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# Chemical species of iodine in some seaweeds
## II. Iodine-bound biological macromolecules

**Xiaolin Hou,[1]* Xiaojun Yan,[2] Chifang Chai[1]**

[1] *Institute of High Energy Physics, Academia Sinica, P.O. Box 2732, Beijing, P.R. China*
[2] *Institute of Oceanology, Academia Sinica, Qingdao, P.R. China*

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The distribution of iodine in various biological macromolecules in *Sargassum kjellmanianum* was studied using neutron activation analysis combined with chemical and biochemical separation techniques. The results indicate that iodine is mainly bound with protein, part of iodine with pigment and polyphenol, and little with polysaccharides, such as algin, fucoidan and cellulose. This result is significant for the mechanism of enriching iodine of algae and utilization of alga iodine.

## Introduction

A very high enrichment factor for iodine was found in marine algae.[1–3] The study on mechanism of enriching iodine in marine algae is important. In addition, algae are becoming a well-received green food, and also an important source of dietary iodine. Nevertheless, the bioavailability of various chemical species of iodine is remarkably different.[4,5] But, few work on this field is available.[6–9] For these reasons, the chemical species of iodine in marine algae were studied. We have determined the contents of total iodine, water soluble iodine, and $I^-$, $IO_3^-$ and organic iodine in water leachate in 14 marine algae.[10] In many seaweeds, more than 50% of iodine exists in water-insoluble form, bound with biological macromolecules. Inorganic iodine ($I^-$, $IO_3^-$) and most iodine-bound low-molecular organic compounds can be easily dissolved in water, whereas iodine-bound organic macromolecules cannot be dissolved by water.

*Sargassum kjellmanianum* is a very common seaweed in the coast of China, only 40% of iodine can be leached by water, and 25% of iodine in water leachate is organic iodine. In the present work, it was selected to study the distribution of iodine in various macromolecular organic compounds using neutron activation analysis combined with chemical and biochemical separation techniques.

In terrestrial plants, the content of iodine in spinach is higher, therefore, this plant was included to compare the difference of chemical species of iodine between marine and terrestrial plants.

## Experimental

### *Sampling and pre-preparation of samples*

*Sargassum kjellmanianum* was collected in April 1997, from Qingdao, China. After washed with sea water in situ to remove mud, sand and attached particles, it was put into clean polyphenol bags, transferred to the laboratory, and preserved at –20 °C. Prior to the experiment, the sample was thawed at room temperature, and ground with a grinder.

Spinach was purchased in Beijing market, and washed with deionized water to remove the attached dust on the leaf surfaces, and ground.

### *Leaching of Sargassum kjellanianum and spinach with various solvents*

For the investigation of leaching ratio of iodine by different chemical solvents, *Sargassum kjellmanianum* and 50 g fresh spinach were leached 3 times with different solvents, with stirring for 2 hours at room temperature or boiling temperature. These solvents include ether, ethanol, 0.1 mol/l HCl, and 0.1 mol/l KOH. The residues leached by HCl and KOH were washed until neutral with deionized water. For the leaching experiment with water, HCl and KOH, 50 g wet *Sargassum kjellmanianum* was used, while leaching with alcohol and ether, 10 g dry algae was used. The leached and original alga samples were dried at 45 °C for the determination of iodine by neutron activation analysis.

* E-mail: xiaolin.hou@risoe.dk

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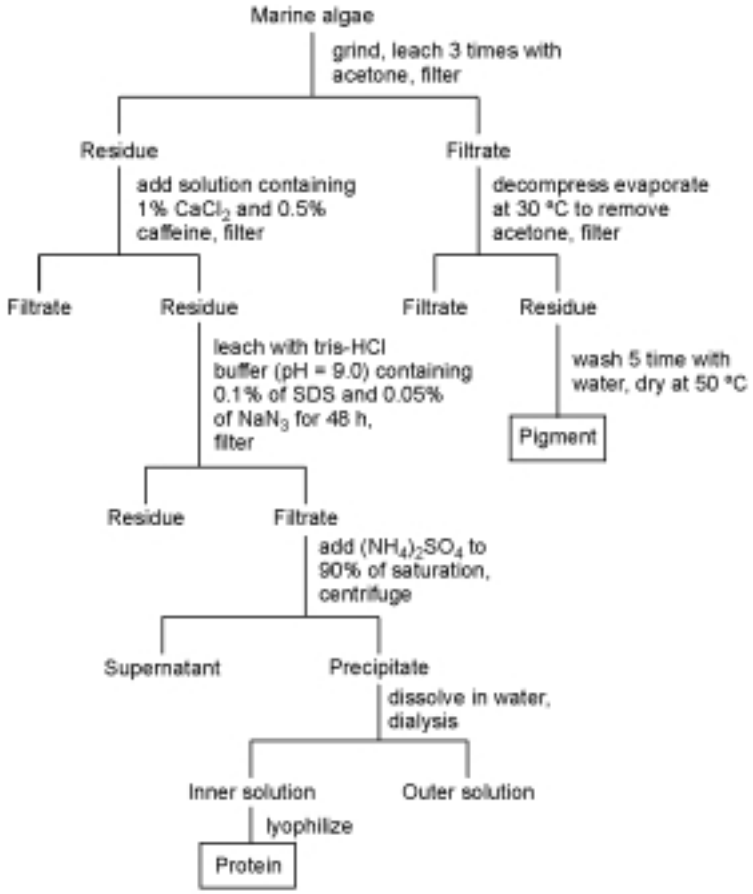


*Fig. 1.* Separation procedure of protein and pigment in marine algae

*Preparation of iodine-bound biological macromolecules*

Iodine-bound protein was leached with Tris-HCl buffer, and precipitated in saturated $(NH_4)_2SO_4$ solution. Separation procedures are shown in Fig. 1. Algal pigments were extracted with acetone.

Fucoidan was leached with boiling water, and precipitated with alcohol. Algin (alginic acid and its salts), a main component of brown algae, was also extracted with boiling deionized water. Ethanol (95%) was added to the leachate with a concentration of 30% to precipitate algin which was separated by centrifugation, washed with acetone and dried at 45 °C. For calculating the percentage of iodine in algin, an aliquot of algae was leached with $K_2CO_3$ solution, algin was separated by precipitating with HCl. The separation procedures are given in Fig. 2.

Polyphenol was extracted from algae with ethanol, and purified with PVPP column chromatography (Fig. 3).

*Determination of the contents of protein and polysaccharide in extraction*

The contents of protein and polysaccharide were determined by the methods described by BRADFORD[11] and LI.[12]

*Epithermal neutron activation analysis for iodine*

The prepared samples and standards were put into a BN shield capsule with wall thickness of 1.8 mm.

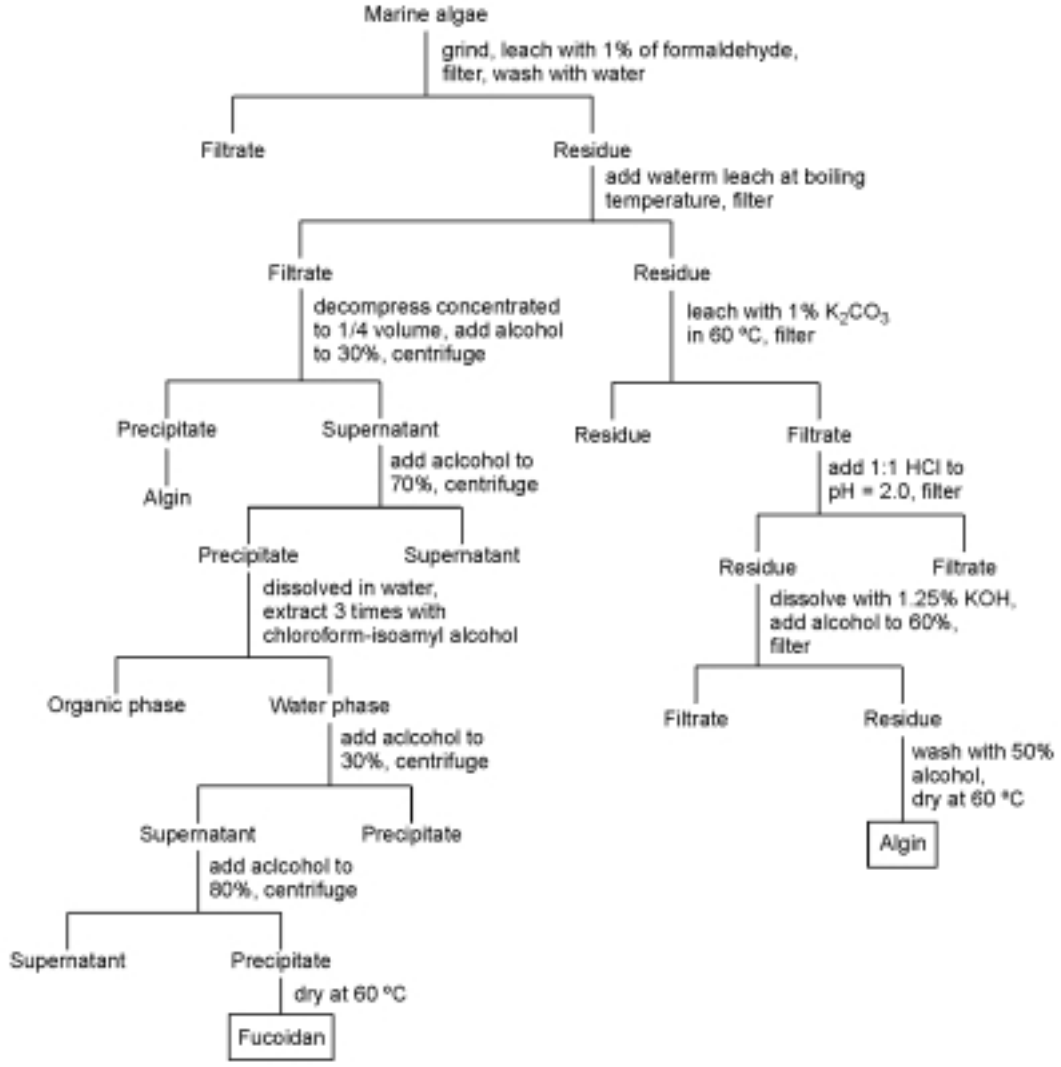


*Fig. 2.* Separation procedure of algin and fucoidan in brown algae

Then, the BN capsule was put into a polyethylene rabbit capsule and heat-sealed. The irradiation was carried out in an inner irradiation site of Miniature Neutron Source Reactor with the epithermal neutron flux of $2.6 \cdot 10^{10}$ n·cm$^{-2}$·s$^{-1}$. After 15-minute irradiation, the samples and standards were decayed for 5–15 minutes, then counted for 600 seconds. The 433 keV γ-rays of $^{128}$I was measured with a PC computer γ-multichannel analysis system. The software CIAE/SPAN developed by China Institute of Atomic Energy was used to analyze the γ-spectra. The concentrations of iodine and bromine in samples were calculated by using the comparative method. The detailed analytical method was described elsewhere.[13,14]

## Results and discussion

The concentration of iodine in *Sargassum kjellanianum* and spinach before and after leaching with various solvents are listed in Table 1. The leaching rate of iodine is the lowest in ether and the highest in KOH. Ethanol is moderate, and close to the extent of leaching in HCl and deionized water. For *Sargassum kjellanianum* and spinach, leaching rates of iodine in various solvents are relatively similar.

The leaching rate of iodine in *Sargassum kjellanianum* in water at room temperature (20 °C) is not significantly different from that leaching in boiling water. This result indicates that leaching by boiling does not decompose iodine-bound biological macromolecules or change the combined states of iodine in marine algae.

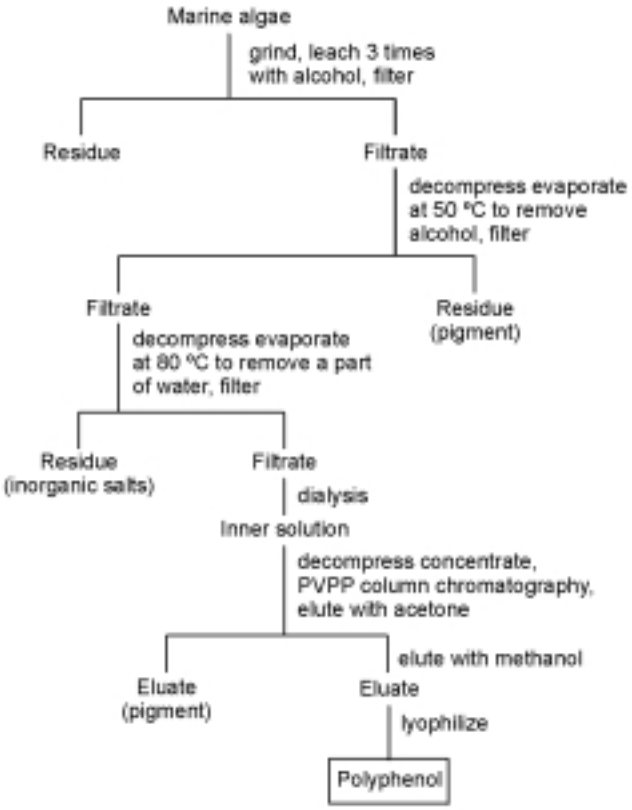


*Fig. 3.* Separation procedure of polyphenol in brown algae

*Table 1.* Leaching rate of iodine with various solvents from *Sargassum kjellanianum* and spinach

| Solvent | *Sargassum kjellanianum* | | Spinach | |
|---|---|---|---|---|
| | Concentration, µg/g | Leaching rate, % | Concentration, µg/g | Leaching rate, % |
| No leaching | 140.6 ± 7.6 | | 0.196 ± 0.043 | |
| Water (20 °C) | 149.7 ± 8.2 | 39.7 | 0.310 ± 0.032 | 29.4 |
| Water (99 °C) | 153.2 ± 5.8 | 40.0 | | |
| Ethanol | 121.6 ± 6.4 | 27.8 | 0.231 ± 0.028 | 43.9 |
| Ether | 129.5 ± 5.2 | 8.9 | | |
| 0.1 mol/l HCl | 151.9 ± 9.2 | 39.2 | 0.334 ± 0.029 | 28.4 |
| 0.1 mol/l KOH | 66.0 ± 3.2 | 93.3 | 0.177 ± 0.017 | 70.3 |

*Table 2.* Content of iodine in biological macromolecules in *Sargassum kjellanianum*

| Sample | Fractions | | Iodine | |
|---|---|---|---|---|
| | Weight, g, dry | Percentage, % | Concentration, µg/g | Percentage, % |
| Total algae | 20.0 | 100 | 153.5 ± 2.4 | 100 |
| Algin | 3.05 | 14.3 | 1.86 ± 0.30 | 0.185 |
| Fucoidan | 0.25 | 1.17 | 6.10 ± 0.25 | 0.05 |
| Protein | 1.30 | 6.5 | 403.2 ± 7.4 | 65.5 |
| Polyphenol | 0.53 | 2.50 | 178.7 ± 3.6 | 3.09 |
| Pigment | 0.35 | 1.64 | 138.7 ± 1.7 | 1.57 |

The contents and distribution of iodine in various biological macromolecules in *Sargassum kjellanianum* are listed in Table 2. Iodine is mainly bound with protein, part bound with pigment and polyphenol, and little with polysaccharide, such as algin and fucoidan.

*Protein in algae*

There are more than thousands proteins in a seaweed. The solvability and amino-acid sequence of these proteins are different, these solvability can be used to separate various protein. For plant proteins, a buffer such as tris-HCl was usually used for extraction.[15,16] We had tried to separate proteins directly by leaching with tris-HCl buffer and precipitating with $(NH_4)_2SO_4$. However, the proteins in leachate cannot be precipitated by $(NH_4)_2SO_4$ because of high viscosity. The main reason is that alginic acid and its Na, K and Mg salts with high content in brown algae can be dissolved by neutral or basic solution and cannot be precipitated by $(NH_4)_2SO_4$. In addition, after leaching, some pigments are also extracted. Thus, we applied acetone to remove the low-molecular weight compounds (i.e., pigment and fat), because of high solvability of these low-molecular weight compounds in acetone. The algin and polyphenol were then fixed with $CaCl_2$ via the formation of insoluble calcium alginate and with caffeine via the combination of polyphenol with caffeine.[17] Afterward, tris-HCl buffer (pH 9.0) was added to leach protein. It was found that protein extracted by this method can be easily precipitated by $(NH_4)_2SO_4$. Furthermore, a few drops of SDS and $NaN_3$ were added into the buffer to destroy the cell walls and protect leachate against bacteria.

The content of pure protein in protein extract is very low (~20%), and other component is mainly poly-saccharide: The content of iodine in various polysaccharide are quite low, thus, iodine is mainly bound with protein, which was calculated be 1405 μg/g protein, that is significantly higher than that in other components. Because only part of protein in algae can be extracted by this method, total protein content in *Sargassum kjellanianum* was calculated by determination of total nitrogen content. The result indicates that the concentration of protein is 6.5±0.3%. Therefore, the iodine content in protein amounts to 65.5% of total iodine content in *Sargassum kjellanianum.* It has been reported that 40% of iodine in *Sargassum kjellanianum* is water soluble, and 26% of iodine in water leachate is organic iodine.[10] Thus, total organic iodine accounts to 72% of total iodine. In this work, besides iodine-bound protein, ~5% of iodine bound with other organic compounds, such as pigment and polyphenol. It means that total organic iodine will reach 70% of total iodine content.

*Pigment and polysaccharide*

Besides protein, the contents of iodine in pigment and polyphenol are also high. *Sargassum kjellanianum* is a brown alga, chlorophyll and many other pigments are also present, such as xanthophyll, carrotene and fucoxanthin. These pigments are soluble in acetone, petroleum ether and methanol, their solubility is very low in water. Acetone is usually used to extract them from algae.[17,18] Besides pigments, other fatty materials, such as vegetable fat, free fatty acid and posphatide can also be dissolved by acetone, ether and other organic solvent. In the experiment, *Sargassum kjellanianum* was leached 3 times with ether at 50 °C for 5 hours, after ether was removed, little of material remained. Thus, pigment is the main compound in acetone leachate, the contents of other low-molecular weight organic compounds are very low.

Because fresh algae was used, inorganic iodine and some other water-soluble materials in alga cell can be included in the leachate. For removing inorganic iodine and some polyphenol, after evaporated acetone, leachate was then filtered, and washed with water.

Phenolic compounds are an important chemical component in marine algae. In brown algae, they are mainly phloroglucinol tannins (brown seaweed polyphenols), their molecular weights range $10^2$~$10^5$ Da.[17] Brown seaweed polyphenols are easily dissolved by ethanol, methanol, ethyl acetone and other organic solvents, ethanol was usually used.[17] In the experiment, besides polyphenols, inorganic salts (include inorganic iodine) and pigments also exist in the leached because of cell solution of fresh algae. Thus, after concentrated by evaporation, the leachate was filtered for removing main inorganic salt and dialysis was followed to remove residual inorganic salts. Meantime, the inorganic iodine was also removed. In addition, pigments were also removed by filter after all alcohol was evaporated because of its low solubility in water. For purifying the polyphenols, PVPP column chromatography was applied to remove residual pigment, the pure polyphenol was then obtained.

*Polysaccharide*

Carbohydrate is a main component of marine algae, it accounts to 30~50% of total dry weight of algae.[3] In brown algae, the carbohydrate mainly includes algin, fucoidan, cellulose and mannitol. Mannitol is a low-molecular weight compound, it is easily dissolved by water, and cannot combine with iodine. Thus, it was not studied in this work.

Algin is a main component in brown algae. It lies in the cell wall, and exists as alginic acid, and its Ca, Mg,

K and Sr salts. In the outer layer of the cell in marine algae, the algin exists as its Ca salt (calcium alginate), and in the inner part, its K, Na, and Mg salts. Alginic acid and its di- and tri-valent metal salts (except Mg and Hg) are insoluble in water, whereas, its K, Na and Mg salts are water-soluble.[3,17] Thus algin is usually extracted by $K_2CO_3$ or $Na_2CO_3$ solution via exchanging insoluble alginate into its soluble Na or K salts, then adding acid into leachate to precipitate algin via transferring sodium alginate or potassium alginate into insoluble alginic acid. However, it is known that alkali solution can hydrolyze some organic macromolecules and release iodine. Thus, it is unsuitable to study the combination of iodine with algin. In this work, water soluble algin was separated by leaching with hot water and precipitating with 30% ethanol. For calculating the percentage of iodine in algin, all algin was separated from algae by leaching with $Na_2CO_3$, and purifying algin, KOH solution was added into crude alginic acid precipitate to dissolve it, and ethanol was applied to precipitate algin via remove water in it.

Fucoidan is a soluble polysaccharide, it belongs to an intracellular component; water or diluted acid was usually used to leach it from algae at higher temperature, and ethanol or quarternary ammonium salt was used to precipitate it from the leachate.[3,17,19] In this work, hot water and ethanol were used to leach and separate fucoidan. Because a part of protein can also be leached and precipitated by hot water and ethanol, a method extracting with chloroform-isoamyl alcohol was applied to remove protein from fucoidan.[20] In addition, for obtaining pure polysaccharide, 1% of formaldehyde was firstly added to remove pigment through fixing some protein absorbed pigments.

*Combination states of iodine in various biological macromolecules*

From Table 2, it is clear that iodine is mainly combined with protein, pigment and polyphenol, whereas the content of iodine in various polysaccharides is very low.

No branched chain and unsaturated C-C bond exists in β-D-mannuronic and α-L-guluranic acid which constitutes alginic acid molecule. Therefore, iodine does not bind with algin via a C-I bond.

Fucoidan consists of L-fucose-4-ester sulfate and a little of xylose, mannose and lactose, it is also impossible to combine iodine combine in fucoidan. However, fucoidan is likely combine with some proteins to form a sugar-bound-protein, iodine can bind with the proteins in this biological macromolecule. However, due to the protein has been removed in the preparation of fucoidan, sugar-bound protein was also removed. Thus, the content of iodine in fucoidan is very low. A little of iodine in fucoidan is likely inorganic or other organic bound iodine wrapped in it.

Brown seaweed polyphenol is a polymer of phloroglucinol, there are many unsaturated conjugated double bonds and replaceable sites in the phloroglucinol. It can combine with iodine and other halogen by halogenation reaction, and produce iodine-bound polyphenols containing C-I bonds. Halogenated phenol, including iodinated phenol, can be easily synthesized in the laboratory.[21] Some bromine- and chlorine-bound polyphenols have been found in brown and red algae.[3,22–24] But iodine-bound polyphenol has not been conformed in algae. From this result, it can be suggested that iodine-bound polyphenols also exist in marine algae. Iodine-bound pigment has not been reported.

Protein consists of various amino-acids, many iodine-containing amino-acids and their derivatives, such as monoiodo-L-tyrosine, diiodo-L-tyrosine, diiodo-L-tyronine, triiodo-L-thyonine and tetraiodo-thyonine, have been found in the leachate or hydrolysate of marine algae.[6–8] The protein fraction extracted in this work is a crude protein, the combination state of iodine in protein needs to be further studied. In the experiment, protein fraction was dialyzed against distilled deionized water using two kinds of dialysis membranes of molecular weight cut-off (MWCO) of 2,000 and 10,000. The results indicate that the contents of iodine and protein after dialysis with 2 different dialysis membranes are not significantly different. Thus, it is suggested that iodine is likely combined with high-molecular protein.

*Leaching rate of iodine with various solvents from Sargassum kjellanianum and spinach*

For water, ethanol and ether, the polarity of water is the highest, and of ether is the lowest. Inorganic iodine ($I^-$, $IO_3^-$) is easily dissolved by polar solvent. Its solubility in water is higher than that in ethanol, and very low in ether. Polyphenols, pigments and fat, are easily dissolved by organic solvent, e.g., polyphenols can be easily dissolved by ethanol, whereas many low molecular weight proteins, polypeptides and polysaccharide are water-soluble. In *Sargassum kjellanianum*, iodine exists mainly as iodine-bound macromolecular protein and inorganic ions, the contents of iodine in pigments and polyphenols are lower. Thus, in the leachate of 3 solvents, iodine content in water leachate is the highest. The main reason of close leaching rates of iodine with $H_2O$ and HCl is that solvability of iodine ions in both of water and diluted HCl are higher, and of macromolecular protein, pigment and fatter materials are very low.

The leaching rate of iodine with ethanol in spinach is higher than that in *Sargassum kjellanianum*. As fresh spinach contains some water and part of water-soluble

iodine can be leached. The high leaching rate of iodine in diluted KOH solution for the two types of samples was found, it is probably because most of biological macromolecules, such as protein, and polysaccharides, can be hydrolyzed by alkali solution, and iodine bound in this organic compounds was removed in alkali solution.

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