LII. ON THE NATURE OF THE CARBOHYDRATES FOUND IN THE JERUSALEM ARTICHOKE.

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In spite of repeated investigations, divergencies still exist as to the nature and relative proportions of the carbohydrates found in the tubers of the Jerusalem artichoke (*Helianthus tuberosus*).

The most detailed account of these carbohydrates has been given by Tanret [1893], who carried out investigations with tubers gathered in September and October. Tanret pressed these tubers in a press, collected the juice, and mixed it with about one-tenth its volume of warm basic lead acetate solution of specific gravity 1.25. After filtering off the precipitate formed, the excess lead in the filtrate was removed by means of the requisite amount of dilute sulphuric acid. Addition of hot concentrated baryta water to the clear filtrate thereby obtained yielded a precipitate, which Tanret describes as the barium compound of inulin. Further precipitates could be obtained from this baryta-containing filtrate by addition of increasing quantities of alcohol. Altogether seven carbohydrates were separated from the artichoke juice (see Table I).

Table I.

	Percentage of carbohydrates ¹	
	Calculated on dry tubers	Calculated on total carbohydrates
Inulin	3.12	20.62
Pseudo-inulin	0.35	2.31
Inulenin	1.25	8.26
Helianthenin	0.83	5.49
Synanthrin	7.26	47.98
Sucrose	1.78	11.77
Laevulose and dextrose	0.54	3.57

¹ In his paper Tanret gives his results in grams of carbohydrates per litre of concentrated juice. His figures have been converted as above for the purpose of comparison with the results of other workers.

The reducing sugars laevulose and dextrose were present in small quantities only. The relative proportions of the various other carbohydrates are not given by Tanret, except in the case of a sample of juice pressed from tubers which had been collected in the autumn, but stored in a cellar till the following June before analysis. In consequence of the prolonged storage of these tubers the figures given by Tanret may not be correct; they are quoted, however, as they represent the most complete analysis available in the existing literature.

Other investigators have arrived at different conclusions with regard to the amount of inulin present in artichoke tubers. Thus König [quoted by Czapeck, 1913], reports the presence of 58 % of inulin, presumably on the total carbohydrates present; while Shohl [1923] records 15.5 % of inulin in the fresh tubers.

The presence of sucrose in the tubers has been reported by Dubrunfaut [1867] and by Colin [1918]. Helianthenin and synanthrin are names given by Tanret to two laevorotatory carbohydrates with the following characteristics.

Table II.

	Specific rotation [α] _D ²⁰	Melting point	Solubility in water in all proportions	Specific rotation after inversion [α] _D ²⁰
Helianthenin	-23.5°	176°	50 %	-70.2°
Synanthrin	-17°	170°		-70.6°

Pseudo-inulin and inulenin are closely related to the inulin proper, but are stated to differ from it in their specific rotations, which are said to be for pseudo-inulin - 32.2°, and for inulenin - 29.6°. On inversion the carbohydrates show a specific rotation of - 85.6° and - 84° respectively.

Of all the carbohydrates isolated by Tanret from the artichoke tubers only sucrose and dextrose give a positive specific rotation. It is to be expected, therefore, that the juice obtained by extracting the fresh tubers with water will show a negative rotation. That the juice is laevorotatory is confirmed by Colin [1918], who finds that an extract of the tubers is strongly laevorotatory when prepared in autumn (September). When taken from tubers collected during early spring, Colin obtained dextrorotatory juice. This undoubtedly implies that considerable changes have occurred in the carbohydrates, changes which, according to Colin, result in an altered fermentability of the tubers. Colin points out that it is necessary to subject autumn-gathered tubers to a preliminary hydrolysis with acids before they can be completely fermented by yeast, while the carbohydrates of the tubers collected in spring may be directly converted into alcohol by yeast without preliminary inversion. That a preliminary hydrolysis of the tubers raises the yield of alcohol obtainable from them has also been observed by Rudiger [1920] and by Windisch [1920].

On the assumption that inulin, which is unfermentable by yeast in the pure state¹, is really present in artichoke tubers, the above observations would

¹ An observation which has been experimentally confirmed by the writers.

seem to indicate that inulin undergoes an inversion during storage of the tubers, resulting in its conversion into a fermentable sugar; unless it be considered justifiable to assume with Tanret [1893] that inulin is fermentable by yeast when present in an already fermenting mixture of other carbohydrates. The experiments on which Tanret bases this assumption were carried out with tubers gathered during the early autumn, but stored in a cellar until June of the following year. In the juice obtained from these tubers Tanret found as much as 1.36 % of inulin, calculated on the tubers, of which he was able to recover only 27.5 % after fermentation of the juice by a yeast, 72.5 % of the original amount having disappeared during fermentation.

As certain investigations carried out by the writers made it desirable to collect further information on the fermentative properties of the carbohydrates of the Jerusalem artichoke, it was thought justifiable to repeat part of the work referred to above and to endeavour to co-ordinate the divergent views of previous workers.

As a preliminary it was decided to determine the rotation of juice pressed from tubers collected both during autumn and spring. The results obtained are given in Table III and show that a change from laevo-rotation to dextro-rotation does take place. The actual figures when expressed as specific rotations of the whole mixture of carbohydrates found in the tubers agree closely with the observation of Colin [1918], who observed a specific rotation not exceeding $+10^\circ$ in tubers stored until Feb.-March. The comparatively strong positive rotation of the carbohydrates in spring juice reaches its maximum about March and has decreased to $+2.12^\circ$ by May, when germination has proceeded for some time and emptied the tubers of a considerable percentage of their carbohydrates.

Table III.

Date of examination	Percentage of carbohydrates		Observed rotation of juice		Specific rotation of carbohydrate mixture in juice	
			$[\alpha]_{D}^{20^\circ}$		$[\alpha]_{D}^{20^\circ}$	
	Total	Reducing	Before inversion	After inversion	Before inversion	After inversion
3. xi. 23	3.2	—	-0.095°	-3.13°	-1.48°	-58.9°
16. xii. 23	5.16	0.64	-0.13°	-6.23°	-1.26°	-61.6°
17. i. 24	(a) 3.43 (a)	*0.31	(a) +0.17°	(b) -4.21°	+1.98°	-61.4°
	(b) 4.29					
27. ii. 24	6.19	0.33	+0.71°	-3.58°	+6.06°	-57.84°
18. iii. 24	5.65	0.41	+0.59°	-0.79°	+4.49°	-57.1°
7. v. 24	5.32	—	+0.261°	-0.764°	+2.12°	-58.6°

* Two different juices (a and b) were used for this determination.

It should be noted that the percentage of reducing sugars in the juice before inversion is very small, even in samples of juice extracted from tubers stored until March. No increase or only a very slight increase in the laevulose and dextrose content has therefore occurred during storage. This is interesting, as it shows that the change in the rotation is not due to an increase in the concentration of a dextrorotatory reducing sugar.

In order to investigate the reason for this change it was decided to attempt the isolation of the various carbohydrates present both in autumn and spring tubers. To do this the method recommended by Tanret was adopted. His method, however, was not found easy to carry out¹, and after several unsuccessful attempts it was decided to proceed with the separation in a somewhat different way, though still adhering to Tanret's method of utilising alcohol of varying strengths for the separation of the individual fractions. The procedure finally adopted was as follows. The freshly-dug tubers were carefully washed and then minced to a fine pulp. The pulp was mixed with its own weight of warm distilled water and the mixture kept at 60°–70° for half an hour. It was then pressed and the juice collected. Basic lead acetate solution of sp.g. 1.25, sufficient to clarify the juice, was added to the extract and the excess lead removed from the clear filtrate with sulphuric acid and sulphuretted hydrogen. The clear filtrate was concentrated *in vacuo* at a temperature not exceeding 42°. Its carbohydrate content was thereby increased to about 20 %. To every 100 cc. of this solution one litre of 95 % ethyl alcohol was added, and the mixture well shaken. The alcohol caused a voluminous precipitate to form when juice obtained from autumn tubers was used. This precipitate was collected on a filter, washed with 95 % alcohol, with ether, and finally dried *in vacuo* over sulphuric acid. When dry it formed a snow-white, light and almost tasteless powder.

For the further purification of this powder it was redissolved in as little warm water as possible, and 95 % ethyl alcohol added to the cooled solution until its alcohol concentration amounted to 50–55 %. The precipitate, after standing overnight, was collected and dried as described above. To the filtrate a further quantity of 95 % alcohol was added to make its concentration equal to about 85 % ethyl alcohol. A precipitate was again formed, which, when dried as already described, formed a granular, sandy-coloured powder. After precipitation of the above two fractions (B and C) the filtrate contained only traces of carbohydrates.

The filtrate from the mixture of clarified juice with alcohol was evaporated *in vacuo* (temperature 42°) to a thick syrup, containing between 40–45 % of carbohydrates. The isolation of these carbohydrates was at first found almost impossible. Finally, however, the following method separated the syrup into two distinct fractions. Three g. of the syrup were poured into 80 g. of absolute alcohol, containing not less than 99.5 % of ethyl alcohol, and the mixture was stirred vigorously for some time. Part of the syrup was thereby converted into a flocculent precipitate, which could be filtered off, while another part formed a gummy substance on the bottom of the container, a third part remaining dissolved in the alcohol. The gum, after dissolution in a very small quantity of water, could again be divided into a flocculent precipitate and

¹ Similar difficulties are also reported by Pringsheim and Lassmann [1922], who were unable to confirm Tanret's results as regards the presence of pseudo-inulin, inulinin, synanthrin and helianthin.

a gum by the addition of the required quantities of absolute alcohol. Repeating this process a sufficient number of times the whole of the syrup could be converted into a flocculent precipitate (fraction D), and a carbohydrate (fraction E) remaining dissolved in this strong alcohol. This latter fraction could be precipitated by the addition of ether to its alcoholic solution, and on separation, formed a gummy and extremely hygroscopic substance, light brown in colour and having a very sweet taste. The flocculent precipitate was not hygroscopic, though extremely soluble in water. It was also light brown in colour and had a sweet taste. The filtrate from the fourth fraction (E) contained traces only of carbohydrates.

The whole of the carbohydrates originally present in the juice had therefore been isolated by the above process.

Subjecting both autumn and spring tubers to the treatment described above the following yields of carbohydrates were obtained (Table IV).

Table IV.

Fraction	$[\alpha]_D^{20^\circ}$	Juice prepared from tubers dug and extracted Nov. 1923		Juice prepared from tubers dug and extracted March 1924	
		Obtained by addition of alcohol	Present as percentage of total carbo- hydrates	Obtained by addition of alcohol	Present as percentage of total carbo- hydrates
B	-34.96°	Insoluble in 55 % alcohol	28.04	Insoluble in 55 % alcohol	1.02
C	-33.42°	Insoluble in 85 % alcohol	13.40	Insoluble in 85 % alcohol	
D	Inactive to -1.233°	Insoluble in 98 % alcohol	38.00	Insoluble in 98 % alcohol	44.7
E	-2.91° in autumn to -24.91° in spring	Insoluble in a mixture of ether and 98 % alcohol	20.56	Insoluble in a mixture of ether and 98 % alcohol	54.28

This table is of considerable interest in several respects. It shows, for instance, that there is a very material difference in the distribution of the carbohydrates in autumn and spring tubers, the latter containing less laevo-rotatory and less insoluble substances than the former. Further, the table appears to explain why artichoke tubers have in some cases been found to contain inulin, while in other cases inulin has been absent.

As seen from Table V fraction B corresponds with the inulin of Tanret; fraction C may be identical with Tanret's pseudo-inulin. These fractions, B and C, disappear from the tubers toward spring and give place to the more readily soluble fractions D and E.

Fractions D and E do not compare with any of Tanret's fractions. They are probably mixtures of carbohydrates, since their specific rotations vary, depending on the season at which they are isolated from the tubers. As both of these fractions yield large percentages of glucose on hydrolysis, the possibility that one of their components is sucrose cannot *a priori* be excluded,

particularly in view of the fact that both Dubrunfaut, Colin and Tanret have isolated sucrose from Jerusalem artichokes.

Table V.

Properties	Inulin	Fraction B	Pseudo-inulin (Tanret)	Fraction C
Specific rotation { Before inversion	-38° to -39°	-34.26°	-32.2°	-33.12°
{ After	-85.7°	-83.42°	-85.6°	-77.94°
Reduction of Fehling's solution	No reduction	No reduction	No reduction	No reduction
Solubility in water	Slightly soluble	Slightly soluble, about 0.062 % at 12°	More soluble than inulin	Readily soluble
Melting point	165°-178°	No sharp melting point between 158-163°	Decomposes at 175°	
Effect on ammoniacal silver nitrate	Reduced	Reduced	—	—
Behaviour towards iodine	Yellow coloration	Yellow coloration	—	—
Melting point of osazone after inversion	205°	205°	—	—
Appearance	White flocculent powder, tasteless	White flocculent powder, tasteless	—	Almost white to light fawn-coloured powder with slightly sweet taste

Attempts have repeatedly been made by the writers to isolate sucrose from these fractions D and E, but complete success has not yet been attained. There are good reasons for supposing, however, that fraction E is a mixture of a small proportion of D with a large proportion of sucrose, while fraction D, as isolated by the above treatment, contains a small quantity of sucrose. If it may be assumed that the whole of the dextrose found in the inverted samples of fractions D and E has been derived from sucrose, the following specific rotations would have to be attributed to the remaining components (Table VI, column 4).

Table VI.

Fraction	Specific rotation of fraction calculated from observed rotation	Proportion of dextrose and laevulose in the inverted fraction	Assuming the dextrose found to have been derived from sucrose the remaining compounds of the fractions would have sp. rotation
D from autumn tubers	Zero	22.8 % dextrose 77.4 % laevulose	-50.94°
D from spring tubers	-1.23°	23.6 % dextrose 76.4 % laevulose	-56.37°
E from autumn tubers	-2.91°	27.9 % dextrose 72.1 % laevulose	-69.0°
E from spring tubers	-24.91°	31.7 % dextrose 68.3 % laevulose	-38.3°

In every case this specific rotation is more negative than that of inulin, probably signifying that the compounds in question are hydration products of inulin, possibly condensation products of laevulose with 6 or 3 laevulose groups, instead of 9 as in the case of inulin [Pringsheim and Aronowsky, 1922].

The question of the fermentability by yeast of the various carbohydrate fractions isolated from the tubers of the Jerusalem artichoke was investigated in a series of experiments the results of which are given in Table VII.

The carbohydrates were dissolved to a concentration of 4 % in a water extract of maize meal, made by digesting 7 parts of maize meal with 100 cc. of physiological saline at 37° for 16 hours in presence of 1 cc. of toluene per litre. The liquid was filtered through an ordinary filter-paper and was then boiled to remove any traces of toluene present¹. The solutions were sterilised by fractional sterilisation in order to avoid the decomposition of the sugars. After inoculation the samples were incubated at 35° for 4 and 10 days respectively before distillation.

The yeast used in the experiments was a pure culture distillery yeast fermenting well at 35°.

An almost complete conversion into ethyl alcohol was obtained with fraction D and E of both autumn and spring tubers. Fractions B and C, on the other hand, remained unfermented.

Table VII.

Fraction	Carbohydrate concentration of solution to be fermented %	Carbohydrate concentration in mash after fermentation %	Duration of fermentation days	Yield of alcohol obtained calculated on carbohydrates present %	Yield obtained: percentage of that theoretically possible
B	4.2	4.04	10	Traces	—
C	4.2	3.85	10	Traces	—
D autumn tubers	4.2	0.47	4	42.5	88.8
D spring „	4.2	0.39	4	43.6	91
E autumn tubers	4.2	0.23	4	44.5	94.6
E spring „	4.2	0.23	4	44.5	94.6
Controls—					
Sucrose	4.2	Less than 0.04	4	47.6	99.1
Laevulose	4.2	Less than 0.03	4	49.5	99.3
Dextrose	4.2	Less than 0.03	4	49.5	99.3

The non-fermentability of the B and C fractions agrees well with what would be expected from a carbohydrate of the nature of inulin, though it makes it rather difficult to explain certain earlier experiments and the fermentation experiments of Tanret.

Tanret in his investigations found that 75 % of the inulin present in the tubers used disappeared during the fermentation, and he suggested that inulin might at least be partly fermentable in the presence of readily fermentable

¹ The extract (after inversion) contained less than 0.2 % carbohydrates in solution.

compounds. A number of experiments have been carried out to determine how far this may be the case, and mixtures of two or more of the fractions isolated from autumn-gathered artichoke tubers have been subjected to fermentation by yeast in the manner already described. Table VIII illustrates the results obtained.

Table VIII.

Fraction	Carbohydrate concentration of mash before fermentation	Carbohydrate concentration of mash after fermentation	Duration of fermentation days	Yield of alcohol obtained calculated on carbo- hydrates present %	Yield of alcohol obtained calculated on the fractions respectively		
	%	%			B+C %	D %	E %
B+C	4.2	3.99	10	2.7	2.7	—	—
B+C+D	4.2	2.42	10	20.4	2.7	38.12	—
B+C+D+E	4.2	1.51	10	33.0	15.3	43.6	44.5
D	4.2	0.47	4	43.6	—	43.6	—
E	4.2	0.23	4	44.5	—	—	44.5

The fractions were present in the proportions in which they were found in the tubers.

It will be seen that a higher yield of alcohol was obtained in the mash where all four fractions were mixed than should have been obtained had the B + C fraction not been attacked. It is true that the yields of alcohol from the fractions D and E are estimated at 43.6 % and 44.5 % respectively. Yet even assuming that both of these gave yields of 50 %, or the absolute maximum obtainable, the alcohol collected from the mixture of all four fractions would still be in excess of what could be obtained had the B and C fractions remained unattacked.

A second series of experiments entirely confirmed these results. It must be assumed therefore that the B and C fractions can be converted into alcohol to some slight extent when present in a fermenting solution of fractions D and E, though hardly perhaps to the extent stated by Tanret.

The fermentation experiments referred to above as being difficult to reconcile with the presence in autumn tubers of the Jerusalem artichoke of large percentages of inulin were carried out during November 1922 with samples of juice pressed from freshly-dug tubers and fermented with yeast after dilution and sterilisation in the cold by filtration through Chamberland filters. After inoculation with a yeast this cold sterilised juice was incubated at 35° for 6 days. A yield of alcohol was obtained equal to 47.5 % of the total carbohydrates present. As the percentage of carbohydrates present was found to be equal to that of the fresh tubers it is clear that the inulin fractions B and C must have been present in the tubers and, therefore, must have been almost completely fermented under the conditions of the experiment.

Tanret has shown, and the writers have confirmed, that a partial conversion of the inulin fractions into alcohol may occur when these fractions are present in a vigorously fermenting mixture of other carbohydrates, but this partial conversion is hardly sufficient to explain the ease with which practically the whole of the carbohydrates of the cold sterilised juice was found to be converted.

That bacteria, possessing inulin-inverting enzymes, had developed in the cold sterilised fermenting juice is out of the question, since a careful bacteriological analysis proved the juice to be free from all micro-organisms except the yeast used as inoculant. A further possibility, that the sterilisation of the juice had caused the B and C fractions to become inverted, does not arise in this connection, since no heat had been used for the purpose.

One is almost compelled to assume, therefore, that the cold sterilised juice had contained inulase, which remained sufficiently active after filtration to convert the B and C fractions into fermentable compounds during the 6 days during which the juice remained incubated at 35°.

Attempts made to confirm experimentally the assumption of the presence of inulase in the tubers have not met with very great success. In one experiment a mixture of the B and C fractions in the proportions in which they occur in the autumn tubers was added to juice collected from March tubers. It was thought that this juice would contain considerable quantities of inulase and would be capable of inverting the B and C fractions when allowed to act on them under the conditions used in the experiment with cold sterilised juice. After incubation at 35° for 8 days, however, in the presence of toluene to check the development of micro-organisms, only insignificant quantities of the inulin fractions had disappeared. The actual figures in this experiment were as follows: 90.6 % of the inulin fractions were recovered from the incubated test sample of spring juice containing the B and C fractions by the usual procedure of isolation, as against 93.6 % from the control experiment which had not been incubated. A satisfactory explanation of the observations on the fermentability of the cold sterilised juice obtained from autumn-gathered tubers therefore cannot be given.

Table IX.

		Percentages of inulin fractions (B + C) recoverable from mashes autoclaved for				
Sample		6 hours (steaming)	1 hour at 20 lbs.	2 hours at 20 lbs.	3 hours at 20 lbs.	5 hours at 20 lbs.
Spring juice + B and C fractions in the proportions found in autumn tubers		99.9	90.9	66.8	30	11.4

Table X.

		Yield of alcohol calculated on the carbohydrates present obtainable from samples of inulin containing juice after heating in the autoclave at 20 lbs. for:				
Sample		6 hours (steaming)	1 hour	2 hours	3 hours	5 hours
Sample juice + B and C fractions in the proportions found in autumn tubers		44.3 %	42.6 %	42.6 %	48.4 %	44.7 %

The control experiment consisting of juice from spring tubers without any inulin fraction added gave a yield of 47.3 % alcohol calculated on the carbohydrates present in the mash.

From a practical point of view this question is not of very great importance. It is of greater importance to decide whether the cooking to which the tubers are usually subjected before fermentation is sufficient to render their inulin fractions fermentable, or whether a special acid hydrolysis of the tubers is essential for this purpose.

A number of experiments have been carried out to investigate this point. Tables IX and X contain the results obtained.

The following figures were actually obtained when calculating the results of the above experiment as the percentage of carbohydrates left after the completion of the fermentation (Table XI).

Table XI.

Amount of carbohydrates left in the mash after fermentation, and calculated as percentage of the initial carbohydrates present

Sample	Steamed for 6 hours	Cooked for 1 hour at 20 lbs.	Cooked for 2 hours at 20 lbs.	Cooked for 3 hours at 20 lbs.	Cooked for 5 hours at 20 lbs.
Spring juice + B and C fractions added in the proportions found in autumn tubers	48.7 %	11.3 %?	22 %	8 %	6.3 %

As a control, the spring juice without addition of the inulin fractions was fermented, and showed 5.7 % of the original carbohydrates remaining after fermentation.

It will be seen that boiling the mashes at a comparatively low pressure for a considerable time is quite sufficient to convert the inulin fractions of the tubers into fermentable carbohydrates, and that an almost full yield of alcohol may be obtained without a preliminary acid hydrolysis of the mashes.

Having ascertained the nature of the carbohydrates found in the tubers of the Jerusalem artichoke it was thought of interest to establish their relationship to the carbohydrates present in the actual parenchymatous tissues of the unripe stalks—the so-called pith. This tissue contains, on an average, 3 % of soluble carbohydrates which can be extracted with warm water. Subjecting this extract to the process used for isolating the carbohydrates of the tubers the following fractions were collected (Tables XII and XIII):

Table XII.

Fraction	Obtained by precipitation			
	with 55 % alcohol (B)	with 85 % alcohol (C)	with 98 % alcohol (D)	with ether and alcohol (E)
Percentage of total carbohydrates }	25.8	4.73	46.27	23.20

The four fractions isolated showed the following properties.

Table XIII.

Fraction	Specific rotation $[\alpha]_D^{20}$		Reduction of Fehling's solution %	Solubility in water	Appearance
	Before inversion	After inversion			
B	-35.4°	-85.6°	1.51	Slightly soluble	Snow-white light powder, tasteless
C	-30.71°	-74.29°	2.41	Slightly more soluble than B	Slightly yellowish sandy powder, slightly sweet
D	-17.91°	-59.39°	12.43	Soluble in all proportions of water	Yellow powder, giving brown solutions, very sweet
E	?	?	?	Soluble in all proportions of water	Light yellow gum giving solutions of the same colour, very sweet

It will be seen that not only are the substances isolated from the pith substantially the same as those isolated from the tubers, but they also appear to occur in the pith in about the same proportions as in the tubers.

All fractions isolated from the pith were tested in the same way as those isolated from the tubers and were found to behave similarly.

SUMMARY.

It has been shown that the autumn-gathered tubers and the unripe pith of the Jerusalem artichoke contain considerable proportions of an insoluble non-fermentable carbohydrate, apparently identical with inulin.

This carbohydrate disappears almost completely from the tubers towards spring and gives way to increased proportions of the more soluble carbohydrates which are already present in the autumn tubers. In addition there would appear to occur towards spring an increase in the dextrorotatory compounds of the tubers. Part of these at least is believed to be sucrose.

Though inulin by itself is unfermentable by yeast the experiments quoted in this report would appear to show that a comparatively mild autoclaving is sufficient to render it fermentable without the use of acid hydrolysis.

The observation of Tanret that inulin may be fermented by yeast when present in a solution of already fermenting carbohydrates has been confirmed, though not to the extent claimed by him.

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