–water, volume ratio 2:2:1, and methyl ethyl ketone–heptane–water, volume ratio 2:2:1). A batchwise Pfaudler distillation unit using Skellysolve B as an extracting solvent was used to obtain a sufficient amount of miscella for subsequent separation of wax from oil in the miscella by the acetone precipitative extraction and the urea clathrate methods. The crude wax yield was increased about 50% by lowering the temperature from 6 to −8 °C in the acetone method. At room temperature, the urea clathrate method yielded more than twofold increase in the crude wax over the acetone method at −8 °C.

For better and fuller utilization of agricultural products, the extraction of economically valuable lipid materials from the sorghum bran has been studied. All the soluble material that is extracted from the bran is referred to as "miscella". The fraction of miscella that remains in the solid state at room temperature after the separation is referred to as "wax" and the other fraction is the "oil". Since the sorghum wax resembles the commercially valuable Carnauba wax (Bunger and Kummerow, 1951) in many of its physical properties, the objective of the study was to develop an economically feasible process for the separation of the wax and oil fractions in the miscella; the desired process must recover the wax fraction more completely and in purer form, with the yield and quality of the oil fraction being of secondary importance. The first

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