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EFFECT OF PROCESSING ON TOTAL STARCH, AMYLOSE AND RESISTANT STARCH CONTENT OF SAGO

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ABSTRACT
Total starch, amylose and resistant starch content were estimated in raw, soaked and boiled sago. The total starch content of raw sago was 81.9±2.7g% and 81.1±3.9g% in large and small pearls, respectively. Amylose comprised 67-72 percent of total starch. Resistant starch content in raw sago was 2.84±0.22 g% and 2.59±0.41 g% in the large and small pearls. Effect of boiling sago in milk and water with two ratios of sago: fluid – 1:2 and 1:4 was examined. Boiling led to loss of both starch and amylose, with loss of the latter being greater. Loss of both total starch and amylose was greater when milk was used for boiling than when water was used and was slightly higher when the amount of fluid used for boiling increased from 1:2 to 1:4. Also, loss in large pearls was more than in small pearls. In contrast, boiling sago resulted in an increase in resistant starch content. The percent increase upon boiling in water, was high varying from 88 to 93%; whereas cooking in milk resulted in a comparatively lower increase of only 8-15%. The results of the study indicate that cooking in water is preferable if resistant starch content of sago has to be increased.

Key words: Amylose, Resistant starch, Total starch, sago.

INTRODUCTION
Starch is quantitatively the most abundant polysaccharide in plants and is a major source of energy. Starch is composed of amylose and amylopectin, with the proportion of these varying from food to food. Until 1982, starch was considered to be completely digestible. However, it is now well established that some part of starch escapes digestion by pancreatic amylase and enters the colon. This fraction is referred to as 'resistant starch (RS) and is a non-glycemic carbohydrate (Alsaffar, 2011). RS has several physiological benefits and is regarded as an important dietary component. RS content is influenced by intrinsic and extrinsic factors as well as processing techniques (Sajilata and Singhal, 2006; Alsaffar, 2011).

In India, among the various foods, sago is one of the high starch foods consumed by large segments of the population. Sago is a processed food starch marketed as small globules or pearls. It is popularly consumed during fasting. Although sago is manufactured from the starchy core of the sago palm such as *Metroxylon sagu* and *M. rumphii*, in India, it is generally obtained from tapioca tuber (*Manihot esculenta*). The pearls are made in sizes varying from 1 mm to 5mm. The pearls are generally soaked in water and then made into different snacks by cooking with different spices. Alternatively a sweet preparation 'kheer' is made by boiling with milk and/or water. Sago contains about 87 percent carbohydrates (NIN, 2009). However, there is little information available about its content of amylose, amylopectin and resistant starch. Hence the present study estimated the content of amylose and resistant starch in raw pearls of two different sizes and examined the effect of the commonly used cooking techniques on the content of these two components.

MATERIALS AND METHODS

PROCUREMENT OF SAMPLES
Large and small varieties of sago were purchased from 6 different markets in Mumbai city. The large variety had a diameter ranging from 2.5 -3.0 mm. The diameter of the small variety was 1mm. Two kilograms of each sample was purchased for studying the effect of various processing techniques as well as analysis of the raw samples. Domestic processes generally used in India for preparation of sago products were studied, that included soaking, boiling in water and boiling in milk.

SOAKING

600 g of each sample was drawn by quartering method and was soaked in water 1:2 w/w and kept at room temperature for 8 hours. There was no excess water after soaking since the pearls had imbibed all the water. The soaked samples were then divided into two equal portions by weight. One portion was kept aside for analysis of the soaked samples.

BOILING IN WATER

From the second portion, 50 gm of the soaked sample was taken by quartering method and boiled in water for two minutes, using two different proportions of sago to water – 1:2 and 1:4 w/w.

BOILING IN MILK

50 grams of soaked sample was boiled for 3 minutes in cow's pasteurized milk containing 3.5%fat, using two different proportions of sago to milk– 1:2 and 1:4 w/w. All samples were cooled to room temperature before analysis. All samples were prepared and analysed in duplicate for total starch, amylose and resistant starch.

CHEMICALS

All chemicals were of analytical grade. Enzymes – fungal α-amylase, amyloglucosidase and bacterial protease were procured from Advanced Enzyme Technologies Ltd, Thane. Glucose oxidase peroxidase (GOD-POD) was procured from SPAN Diagnostics, Mumbai.

ESTIMATION OF STARCH AND AMYLOSE

These were estimated by the method given by Gibson et al., (1997). The assay was based on the precipitation of amylopectin with Concavalin A in a solubilized, lipid-free sample. The amylose remaining in the sample was hydrolyzed to glucose that was estimated by GOD –POD method. Total starch was estimated in a separate aliquot prior to treatment with Concavalin A.

ESTIMATION OF RS:

This was done by the method given by Parchure and Kulkarni (1997). It was estimated in terms of glucose after enzymatic hydrolysis and removal of digestible starch followed by solubilization of RS in alkali and its hydrolysis by amyloglucosidase.

DATA ANALYSIS

Paired t-test was used to determine whether there were significant differences between the raw and processed sago samples. Karl Pearson's correlation coefficient was calculated to determine whether there was a correlation between the amylose and RS content.

RESULTS AND DISCUSSION

TOTAL STARCH AND AMYLOSE CONTENT AND EFFECT OF PROCESSING

Total starch content of large and small pearls was similar, with the mean total starch content in the large pearls being 81.96 ± 2.68 g% and 81.14±3.96 g% for the small pearls. The values observed in the present study are similar to those reported in the nutrient database for Indian foods (NIN, 2009). Also, amylose content of the large pearls (59.57± 1.29 g%) did not differ from that of the small pearls (54.76 ± 5.35 g %). Amylose comprised 72.79% of the total starch in the large pearls and 67.69% in the small variety. Uthumporn and coworkers (2008) have also reported that sago starch contains high amount of amylose compared to other sources of starch. The high amylose content of sago would favour gelatinization of the starch, which is important for gelation of starch and other rheological properties (Li et al., 2008).

The effect of soaking and boiling on total starch and amylose content was examined (Table 1). Soaked samples of small and large pearls did not differ significantly in the total starch content. There was very little change in the total starch and percent amylose after soaking, with the loss of total starch being 2.17% for the large pearls and 1.19 % for the small pearls. Loss of amylose upon soaking was negligible (0.05%) in the large pearls and was higher for the small pearls (3.70%).

Effect of boiling was examined using two fluids – water and milk, each in two different proportions with sago: fluid ratios being 1: 2 and 1:4. When sago was boiled in water, starch content was higher in the large pearl variety than in the small pearl variety (Table 1). Loss of total starch in the large pearls was 10.70%, which was slightly but not significantly higher than that in the small variety (9.37%), when the sago:water ratio was 1:2. When the amount of water was increased to a sago:water ratio of 1:4, the loss of total starch in the small pearls was slightly higher (10.22%) than that observed for the large variety (9.44%).

When sago was boiled in milk with a sago: milk ratio of 1:2, the starch content was higher in the large pearls than the small pearls (Table 1). There was a small loss in starch content – approximately 3 to 4 percent. When the amount of milk used for boiling was increased to sago:milk ratio of 1:4, the starch content was much lower as compared to when the sago:milk ratio was 1:2, with not much difference between the large and small pearl varieties.

Comparison of total starch content and loss of starch when the two fluids were used, indicated that starch content of sago boiled in milk was significantly lower ($p<0.05$) as compared to the sago boiled in water at both ratios and in both varieties. The loss of starch when sago was boiled in water was much higher, almost three times more compared to when the starch was boiled in milk.

Amylose content of the small pearls was lower than that of the large pearls when the sago: water ratio was 1:2, although this difference was not statistically significant. When the sago: water ratio was increased to 1:4, the difference between the two varieties was less. Further, when sago:water ratio of 1:4 was used, the amylose content was approximately half the amylose content as compared to when the ratio was 1:2, in both small and large pearl varieties. When the sago was boiled in milk, the trend was similar to that observed when sago was boiled in water. However, amylose content was lower in the sago pearls boiled in milk, regardless of the size of the pearls and the amount of milk used.

Amylose as a percentage of total starch did not differ when either milk or water was used for boiling the sago, within the variety of sago (Table 1). However, in the large pearls, amylose comprised approximately 70 percent when the sago to fluid ratio was 1:2; whereas in the small pearls, the percent amylose was slightly lower (approximately 66%). When the ratio of sago:fluid was 1:4, the reverse pattern was observed, with amylose as a percentage of total starch being higher in the small than in the large variety. These results indicate that the loss is slightly more in the large pearls when more amount of fluid is used for cooking. Loss of amylose was higher than the loss of total starch for both varieties at both sago: water ratios. For the large pearls, amlyose loss was slightly higher (13.67% for 1:2 sago: water ratio and 13.42 % for 1:4 sago: water ratio) compared to the small pearls (10.36% for 1:2 sago: water ratio and 10.48% 1:4 sago: water ratio).

Table 1: Total Starch and Amylose Content of Raw and Processed Sago

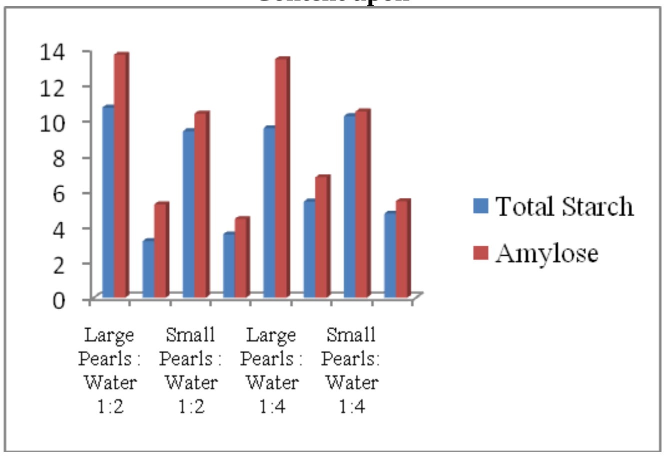
Sago Samples	Large Pearls			Small Pearls		
	Total Starch	**Amylose**	**Amylose as % of total starch**	**Total Starch**	**Amylose**	**Amylose as % of total starch**
Raw(g/100g)	81.9±2.7	59.6±1.3	72.8	81.1±3.9	54.8±5.3	67.7
Soaked(g/100g) Content in Equivalent amount of raw pearls	28.9±0.9 29.5±0.9	20.4±1.5 20.4±2.3	70.6 -	28.1±1.5 28.4±1.4	18.5±0.9 19.2±1.0	65.8 -
Boiled in water						
Sago:water 1:2 Content in Equivalent amount of raw pearls	15.2±10.7 17.1±0.6	10.7±0.1 12.5±0.3	70.0 -	12.3 ± 0.3 13.5±0.7	8.2 ± 0.8 9.1±0.9	66.0 -
Sago:water 1:4 Content in Equivalent amount of raw pearls	8.2±0.2 9.1±0.3	5.7±0.1 6.6±0.1	69.9 -	6.8±0.3 7.5±0.4	4.9±0.2 5.5±0.1	73.0 -
Boiled in milk						
Sago:milk 1:2 Content in Equivalent amount of raw pearls	13.9±2.2 14.4±0.5	9.9±1.5 10.5±0.1	71.0 -	11.9±2.5 12.3±0.6	7.9±1.1 8.3±0.8	66.9 -
Sago:water 1:4 Content in Equivalent amount of raw pearls	6.5 ±0.5 6.8±0.2	4.3 ±0.4 4.6±0.1	65.8 -	6.5±0.6 6.8±0.3	4.7±0.2 5.0±0.4	72.8 -

The results of the present study are comparable to the findings reported by Rashmi and Urooj (2003) on the starch content of raw and boiled rice. When starch is heated in sufficient water, gelatinization occurs and the molecular and crystalline order within the starch granule is lost. The granules absorb water and swell. Eventually there is fragmentation of granules that causes a decrease in total starch content (Sablani et al., 2007). Further, cooking in excess fluid alters the structure of the starch granule, leading to the solubilization of starch (British Nutrition Foundation, 1990). The results of the present study suggest that these phenomena occur slightly more in the large pearls than in the small pearls.

The decrease in amylose content observed in the present study can be attributed to leaching out of amylose from the swollen starch granule into the cooking fluid. The leached –out amylose forms a three-dimensional network that leads to the formation of starch paste (Li et al., 2008). The extent of leaching depends on the type of starch and the conditions used for cooking. Leaching out of amylose tends to be less in amylopectin-rich waxy starches (Sasaki, 2005). In the present study, sago contained almost 70% of starch as amylose and hence the loss of starch was high.

When milk was used as a cooking medium, loss of total starch and amylose was lower compared to when water was used as the cooking medium (Figure 1). The protein and lipids present in the milk may have coated the sago pearls thus reducing their water holding capacity and affecting gelatinization (Mangala et al., 1999). Debbet and Gidley (2007) observed that the presence of surface proteins and lipids on maize granules limited expansion and prevented dissolution, thus reducing the rate of formation of solubilized starch and the loss of starch. When sago is boiled in milk, it is possible that the absorption of fluid by the pearls, their swelling and eventual fragmentation is less, thereby resulting in lower loss of starch on boiling.

Figure 1: Percent Decrease in Total Starch and Amylose Content upon



Boiling in Water and Milk

RESISTANT STARCH (RS) IN RAW AND PROCESSED SAMPLES

RS content of raw sago for both large and small varieties was low, comprising only 3% of total starch (Table 2). RS content of soaked sago as a percentage of total starch did not differ from that of raw sago. Boiling in water with sago: water ratio of 1:2, resulted in a significantly higher RS content irrespective of the size of the sago pearls. In contrast, when the sago: water ratio was 1:4, the RS content of the boiled sago was lower as compared to the former.

When sago was boiled in milk, the RS content was significantly lower than when sago was boiled in water. RS comprised approximately 4% of total starch, as against the content being 33 to 56 percent of total starch when water was used as the medium of cooking.

The percent increase in RS content after cooking was calculated (Figure 2). The percent increase in RS content was 91.6% for the large variety and 93.7% for the small variety when the grain: water ratio was 1:2. When the water content was increased with the grain: water ratio being 1:4, the percent increase in RS content was slightly lower (88.5% for the large variety and 90.6% for the small variety).

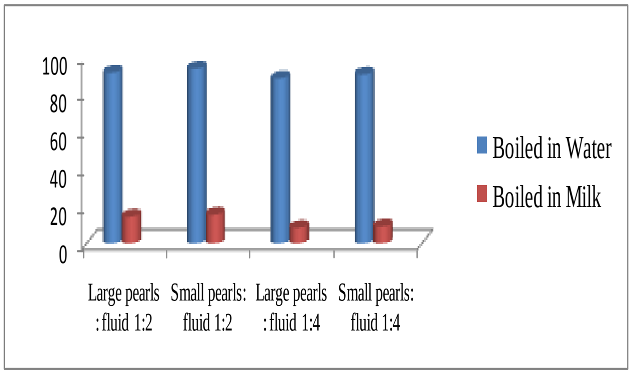
Table 2: Resistant Starch in Raw and Processed Samples of Sago

Sample	Large Pearls		Small Pearls	
	Resistant Starch (g)	% of total starch	Resistant Starch (g)	% of total starch
Raw(g/100g)	2.84±0.22	3.5	2.59±0.41	3.2
Soaked(g/100g) Content in Equivalent amount of raw pearls	1.04±0.12 0.99±0.08	3.6 -	0.93±0.08 0.91±0.14	3.3 -
Boiled in water				
Sago:water 1:2 Content in Equivalent amount of raw pearls	7.05±0.12 0.59±0.04	46.3 -	6.88±0.19 0.43±0.07	56.0 -
Sago:water 1:4 Content in Equivalent amount of raw pearls	2.78±0.10 0.32±0.02	33.7 -	2.55±0.17 0.24±0.04	37.7 -
Boiled in milk				
Sago:milk 1:2 Content in Equivalent amount of raw pearls	0.66±0.04 0.49±0.04	4.0 -	0.45±0.05 0.39±0.06	3.8 -
Sago:water 1:4 Content in Equivalent amount of raw pearls	0.26±0.01 0.24±0.02	4.0 -	0.24±0.01 0.22±0.03	3.7 -

When milk was used as a cooking medium, the percent increase in RS content was significantly lower than when water was used, for both the varieties and both the ratios of sago: fluid. The percent increase in RS content of samples cooked in milk (sago: milk ratio of 1:2) was 14.3% for the large variety and 15.4% for the small variety. The percent increase in RS was lower when more milk was used (grain: milk ratio 1:4), with the increase in the large variety being 8.3% RS and 9.1% in the small variety.

The observations in the present study are similar to those reported by Mahmood *et al.,* (2006) who observed that upon boiling tapioca and other roots, RS content increased by 7.2 – 28.7%. A similar trend was reported earlier by Parchure and Kulkarni (1997) for rice and amaranth starch. The increase in RS content could be attributed to the process of starch retrogradation and recrystallization of amylose, when samples were cooled to room temperature after cooking. Xu and Sieb (1993) reported that swollen tapioca granules yielded enzyme-resistant starch, owing to the formation of retrograded starch. Thermal processing of cassava starch has also been reported to result in formation of starch resistant to the action of α - amylases due to crystalline regions formed by recrystallization (Charles *et al.*, 2005).

Figure 2: Percent Increase in Resistant Starch upon Boiling Sago in Water and Milk



The results of the present study indicate that the amount of RS formed varies when different fluids are used for boiling. The increase in RS content was much lower when milk was used to boil the sago as compared to when water was used. This can be explained by reports in the literature about the effect of proteins and lipids on gelatinization. Both these components hinder gelatinization. Mangala *et al.,* (1999) reported that deproteinization and defatting increased the RS content in rice and finger millet samples. Escarpa and coworkers (1997) stated that retrogradation can be hampered because of inability of amylose to recrystallize owing to the formation of starch – protein bonds, thus possibly reducing RS formation. Also a lower ability to retain water when the sago is cooked in milk could lead to reduction in gelatinization and thereby subsequent retrogradation. Jane and Tobyt (1984) and Cui and Oates (1999) reported that amylose can complex with fatty acids and monoglycerides. Copeland *et al.*, (2009) and Putseys *et al.*, (2010) reported that the amylose – lipid complexes can be formed when starch is gelatinized in the presence of lipids. Since, milk is a good source of both proteins and lipids; RS content was lower when sago was boiled in milk.

CONCLUSION

RS formation upon cooking in water suggests that this method of preparation can help increase the RS content in starchy foods, making them healthier. In recipes where sago is cooked in milk, it may be advantageous to first cook the sago pearls in water to increase the RS content, and the milk can be added thereafter.

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