THE AMYLOSE AND THE AMYLOPECTIN CONTENTS OF RICE AND THEIR INFLUENCE ON THE COOKING QUALITY OF THE CEREAL

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INTRODUCTION

HUNDREDS of varieties of rice are known to exist. These varieties exhibit on cooking marked differences in quality. Attempts to correlate quality of a rice with its chemical composition have not so far been successful. According to B. S. Rao,[1] the differences in quality are mainly to be attributed to differences in colloidal structure. Rao has shown, that the extent of swelling of any variety of rice on cooking, is an index of its quality. He has developed a convenient and accurate method of estimating the swelling. This swelling is quantitatively expressed as the " Swelling Number " (S.N.) of a rice. The swelling number, is the weight of water imbibed by 100 grams of rice when cooked in water at 98° C. under standard conditions. It has been found that it is possible to alter the swelling number of rice by processing, *e.g.*, by par-boiling.

The starch of rice, like all other starches, consists of amylose and amylopectin. W. N. Haworth, *et al.*,[2] have confirmed that amylose is composed largely of unbranched chains of 100 or more glucose members, while amylopectin has a laminated or branched structure, composed of unit chains of about 20 glucose residues. Amylose produces a blue colour, while amylopectin yields reddish coloration, with iodine.

The present investigation was undertaken to find out if there was any relationship between the swelling number of a rice and its amylose and amylopectin contents. It was found that there were varietal differences in the amylose and the amylopectin contents of rice. Whether processing of rice (which brings about a change in the swelling) would also affect the amylose-amylopectin ratio, was determined.

EXPERIMENTAL

(A) *Fractionation of Amylose and Amylopectin from Rice*

Pure amylose and amylopectin samples were required for the standardisation of the method of analysis. These fractions were obtained from rice,

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The more important methods that have been employed, from time to time, for the fractionation of starch are the following:—

(1) *Aqueous leaching.*[3, 4, 5]—This method depends on the extraction of the swollen granules, with successive portions of water, until all the soluble portion is removed. The insoluble portion is separated by sedimentation, filtration or centrifugation. This method is tedious, sterile conditions are difficult to maintain, and the separation is imperfect.

(2) *Electrophoresis.*[6, 7]—This method depends upon the polarity imparted to one or the other of the fractions, by the presence of non-carbohydrate substances. The separation is slow and incomplete. Furthermore, there is a considerable amount of degradation of the starch.

(3) *Selective retrogradation.*[8, 9]—When a starch sol is allowed to stand, the amylose fraction slowly retrogrades and precipitates from solution. This method is usually employed on starches solubilized by hydrolytic action. Fractionation is superficial, since the amylopectin fraction acts as a protective colloid. Moreover, considerable co-precipitation occurs.

(4) *Selective adsorption.*[10, 11, 12]—Some adsorbents like cotton, cellulose pulp, charcoal, activated alumina, bentonite and fuller's earth, preferentially adsorb the amylose fraction which can be eluted with hot water. Although this method involves no chemical degradation and yields relatively pure amylopectin fraction, the amylose fraction is badly contaminated with amylopectin.

(5) *Enzymic method.*[13, 14]—The amylose fraction can be removed through hydrolysis with various enzymes. But it is now recognised that amylopectin is also affected to an extent of about 50%. Consequently this method is also unsuitable.

(6) *Selective precipitation.*[15]—The only suitable method seems to be selective precipitation by polar organic substances. This method, which has been developed by Schoch, gives without any chemical degradation, both fractions of maximum purity. In the present investigation, the method of Schoch[16] has been employed.

The fractionation involves dispersion of starch by autoclaving, precipitation of the amylose by the addition of butyl alcohol, separation of the precipitate by centrifuging and the final purification of amylose by " Recrystallisation ".

A mixture of amyl alcohol and butyl alcohol is found to give better yields than butyl alcohol and is therefore preferred. During autoclaving, the fat gets hydrolysed and develops acid, which aids glucosidic hydrolysis

of starch. Consequently, defatted starch is preferred in all the physico-chemical investigations. Schoch recommends methyl alcohol as the best defatting agent for the cereal starches.

100 g. of powdered rice (fully polished) was extracted in a Soxhlet with methyl alcohol for 24 hours. The defatted starch granules were dried in air and finely powdered to pass through 200 mesh.

20 g. of the defatted rice flour was added to 75 ml. of water at 70° C. and kept at this temperature for 10 minutes. This suspension was slowly added with constant mechanical stirring to a boiling mixture of one litre of water and 100 ml. of butyl alcohol, heated on the steam-bath. The paste was then autoclaved for 3 hours at 18–20 lb. pressure (124–25°).

The hot starch sol was passed through the Sharples continuous-flow supercentrifuge to remove traces of cellular material and other impurities. To the clear sol was then added 100 ml. of a hot, 1 : 1 mixture, of butyl and amyl alcohols. The solution was allowed to cool slowly to room temperature (without agitation), over a period of 36 hours. To effect slow cooling, the beaker was kept wrapped in flannel.

When the temperature was about 50° C., the precipitated fraction separated out as a gelatinous floc. The mixture was then stirred at room temperature for about 3 hours to break up the mass and refrigerated overnight. The starch mixture was then centrifuged at about 3,000 r.p.m. in a centrifuge. The supernatant liquid was decanted carefully. The precipitated material was again suspended in cold water (previously saturated with butyl alcohol) and then centrifuged. The washing was repeated, until the supernatant liquid indicated no turbidity with methyl alcohol, added in excess.

The crude fraction was further purified by repeated " Recrystallisation " from boiling water in the presence of excess of butyl alcohol. The purified amylose fraction obtained in this way, was preserved under methyl alcohol.

The amylopectin fraction could readily be removed from the centrifuge by flocculating it with excess of methyl alcohol. The wet precipitate was triturated with fresh portions of methyl alcohol, to effect its dehydration. The purified amylopectin fraction obtained in this way was also preserved under methyl alcohol.

(B) *Analytical Procedure*

Amylose is stained blue by iodine while amylopectin is stained purple to red. F. L. Bates[17] and others attributed this striking difference in behaviour to a difference in the ability of amylose and amylopectin to bind

iodine in complex formation. So they titrated solutions of amylose and amylopectin with iodine potentiometrically. Their results indicate that the " activity " of iodine in an amylose solution remains constant upon addition of iodine until complex formation is complete, while there is a continual rise in the iodine activity, when iodine is added to an amylopectin solution. Starches containing both components show an inflexion in the curve: potential *vs.* Iodine titre. This inflexion point is a measure of the amylose fraction present in starch.

In the method developed by Bates and co-workers,[17] the starch sample is dissolved in dilute potassium hydroxide solution, neutralised with hydriodic acid and titrated potentiometrically with a solution of iodine in potassium iodide. Since hydriodic acid requires frequent redistillation over red phosphorus, Schock, *et al.*,[18] have modified the process by employing hydrochloric acid to neutralise the alkaline starch solution. The titration is then carried out with a solution containing iodine, potassium iodide and potassium chloride. They point out that completely defatted cereal starches have to be used, in order to avoid interference due to the adsorption of fatty acids on starch. In the present investigation, Schoch's method, in its modified form, was employed.

Very dilute solutions—0·01 to 0·04%—have to be employed as it is essential that starch should be in a completely dispersed state. As the amount of iodine taken up by starch is influenced by the iodide concentration, it is necessary to keep the iodide concentration constant, to obtain concordant results.

0·01 to 0·04 g. of starch was dispersed in 10 ml. of 0·5 N potassium hydroxide and slightly warmed to get an effective dispersion. The solution was diluted with distilled water, and made neutral to methyl orange by the addition of 0·5 N hydrochloric acid. 10 ml. of 0·5 N potassium iodide was then added and the solution made up to 100 ml. with distilled water.

The solution thus prepared, was slightly acidic and 0·05 N with respect to potassium chloride and potassium iodide. This solution was titrated potentiometrically with an iodine reagent 0·001 N to iodine, 0·05 N to potassium iodide and 0·05 N to potassium chloride. The potentiometric titrations were carried out with a vernier type potentiometer which could read to a tenth of a milli-volt.

Using the above procedure, different varieties of rice were analysed. The results are given in Table I. Cured, Calcured and Parboiled forms from the same sample of " Madivala Paddy " were also analysed to find out whether the different methods of processing the cereal, affect the amylose and amylopectin contents in rice.

Calcured Rice.[19]—Paddy was soaked in a 0·2 molar solution of calcium chloride (pH adjusted to 4·5) at 65° C. for 2 hours. The paddy was removed from this hot solution and steamed for 10 minutes to partially gelatinize the starch. The paddy was then dried.

Cured Rice.—This was obtained in the same way as indicated above for calcured rice, but water was used for soaking the paddy.

Parboiled Rice.—Parboiled rice was prepared by soaking the paddy in cold water for 24 hours and boiling the paddy with water for half an hour. The paddy was then dried.

All the samples of paddy were husked and the rice fully polished. The samples of rice were defatted by extraction with methyl alcohol in a Soxhlet for 24 hours.

Standardisation of the Analytical Procedure

Fig. 1 shows the changes in potential when 0·001 iodine is added to solutions of amylose and amylopectin in 0·05 N potassium iodide solution.
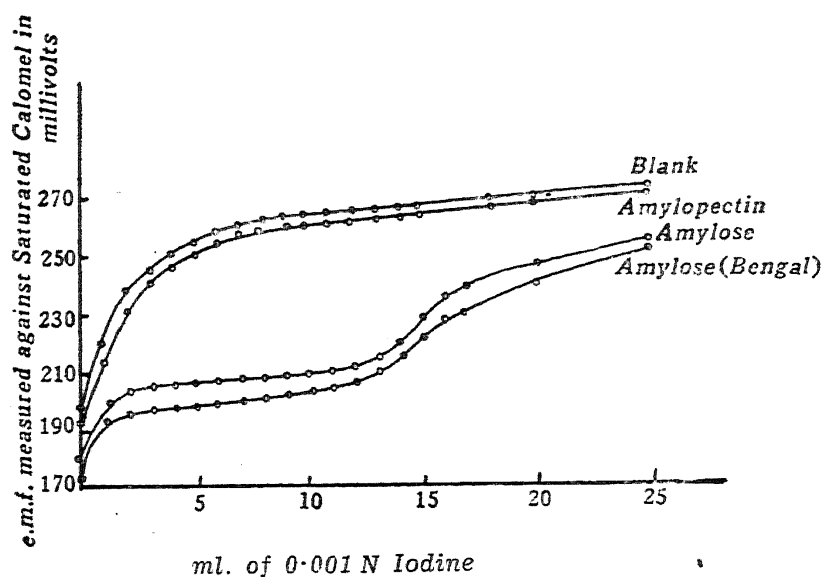

ml. of 0·001 N Iodine

FIG. 1

The curve for the potassium iodide solution, no starch being present, indicates the concentration of free iodine, corresponding to any potential. The curve for amylopectin shows that all the iodine adsorbed is bound by amylopection. For amylose, there is an inflection point and at this stage, all the iodine present, is in the form of a complex with amylose. On further addition, the iodine is merely adsorbed, as can be deduced from the proportional increase in the concentration of free iodine. The value of bound iodine is obtained by subtracting from the total iodine, the free iodine corresponding

to any potential. In Fig. 2 the number of grams of iodine bound per gram of amylose have been plotted against the concentration of iodine. Estimated
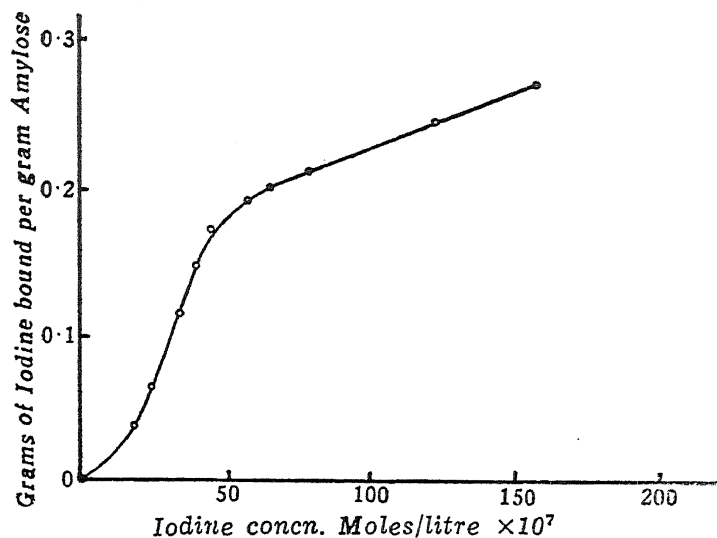

FIG. 2

in this way, amylose from rice is found to take up 18·2% of iodine. An amylose sample, supplied by Bengal Immunity Research Institute, has been found to take up 18·4% of iodine.

By determining the inflection point obtained with a mixture of amylose and amylopectin, the amylose content of the sample can be computed. The values of amylose in various samples of rice are given in Table I. The titration curves obtained with a few typical samples of rice are indicated in Fig. 3.

TABLE I. *Effect of Time of Cooking on the Swelling of Different Samples of Rice*

Sl. No.	Duration of cooking (in minutes)	Swelling number of "Madivala Sanna"	Swelling number of "C. Sanna"*	Swelling number of "D.S. Medium"†	Swelling number of "D.S. Coarse"‡
1	20	255	240	225	200
2	30	305	285	240	225
3	45	349	323	282	250
4	60	396	362	320	276
5	90	452	420	365	305

* "C. Sanna"—Coimbatore Sanna; ‡"D. S. Medium"—Dabbalsala medium; †"D. S. Coarse"—Dabbalsala coarse.

B3

e.m.f. measured against Saturated Calomel in millivolts
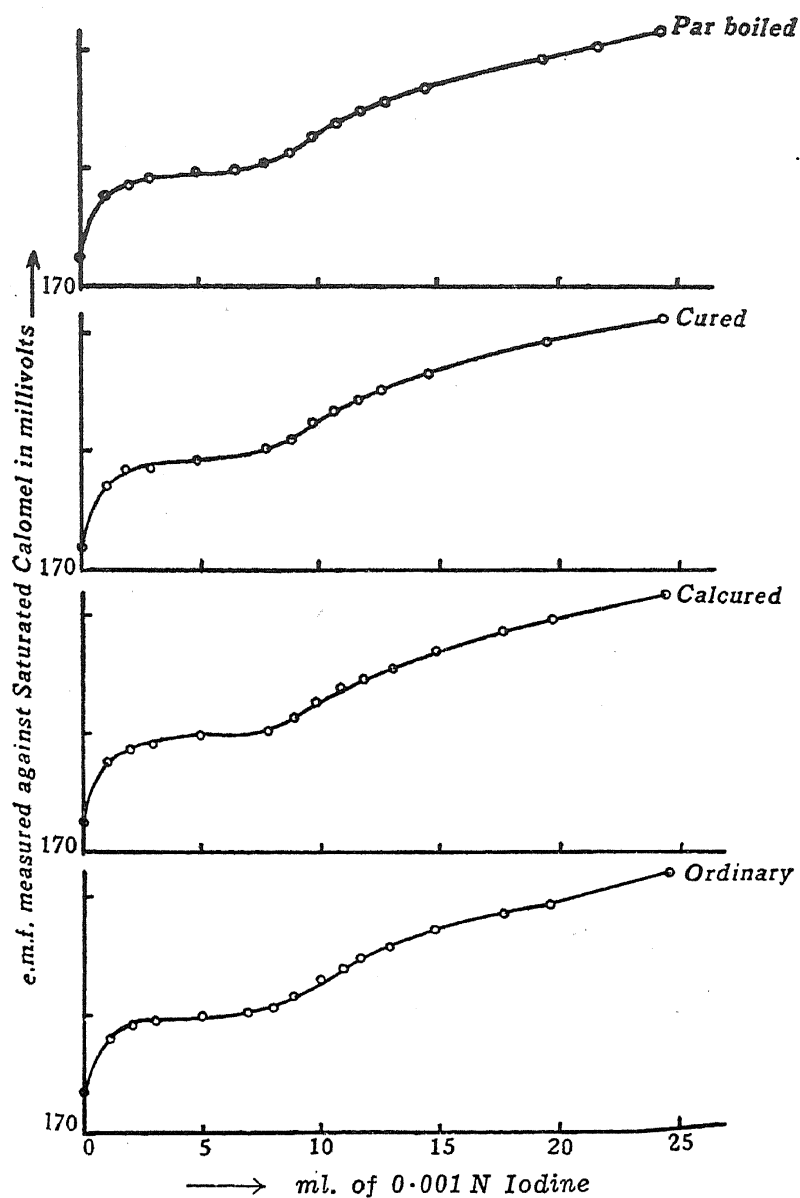
ml. of 0·001 N Iodine

Fig. 3

(C) Swelling Numbers

The " Swelling Numbers " (S.N.) of different varieties of rice were determined by the procedure described by B. S. Rao.[1] The apparatus consisted of a three-necked flask through one neck of which was attached a water-cooled condenser and through the other a pyrex test-tube containing 10 ml. of distilled water. A thermometer passing through a rubber-stopper was fitted into the third neck. The test-tube was loosely plugged into the neck with cotton wool. When the water in the test-tube just began to boil, a known weight (1 to 1·3 g.) of the fully polished rice sample was intro-

duced through a wide-mouthed funnel. The funnel was removed and the cotton plug was inserted.

Exactly, 30 minutes later, the test-tube was withdrawn from the steam-bath and filled with ice-cold water. The water was drained off by transferring the contents of the test-tube into a perforated thimble of porcelain. The thimble was then kept for about five minutes in ice-cold distilled water. It was withdrawn, and after the water had drained off, the rice was spread over moist chamois leather kept on a porcelain plate and the excess of moisture was removed by placing over the rice another piece of moist chamois leather and applying gentle pressure. Moist chamois leather was employed, as it was found to be the most convenient way of removing surplus water. The swelling numbers could be determined with an accuracy of 1%.

The effect of time of cooking on the swelling of rice, was studied by cooking a sample for different periods. The swelling number could not be determined beyond 90 minutes as there was disintegration in the rice grain. Table I indicates the effect of time of cooking on the swelling numbers of different samples of rice.

TABLE II. *Moisture, Amylose Content and Swelling Number of Various Samples of Rice*

Sl. No.	Rice sample	Moisutre %	Swelling number	% of Amylose
1	"Madivala Sanna"	14·8	305	17·5
2	"Mysore Kaddi"	15·0	304	17·5
3	"B. 1399 Putta Sanna"	15·0	292	15·0
4	"Chikka Dabbalasala"	15·3	242	7·0
5	"Makka Dabbalasale"	15·0	272	12·0
6	"Tolaga"	15·6	266	10.5
7	"C. Sanna"	15·1	285	14·5
8	"Siddasale"	15·4	260	10·0
9	"Kumbri Halaga"	15·0	260	10·0
10	"Neerudabbalasale"	15·0	258	10·0
11	"S. 661"	15·4	275	12·5
12	"C.S. Fine"	15·3	305	17·5
13	"S. 749"	15·4	292	15·0
14	"Dabbalasala (Medium)"	14·7	240	7·0
15	"Dodda Hegge"	15·5	282	14·0
16	"Rathachoodi"	15·2	300	17·0
17	"Dabbalasala (Coarse)"	15·0	225	4·5
18	"B. 281 Koppa"	15·4	265	10·5
19	"Makki D.S. Paddy"	15·6	286	14·5
20	"Bilikan Hegge"	15·0	260	10·0
21	"Varedabbalasale"	15·2	256	9·5
22	"Dodda Dabbalasala"	15·2	200	0·0

(D) *Estimation of Moisture*

Moisture was estimated by employing the Carter-Simon rapid moisture tester. The oven was kept at 155° C. Drying time was 30 minutes. The moisture content was calculated on the basis of weight of " dry " rice, present in paddy, taking into account the weight of husk.

In Table II are given the moisture and amylose contents and the swelling numbers of various samples.

It is interesting to note that the coarsest variety of rice investigated, has no amylose at all. This rice is similar to the waxy rice reported by Schoch.[16] Superior varieties are richer in amylose. No marked difference is noticed in the moisture content of different samples of paddy under identical conditions of humidity.

DISCUSSION

The experimental results recorded in this paper, indicate that there is a close correlation between the " Swelling Number " of raw polished rice and its amylose content. The higher the percentage of amylose in a rice, the greater is the swelling. According to modern views, amylose is a linear chain polymer, while amylopectin is a branched chain polymer. During its growth in the rice plant, the rice grain seems to acquire its amylopectin by the action of certain enzymes on amylose. Haworth[2] has discussed the relationship between amylose and amylopectin. It is of interest to note that the amylose content of rice does not alter when it is cured.

Rice having a high swelling number is liked by consumers as such rice is soft when cooked. The swelling number may therefore be considered to be an index of the quality of rice. The determination of swelling numbers is easily carried out and highly reproducible values are obtained. The correlation between swelling number and the amylose content of rice is of significance. It may be pointed out that the swelling number is an index of the rigidity of structure of the rice grain. If the grain has comparatively a loose structure, the swelling number is high. Amylose, being a linear chain polymer, makes the structure of the rice grain less rigid and this accounts for the higher swelling number of varieties having higher amylose content. The looseness of structure caused by amylose may however be modified by the curing of rice, as in parboiling, where the paddy, on soaking in water, is subjected to " wet heat ", *i.e.*, the soaked paddy is boiled or steamed for 15 to 20 minutes.

B. S. Rao has emphasised the fact that rice is to be looked upon as a colloidal system analogus to the hydrogel of silicic acid. It is well known that when the hydrogel of silicic acid is subjected to " wet heat " that is,

heated under conditions in which there is no loss of moisture, the hydrogel acquires rigidity of structure and becomes a Xerogel. In fact, this is the technical method for the production of silica gel on the large scale. Similarly, when the hydrogel of starch (containing both amylopectin and amylose) is subjected to wet heat, we get a product having greater rigidity. The rigidity of structure in the rice grain can, in fact, be controlled. For instance, in the case of rice parboiled by the indigenous process, the swelling number drops to 265 (Table III) while rice that has been subjected to wet heat under controlled conditions, can be made to yield a product having practically the same swelling number as raw rice.

TABLE III. *Swelling Number and Amylose Content of Processed Rice from " Madivala Sanna "*

Sl. No.	Rice sample	Swelling number	Amylose content
1	"Raw polished"	305	17·5
2	"Cured with water"	304	17·5
3	"Calcured"	305	17·5
4	"Parboiled" (Indigenous method)	265	17·5

The fact that the rigidity of the rice grain can be controlled by wet heat treatment is of considerable practical importance as it enables one to get rice, in which vitamins are fixed in the endosperm, and which has the desired softness in cooking. It may be pointed out that rice cured by the indigenous " parboiling " process is generally " overcured ". This partly accounts for the unpopularity of parboiled rice in certain parts of the world.

SUMMARY

1. Amylose and amylopectin fractions have been isolated from rice by a slight modification of the butanol fractionation method of Schoch. This method has been found to give good yields.

2. The amylose and the amylopectin ratios of the different varieties of rice have been estimated by the potentiometric titration of starch solutions with iodine.

3. The cooking quality of the different varieties of rice has been determined by employing the " Swelling Number " method of B. S. Rao.

4. It has been found that with an increase in the amylose content, the swelling number of rice increases.

5.   No change in the amylose content takes place, when rice is parboiled though there is a marked fall in the Swelling Number.   Other ways of curing rice do not also affect the amylose content.

ACKNOWLEDGEMENT

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