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Research article

Extraction and Properties Evaluation of Chitin and Chitosan Prepared from Different Crustacean Waste

Nafisa Nawar Tamzi*, Mohammad Faisal, Tahsin Sultana and Subrata Kumar Ghosh

Department of Fishing and Post-Harvest Technology, Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University, Khulshi, Chattogram-4225, Bangladesh

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**Corresponding author:*

Cell: +8801778000559
Email: nafisacvasu1@gmail.com

A B S T R A C T

Chitin and chitosan are the most widely used raw material as biocompatible product that is naturally available in different crustacean shells. The aims of this research were the production of chitin and chitosan from crustacean waste, and analyze their quality based on proximate composition, WBC, FBC, solubility, pH, and DDA. Chitin and chitosan were prepared from the waste of three crustacean species viz., *P. monodon*, *M. rosenbergii*, and *S. serrate* through a chemical treatment. The yield of prepared chitin and chitosan were varied from 10.43-12.32% and 4.57-5.78% respectively, with pH ranged from 7.1-7.9. The physical appearance of chitin and chitosan, based on color was found yellowish white and bright off-white respectively. Chitosan from *M. rosenbergii* shell was showed high water binding capacity (420%) and fat binding capacity (276%) than chitosan prepared from other shells. The solubility of chitin in 1% acetic acid solution was very low (20-39%) due to the presence of the acetyl group. The degree of deacetylation of chitosan was determined by the acid-titration method and it was found high (87%) in chitosan prepared from *M. rosenbergii* shell. The DDA value obtained from chitosan was high ranged from 69-87%, while the solubility of chitosan achieved up to 96%. For determining the quality changes both chitin and chitosan were stored at ambient temperature in air-tight condition. There was a little much changes in moisture and solubility remain unchanged in chitin and chitosan structure. The quality of chitin and chitosan prepared from *M. rosenbergii* shell showed the better quality among the above mentioned three sources. Beside this, the present research result also indicates that the chitin and chitosan prepared from different crustacean waste could be utilized as a raw product for different food and pharmaceutical industry.

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1. INTRODUCTION

The fishery is an essential industry in Bangladesh and plays a major role in alleviating protein deficiency, malnutrition, generating employment, and foreign exchange earnings. Fish and fishery products are considered most consumed as food in almost all regions around the world and a highly consumed food by the population because of its availability, flavor, nutritional value, and palatability. Currently, a large number of fish processing plants (around 100) are installed to produce a fishery product for exporting to other foreign countries (DoF, 2019). Shrimp, prawn, and crab are considered important aquaculture products that contribute to the foreign exchange earnings of the country. In the last decade, the yearly export of frozen shrimp and crabs in Bangladesh ranged between 15,000 and 26,000 metric tons (Saha*et al*., 2005).

During production in the processing plant, a large amount of wastes (around 40-80%) produced as bio-waste (Suparno and Poernomo, 1992; Irianto and Giyatmi, 1997). This fishery waste contains head, shell, tail, and viscera (Khan and Nowsad, 2013) that haslow commercial value. Year after year production of fishery waste is increasing worldwide with the volume of exportable frozen products. The mass amount of these wastes creates environmental pollution and becoming a global concern that is affected by several biological, technical, and operational factors (Kim and Mendis, 2006; Arvanitoyannis and Kassavetia, 2008). Every year around 30,000 tons of shrimp and prawn wastes are dumped by the processing industries of Bangladesh having no economic value (Nowsad, 2005). However, these dumped wastes can significantly contribute to the economy if those are utilized properly to produce different by-products such as chitin and chitosan. Chitin is a naturally occurring biopolymer that has a highly linear structure and a versatile environment-friendly modern material. It is a white, hard, inelastic, nitrogenous polysaccharides, found in the exoskeleton of invertebrates (Dutta and Tripathi, 2004). Chitin is composed of β (1-4)-linked 2-acetamido-2deoxy-β-D-glucose whereas, chitosan (principle derivation of chitin) composed of α (1-4)-linked2-amino -2-deoxy-β-D-glucopyranose. Shrimp and crab shell contains 8-10% and 24-29% chitin respectively, which is an expensive ingredient used in many foods, cosmetics, and pharmaceutical products (Suparno and Poernomo, 1992; Djaeni, 2003).

Chemical and enzymatic treatments are done to prepare chitin and chitosan from crustacean shell; yet, no standard method has been adopted (Younes and Rinaudo, 2015). During chitin preparation some major constitutes calcium carbonate, potassium phosphate and protein are need to be removed. Both demineralization and deproteinization are methods, used for removing protein, minerals, lipids, and other pigments from the shell. After the production of chitin and chitosan, quality maintenance is an important factor. Proper storage and maintenance of quality can increase the shelf life of chitin and chitosan.
In Bangladesh, annually about 100-200 tons of chitin and chitosan are imported from other countries mainly for food and medicine industries (Islam et al., 2016). Commercially production of chitin and chitosan within the country can be reduced dependency on imports for these valuable products. The attempts of the studywasto produce good quality chitin and chitosan from different crustacean waste and identify good chitin and chitosan producing crustacean shell. Through this technique, owner of the shrimp processing industry canproperly use the unutilized crustacean waste instead of dumping.

2. 2. MATERIALS AND METHODS

Collection of raw materials

Shrimp (*Penaeusmonodon*), prawn (*Macro-brachium rosenbergii*) and crab (*Scylla serrata*) shells were used as raw material for chitin and chitosan preparation. The raw shrimp and prawn waste were collected from a processing plant which is located in Chattogram. For crab waste, freshly crab was collected from market and seperated their shell with knife. After collection, the crustacean wastes were washed with potable water for two times and stored at -18 to -20℃ till needed. The study was performed in the Nutrition and Processing Laboratory; Disease and Microbiology Laboratory of Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University, Chattogram.

Preparation of chitin from crustacean waste

The chitin was prepared through two steps: (i) demineralization and (ii) deproteinization. Firstly clean crustacean wastes were taken in a beaker for demineralization. In this regard 1.75N of HCl was added in this beaker and kept overnight at room temperature. Then the soaked samples were washed with distilled water until the neutral pH (pH6.8-7.4) was achieved. After that allowed to dry for few hours.

After drying, the demineralized samples were soaked in 1.25M NaOH solution for deproteinization purpose. Then heated at 80℃ temperature for 2 hours. After that, the residue washed with distilled water till neutral pH was achieved. The whole process was continued for three times. The purified samples were dried using hot air oven at 70℃ for 8 hours. The dried samples were named as chitin and grounded to make small flakes.

Preparation of chitin from chitosan

Chitosan was prepared from chitin through deacetylation process. The chitin samples were

soaked in 40% of NaOH at 100°C for 2 hours. Then the samples were drained and washed until neutral pH was achieved. This process was continued for 3 times to obtain pure chitosan. Finally, chitosan samples were dried in hot air oven at 80°C for 8 hours.

Nutritional profile analysis

The proximate composition (protein, lipid, moisture and ash) of raw crustacean waste, chitin and chitosan were analyzed accordng to AOAC method (AOAC, 2016) with certain modifications.

Microbial quality assessment

Microbiological analysis was also conducted according to AOAC (AOAC, 2016) and FDA BAM method (FDA BAM, 2007) with certain modifications. Presence of total viable bacteria in raw crustacean waste were determined through consecutive decimal dilution method.

Properties analysis of chitin and chitosan

Determination of yield

The yield of chitin and chitosan was determined according to the formula:

$$Yield\ (\%) = \frac{Dried\ chitin\ or\ chitosan\ weight}{Raw\ crustacean\ shell} \times 100$$

Determination of water binding capacity (WBC)

Water binding capacity of chitin and chitosan are determined according to Knorr (1982) with some modifications.

$$Water\ binding\ capacity\ (WBC\ \%) = \frac{Water\ bound\ (g)}{Initial\ sample\ weight\ (g)} \times 100$$

Determination of Fat binding capacity (FBC)

Fat binding capacity was determined according to Knorr (1982) with some modifications.

$$Fat\ binding\ capacity\ (FBC\ \%) = \frac{Fat\ bound\ (g)}{Initial\ sample\ weight\ (g)} \times 100$$

Determination of degree of deacetylation (DDA)

The degree of deacetylation was measured through acid-base titration method (Domard and Rinaudo, 1983) with some modifications. This is determined through following formula:

$$Degree\ of\ deacetylation\ (\%) = \frac{M \times 0.0994}{C1V1 - C2V2} \times 0.016$$

Here,

C1= Concentration of standard HCl aqueous solution (mole/L)

C2= Concentration of standard NaOH aqueous solution (mole/L)

V1= Volume of standard HCl aqueous solution used to dissolve chitosan (ml)

V2= Volume of standard NaOH aqueous solution during titration (ml)

M= Weight of chitosan

0.016 is the equivalent weight of NH_2, and

0.0994 is the proportion of NH_2 group by weight in chitosan

Solubility determination

Prepared chitin and chitosan were dissolved at 1% acetic acid solution. The concetration was determined according to Puvvada *et al.,* (2012) with some modifications.

$$Insoluble\ (\%) = \frac{Insoluble\ (g)}{Sample\ weight\ (g)} \times 100$$

$$Solubility\ (\%) = 100 - \%\ of\ insoluble$$

Storage of chitin and chitosan

After production of products (chitin and chitosan) were packed in sealed packet and kept in ambient temperature.

Statistical analysis

The data analysis was examined through one-way analysis of variance (ANOVA) and Duncan's multiple range tests using SPSS software at 95% confidence level. The significance level was set for analysis $p \leq 0.05$.

3. RESULTS AND DISCUSSION

Yield determination of chitin and chitosan

Chitin and chitosan were prepared from shrimp, prawn, and crustacean shell by chemical treatment. The process involved in chitin and chitosan preparation viz., demineralization, depro-teinization, and deacetylation. In this study yield of chitin was found from different shells varied from 12.19 to 12.32% (Table 1). The extraction rate of chitin and chitosan mainly

depends on the demineralization process because the remaining minerals content in chitin and chitosan increased the yield. According to Hossain and Iqbal (2014), a lower concentration of HCl could not remove minerals from the shell and which increased the yield. The yield of chitosan was lower than chitin produced from the different shells. Crab shell was showed high yield (5.78 %) chitosan than that of prawn (4.57%) and shrimp (5.62%) shell. During the deacetylation process, a high concentration of NaOH with extended heating time reduced the acetyl group from chitin, subsequently decreased the chitosan yield compared to chitin.

Table 1: Yield of processed chitin and chitosan from crustacean shell

Sample	Yield (%)	
	Chitin	Chitosan
Shrimp shell	12.19	5.62
Prawn shell	10.43	4.57
Crab shell	12.32	5.78

Quality analysis of raw crustacean shell

The moisture content of raw crustacean waste below 30% with high protein and ash content has been presented in the table 2. During chitin and chitosan preparation, protein and ash content of raw crustacean shell is an important indicator of the effectiveness of the deproteinization and demineralization process. From these three crustacean shells, shrimp shells contain 43.50±0.55% protein which was higher than other shells. An extremely high amount of protein increased the utilization of these shells in human food also (Islam et al., 2016). In this fresh raw material, microbial load recorded less than 10^6 CFU/g. The values of these properties indicate the good quality raw material, which assures the production of the high-quality final product.

Proximate composition of chitin and chitosan

Moisture is an important factor in the finished product which indicates the ultimate standard quality of this product. The moisture content of purified chitin from different crustacean waste was varied from 10.25-13.37%. In the current study, it was found that chitin contains higher moisture than chitosan. From the previous study, the higher moisture content in the chitosan increasing the degree of chitosan polymer damage via hydrolysis reactions (Viljoien et al., 2014). The moisture content of crab chitosan was reported 8.65±0.11% which was lower than the other two types of chitosan prepared from *P. monodon* and *M. rosenbergii*. Maintenance of moisture around 10% can extend the shelf life of chitosan (Nouri et al., 2016).

In the raw crustacean shell, the crude protein content was varied from 30.48-43.50%, whereas, the produced chitin and chitosan protein content was varied from 2.67-4.7% and 2.31-3.50% respectively.

Table 2: Quality analysis of raw crustacean waste

Sample	Protein (%)	Lipid (%)	Moisture (%)	Ash (%)	SPC (CFU /g)
P. monodon shell	43.50 ± 0.55^a	3.67 ± 0.37^b	22.31 ± 0.20^c	23.65 ± 0.24^c	$2.88\times10^5\pm0.12^a$
M. rosenbergii shell	38.27 ± 0.11^b	4.78 ± 0.20^a	25.54 ± 0.10^a	25.98 ± 0.19^b	$2.53\times10^5\pm0.10^b$
S. serrata shell	30.48 ± 0.13^c	2.87 ± 0.06^c	23.49 ± 0.14^b	26.83 ± 0.12^a	$2.37\times10^5\pm0.10^b$

Means ± Standard deviation

Different letters indicate values in the same column are significantly different ($P<0.05$)

The minimum protein content was observed in crab chitin and chitosan. From this lower amount of crude protein, it was found that increase the effectiveness of deproteinization during production. The lipid value was found nil in both chitin and chitosan.

The ash content of chitin and chitosan from different crustacean shells were varied 1.18-1.93%and0.95-1.17% respectively. There is a correlation between the ash content of the final product and the demineralization process. It was reported that high-quality chitosan contains an ash value of around 1% (No and Meyers, 1995). The good quality of chitin and chitosan also depends on the pH values. The pH content of chitin was varied from 7.1-7.5 and chitosan was 7.8-7.9. It was reported that commercial chitosan contains a pH value of around 8 (Renuka et al., 2019). The values of the proximate composition of chitin and chitosan are shown in figure 1 (A, B and C).

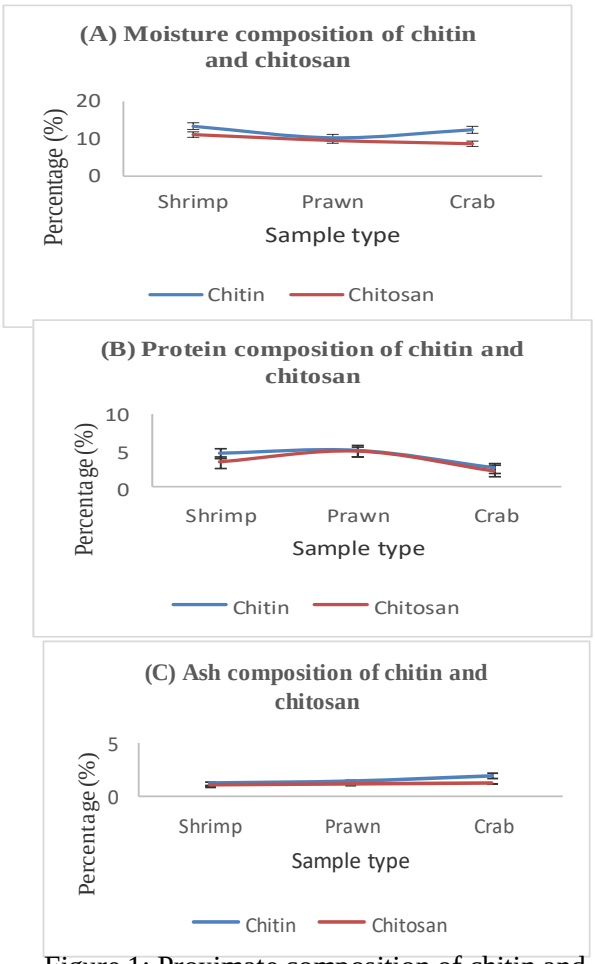

Figure 1: Proximate composition of chitin and chitosan in dry weight basis (A,B and C)

Properties analysis of chitin and chitosan

The characteristics of chitin extracted from *M. rosenbergii* shell was contained off-white color while chitosan was showed bright off-white (Table 3). Chitin and chitosan were prepared from *P. monodon* and *S. serrata* shell both were yellowish-white and off-white respectively. This was happened due to deacetylation of chitin to produce chitosan remove yellowish-white color to bright color during removal of an acetyl group. From this study, it became clear that chitin and chitosan having. It was reported that *P. monodon* shells might brighter color produced from *M. rosenbergii* shell contain higher initial redness which becomes reddish during heat treatment due to the chemical binding of pigments (Islam et al., 2016). In the same way, this might happen for chitin and chitosan prepared from *S. serrate* shell.

Table 3: Characteristics (color) of chitin and chitosan

Sample	Chitin	Chitosan
*P. monodon*shell	Yellowish white	Off-white
M. rosenbergii shell	Off-white	Bright off-white
S. serrate shell	Yellowish white	Off-white

Water binding capacity (WBC) and Fat binding capacity (FBC) analysis

The water-binding capacity was higher in chitosan than chitin (Figure 2). Water binding capacity of chitin was prepared from different crustacean shells were varied from 111-114% whereas for chitosan were varied from 306-420%. Chitin and chitosan produced from *M. rosenbergii* both showed WBC 110% and 420% respectively, which were higher than *P. monodon* and *S. serrate* shell. The water absorption of chitin and chitosan depend on differences in crystallinity of the products, particle size, and amount of salt-forming groups and protein content of the materials (Rout, 2001; Knorr, 1982). Both water binding capacity and fat binding capacity have a very high negative correlation with the other physiological characteristics viz. viscosity and molecular weight, degree of deacetylation, and moisture content (Rout, 2001). We also observed the fat binding capacity, and chitosan showed high efficiency of fat binding. The fat binding efficiency of chitosan was varied from 215-276% (Figure 3). Fat binding capacity depends on the changing of the sequence of steps and it was observed that when demineralization is conducted before deproteinization FBC is increased (Rout, 2001).

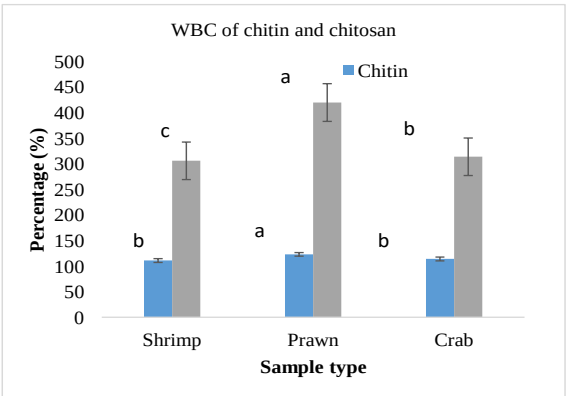

Figure 2: Water binding capacity of chitin and chitosan

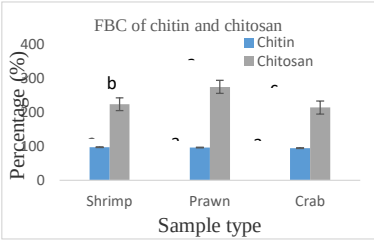

Figure 3: Fat binding capacity of chitin and chitosan

Solubility analysis

Solubility is an important factor in the quality determination of chitosan because high solubility indicates good quality chitosan. It is difficult to dissolve chitosan in water, alkaline solution, or organic solvents but the presence of an amino group in chitosan leads to increase dissolving ability in dilute aqueous acid solution (Esam et al., 2009). The solubility of chitin obtained from different crustacean shell was varied from 20-39% and showed lower solubility compare to chitosan. This happened chitin contains a high amount of acetyl group and solubility depends on the rate of removal of the acetyl group from chitin to produce chitosan. *M. rosenbergii* shell produced chitosan contain 96% solubility which was higher than other chitosan produced from *P. monodon* and *S. serrate* shell. There is a study performed by Patria (2013), was obtained solubility ranged from 17.43-95.29% was found similar to the present study.

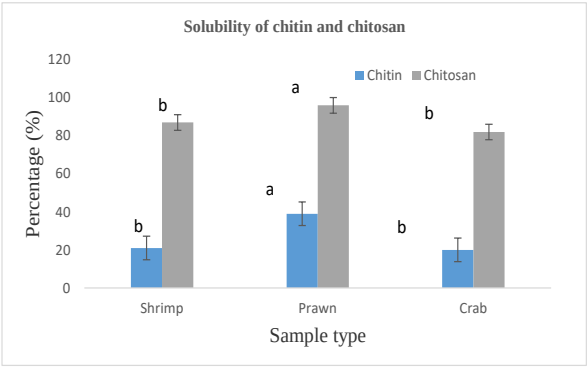

Figure 4: Solubility of chitin and chitosan

Relationship between solubility and degree of deacetylation

The solubility of chitosan was increased with the increase of the degree of deacetylation has been presented in table 4. Chitosan was showed both hydrophilic and hydrophobic because of the presence of glucosamine and N-acetylglucosamine in the chitosan structure. During the deacetylation, the acetyl group was only removed from the chitin and only the amine group remain in the prepared chitosan. Amine group contains hydrogen ion on the other hand acetic acid containing a carboxyl group that facilitates the dissolution of chitosan through hydrogen interaction between the carboxyl group and the amino group (Patria, 2013). The high rate of the degree of deacetylation depends on the high alkali concentration and heating time. The degree of deacetylation of chitosan produced by *P. monodon*, *M. rosenbergii*, and *S. serrate* shell were 72%, 87%, and 69%, respectively. Chitosan from *M. rosenbergii* shell was showed 87% DDA while the solubility was 96%. The similarities were also found for the other two chitosan prepared from *P. monodon*, and *S. serrate* shell. This might be happened due to a lower degree of deacetylation which means the presence of the acetyl group in chitosan. It was reported that lower solubility and degree of deacetylation values suggested incomplete removal of protein and acetyl group from chitosan (Brine and Austin, 1981).

Table 4: Solubility and degree of deacetylation of chitosan

Sample	Solubility (%)	DDA (%)
P. monodon shell	87±2.00^{b}	72±2.00^{b}
M. rosenbergii shell	96±2.65^{a}	87±2.00^{a}
S. serrate shell	82±2.65^{b}	69±3.61^{b}

Results are means ± Standard deviation
Values in the same column are significantly different ($P<0.05$)

Changes of quality during storage

Changes in quality of chitin and chitosan were determined on the basis of changes in moisture and solubility composition. After production, chitin and chitosan were stored in air-tight packed and stored at room temperature. During storage, maintenance of moisture content of chitin and chitosan is important due to its hygroscopic in nature. After 8 months of storage of chitin, there were little changes in moisture content while, solubility remains unchanged. Changes of moisture content of chitin produced by *P. monodon*, *M. rosenbergii*, and *S.*

serrate shell were showed 0.33%, 0.25%, and 0.5% respectively (Table 5).

During chitosan storage solubility also remain unchanged which indicates the quality of chitosan is in good condition. After 8 months storage changes moisture of chitosan from *P. monodon* was 0.84%, *M. rosenbergii* was 0.5%

and *S. serrate* was 0.39%. On the basis of changes of moisture and solubility, both chitin and chitosan were showed good quality during storage time. The shelf life of chitosan produced from *P. monodon* and *M. rosenbergii* were 12 months in terms of pH, solubility, and moisture content (Islam et al., 2016).

4. CONCLUSIONS

The chitin and chitosan obtained from different crustacean wastes were showed good quality with an average yield of 11.65% and 5.32% respectively. The quality of chitin and chitosan was evaluated based on proximate composition, pH, FBC, WBC, and DDA. The moisture and ash content of chitin and chitosan were ranged from 8.65-13.37% and 0.95-1.93% respectively that indicates good quality of chitin. In this study, the solubility of chitosan was high compare to chitin and it ranged up to 96% while chitin ranged up to 39%. Chitosan prepared from prawn shells have high solubility with a high degree of deacetylation (87%). From the above findings, we found that *M. rosenbergii* shell is an excellent source of chitin and chitosan than the other two sources mentioned in this study.

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Table 5: Quality changes of chitin during storage

Storage time	***P. monodon* shell**		***M. rosenbergii* shell**		***S. serrate* shell**	
	Moisture (%)	Solubility (%)	Moisture (%)	Solubility (%)	Moisture (%)	Solubility (%)
0 days	13.37±0.13	21	10.25±0.12	39	12.43±0.07	20
8 months	13.7±0.04	21	10.5±0.04	39	12.93±0.03	20

Table 6: Quality changes of chitosan during storage

Storage time	***P. monodon* shell**		***M. rosenbergii* shell**		***S. serrate* shell**	
	Moisture (%)	Solubility (%)	Moisture (%)	Solubility (%)	Moisture (%)	Solubility (%)
0 days	11.1±0.12	87	9.52±0.11	96	8.65±0.11	82
8 months	11.94±0.04	87	10.02±0.03	96	9.04±0.04	82

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