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EFFECT OF PROCESSING ON CONTENT AND MOLECULAR PROPERTIES OF ENZYME-RESISTANT STARCH FROM KODO AND KUTKI

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Abstract: *Kodo (Paspalum scrobiculatum) and Kutki (Panicum sumatranse) are minor millets consumed by millions of tribal in India. Minor millets are nutritious having hypoglycaemic and hypocholesterolemic properties but constitute a comparatively less studied food class. This study investigated isolation and formation of α – amylase resistant starch from Kodo and Kutki by different processing conditions such as roasting, gelatinization and retrogradation and characterized by X- ray diffraction and Fourier Transform Infrared spectrophotometry. Starch was isolated from defatted millet flour by alkaline steeping method. Total starch content in Native Kodo and Kutki was found to be 82.05% and 73.23% respectively. Resistant starch content was found to be 3.7% and 3.5% in gelatinized kodo and kutki respectively. In roasted and retrograded samples resistant starch czontent was found to be significantly increased. FTIR spectra of native kodo and kutki starches exhibited a similar pattern with maximum absorbance peaks near 3300, 2900, 2000, 1640, 1400, 1000, 930, 860, 700, 575 cm-1. The XRD analysis of millet starches showed altered peak intensities in X-ray diffractogram. Native starch of both the samples showed A-type crystalline pattern. Detailed knowledge of characteristics of Kodo and Kutki starches may facilitate their applications in food industries as a potential food ingredient. This in turn offers a big support to restore minor millet cultivation and consumption.*

Keywords: *Kodo (Paspalum scrobiculatum), Kutki (Panicum sumatranse), isolation, hypoglycaemic and hypocholesterolemic.*

Introduction: In India the most common grains consumed by large number of population for their energy source are wheat and rice. Kodo and kutki are minor millets consumed by tribal as a staple food. Minor millets are considered to be nutritious than wheat and rice owing to their health benefits such as high dietary fiber, protein and vitamin content, hypoglycaemic and hypocholesterolemic properties [1, 2]. Starch in minor millets is reported to digest slowly and releases glucose over a longer period of time [3, 4]. Starch is the most important glycaemic carbohydrate in the diet of large number of population in the world [5]. The release of high amount of glucose from starchy foods may contribute to obesity, diabetes and other metabolic disorders [6]. To tackle such problems, it is long recommended to eat fibre rich products. Resistant starch (RS) has gained interest as a functional food ingredient because it escapes digestion in the small intestine and act like a dietary fiber by fermenting in the colon [7]. Resistant starch is defined as a fraction of starch that resists digestion in the small intestine of healthy individual and passes to the large intestine where it is a substrate for bacterial fermentation [8]. RS have significant implications for human health as it lowers the risk of colon cancer, obesity and cardiovascular diseases [9]. Therefore it is of interest to increase RS content in diet [10]. Considering the importance of RS to human health and its suitability as an ingredient, this study aimed to evaluate the content of total starch, resistant starch and digestible starch in kodo and kutki. Most starches used in food industries as a food ingredient are chemically modified because of the poor functional properties of native starches. Starch properties can be modified by using different processing conditions; the nutritional quality of starch then

becomes a concern [11]. Different forms of starch structure have an impact on its digestibility therefore there is a need to analyse starches in the form in which they are eaten. [12, 13] Understanding the functional properties of starch such as pasting, gelatinization and retrogradation are important for their use in food products and industrial applications [14]. In the present study, we have investigated the effect of processing such as roasting (dry heat), cooking (gelatinization) and cooking and storing at -20□C for 30 days (retrogradation) on the molecular properties and content of resistant starch (RS).

Methods and Material

Processing

Native (Raw): 150 gm raw Kodo and Kutki millet seeds were washed, dried and milled to fine flour and taken for further analysis.

Roasting (Dry Heat): 150 gm raw seeds were roasted on a low flame with occasional stirring until the colour changed to light brown and then grinded in a blender to a fine flour as described.

Cooking (Gelatinization): 250 gm seeds of Kodo and Kutki were cooked for 30 min without pressure, drained and dried at 60ºC, milled to fine flour and stored at ambient temperature in airtight container until further analysis was performed.

Cooking and Storing at -20□C for 30 days (Retrogradation): Part of the cooked samples was stored at -20ºC for 30 days, for comparative analysis. The samples were then thawed to ambient temperature, dried and milled as described above.

Isolation of Starch: Starch was isolated by alkaline steeping method described by Acˇkar *et.al* with minor changes [15]. Defatted millet flour (100g) was suspended in 0.5% sodium hydroxide solution (NaOH) for 1 hr. The alkaline suspension was centrifuged at 5000 rpm for 20 min. The residual starch was repeatedly washed with distilled water until white coloured starch was obtained. The starch was suspended in distilled water and neutralized with 1M HCl and centrifuged again. Upper brown layer was removed and white starch was passed through 300 mesh sieve. The starch pellet was air dried and passed through 250 mesh sieve. In the present study the resultant starch is referred to as total starch (TS).

Proximate Analysis: Standard AOAC methods were used for determination of moisture, ash, proteins and fat content.

Total Starch Analysis: Total starch content of native and processed Kodo and Kutki were determined by enzymatic method according to Goni. et.al [1997). 50 mg Starch samples were dispersed in 2M KOH and shaken for 30 min at room temperature. Dispersed starch solutions were neutralized by acetic acid. The starch samples were incubated with amyloglucosidase (300U/ml) for 45 min at 60ºC and pH adjusted to 4.5 to hydrolyse the starch. The glucose released was determined by GOD/PAP method. The total starch was calculated as glucose X 0.9.

Formula:

$$\text{Total Starch \%} = \frac{\text{Conc. of Glucose} \times 0.90}{\text{Weight of starch (dry basis)}} \times 100$$ [16]

Resistant Starch Analysis: The resistant starch content of native and processed Kodo and Kutki were determined by direct method of Goni et.al. [1996). 100 mg of finely ground samples were incubated with a solution containing pepsin (20 mg) at 40ºC for 45 min to remove proteins. Tris maleate buffer (0.2M, pH 6.9, 4mM CaCl2) containing pancreatic α amylase (40 mg) was added and incubated at 37ºC for 16 hrs. The reaction mixture was centrifuged and the residue was solubilised in 2M KOH and RS was hydrolysed by amyloglucosidase as described in TS analysis. [17]

Digestible Starch Analysis: Digestible starch content was calculated as difference between total starch (TS) and resistant starch (RS).

Digestible Starch = TS - RS

X-Ray Diffraction: The crystalline structure of starch was studied by X-ray diffractometer (X'Pert Pro X-ray diffractometer). X–ray generator running at 45 KV and the current was 40 mA with Cu K α radiation (A°) at 1.54060 A°. The measurement temperature was set to 25 °C of the diffraction angle 2θ and a scanning region with start position at 10.0 and end position at 99.99 which covered all the significant peaks of starch crystallites. The scanning speed of the goniometer radius was 240 mm and specimen length was 10 mm.

FTIR: FTIR absorption spectra were recorded in the spectral range 400–4000 cm^{-1} with (Perkin Elmer Spectrum-1) spectrometer. The spectra of native, processed and enzyme resistant starches were obtained after pelletizing in KBr with the scan repeat of 12 hrs.

Results and Discussion

Results of proximate analysis of native kodo and kutki starches are shown in Table 1. The obtained values are in agreement with the values reported in literature. It is reported that the protein content of millets vary between 9-14% and carbohydrate between 70-80% [18]. Effect of processing such as roasting, cooking without pressure and storage post cooking (-20□C/ 30 days) on the resistant starch content were carried out and the results are shown in table 2. The total starch (TS) content ranged between 73.2-83.4%. TS content was found to be higher in kodo as compared to kutki. Processing did not affect the total starch content of both the samples. This indicates that different processing treatments may induce structural changes but do not affect the total starch content when evaluated enzymatically. The high RS content of native starches i.e. 16.2% and 13.7% in kodo and kutki may be attributed to the ordered crystalline structure and compact and dense granule which make them less accessible to α–amylase digestion. There observed a sharp increase in resistant starch (RS) content of roasted samples. This may be due to less gelatinization occurred during dry heating and the granules remained intact. Low RS content in the gelatinized samples confirmed the disruption of starch granules and became more accessible to enzymatic digestion. Dunder et.al reported the starch granules during gelatinization gradually and irreversibly destroyed [19]. Englyst et.al reported that starch digestibility increases due to gelatinization and reduces after retrogradation. [20]. RS content was found to be significantly increased after storage (-20□C/30 days) in both the samples. The results are in agreement with the results of Patricia M. Rosin et.al [21]. This may be attributed to the recrystallization during storage and formation of ordered structure which is less accessible to α-amylase digestion. [11, 22]

Table 1 Proximate analysis of Kodo and Kutki (g/100g) %

Sample	Moisture	Ash	Fat	Protein	Carbohydrate
Kodo starch (Native)	4.53 ± 0.29	0.16 ± 0.01	0.58 ± 0.02	8.7 ± 0.32	85 ± 0.81
Kutki starch (Native)	4.36 ± 0.08	0.27 ± 0.03	0.84 ± 0.03	16.40 ± 0.36	78 ± 0.81

Values are means of triplicates ± SD.

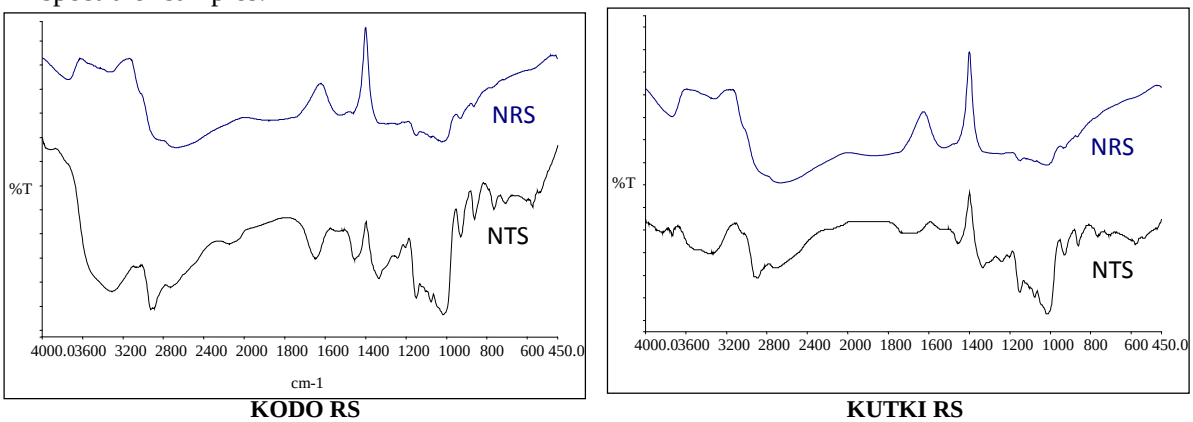
Table 2 Total Starch, Resistant starch and digestible starch contents of Kodo and Kutki millet (% dry weight of starch)

Sr. No	Treatments	TS (Total starch)	RS (Resistant Starch)	DS (Digestible Starch)
1	Kodo Native	82.05 ± 3.4	16.23 ± 0.7	65.80 ± 3.4
2	Kodo Roasted	81.07 ± 1.4	12.5 ± 0.4	68.55 ± 1.6
3	Kodo Gelatinized	82.4 ± 2.1	3.7 ± 0.3	78.6 ± 2.0
4	Kodo Retrograded	81.4 ± 1.5	11.21 ± 0.5	64.7 ± 2.6
5	Kutki Native	73.23 ± 0.8	13.7 ± 1.0	59.4 ± 0.8
6	Kutki Roasted	75.5 ± 1.9	9.9 ± 0.7	65.6 ± 2.0
7	Kutki Gelatinized	75.7 ± 1.7	3.5 ± 0.4	72.2 ± 1.8
8	Kutki Retrograded	74.6 ± 1.3	9.1 ± 1.0	63.1 ± 1.6

Values are means of triplicates ± SD.

Statistics: Results were expressed by means of triplicates ± SD. Comparisons of means were performed by one way analysis of variance (ANOVA) followed by Tukey's multiple comparisons using *Analyse-it* (version 2.30) software. Different treatments show significant differences in RS content. ($p<0.05$)

FTIR: The structural modification induced by different processing may have effects on the vibrations of the molecular bonds. Such structural modifications can be revealed by FTIR measurements. [23] The present study compared the spectra of total starch (TS) and resistant starch (RS). Figure 1 showed the IR spectra of samples.



KODO RS **KUTKI RS**

Figure 1 Infrared Spectra of total starch versus enzyme- resistant starch, (NTS: Native TS, NRS: Native RS)

IR spectra demonstrated main peaks with maximum absorbance near 3300, 2900, 2000, 1640, 1400, 1000, 930, 860, 700, 575 cm-1 for kodo and kutki starch samples after processing. IR spectra obtained from native starches showed 1150 cm^{-1} C-O, C-C stretching with some contributions at 1019, 1076, 1103 for kodo and 1077, 1014 for kutki. We observed clear band at 930 cm^{-1} in total starch samples of both kodo and kutki which is a characteristic of α–1,4 linkage. These results are in agreement with the results of Alica et.al. [15]. The maximum bands near 995–1100 were obtained in retrograded starch of kodo and kutki which indicates higher degree of order in structure after storage at -20□C for 30 days. This result is in agreement with Zhou et.al. [24] The absorbance in IR spectra for starches have been assigned and matched with vibrational modes of bonds in molecular structure of starch. [14]

XRD: XRD is a widely used technique to study the crystalline patterns of starch [25]. The X-ray diffractogram of enzyme resistant starches are presented in figure 2. Native kodo and kutki starch showed A-type crystalline pattern with major reflections at 2θ 15□, 17□, 18□, 23□, 30□ and 33□ whereas kodo native TS showed additional peak at 10□ which is not observed in native kutki starch. Marina et.al obtained the diffraction pattern from native high amylose rice starch and was classified as an A pattern which observed peaks at 15.0□ 17.5□ and 23.2□ of diffraction angle 2θ [26]. Both the native starches showed the similar diffraction pattern with altered peak intensities. Most of the cereal starches possess A pattern. These reflection peaks are in agreement with those reported for native rice starch and cereal starches. Ornanong *et al*. (2006) reported that the X-ray diffractogram of the native rice starch showed an A-type crystal pattern with strong reflections at 14.9□, 17.8□ and 22.8□ of diffraction angle 2θ for all strains of rice. Kodo native RS showed only 4 peaks with major reflections at 15.8□, 18.07□, 20.3□ and 23.2□ which confirmed the A pattern. Roasting did not show any change in diffraction pattern in both the samples and observed A type crystalline pattern. Resistant starch from roasted kodo gave C type pattern with diffraction peak at 11.8□ and few other peaks at 15.3□, 18.0□, 23.4□ and 39.0□. Crystalline peaks of gelatinized kodo starch became smaller and even disappeared in comparison with native starch. Gelatinized kodo TS exhibited C type crystalline pattern. Loss of peaks in kodo gelatinized RS is a result of birefringence loss. Gelatinized kutki TS showed strong reflections at 19.7□ which is a characteristic of V type crystalline pattern. Loss of crystalline peaks were also observed in gelatinized kutki starch and showed reflection at 19.4□ which gave V pattern. R.C. Erlingen reported that level of crystallinity of RS increases with increasing storage time [27]. Retrograded starches from both the samples showed C pattern. RS from retrograded kodo starch showed B type crystalline pattern, whereas kutki showed A pattern. These results are in agreement with the results of R.C. Erlingen et.al. In the study of Jirapa et.al X-ray diffractogram profile of RS and 121□C preheated starch showed V-type diffraction pattern. They suggested that when starches are heated, debranched and retrograde showed crystalline V structure. [28]

X-ray Diffractogram of Enzyme Resistant Starch from Kodo and Kutki

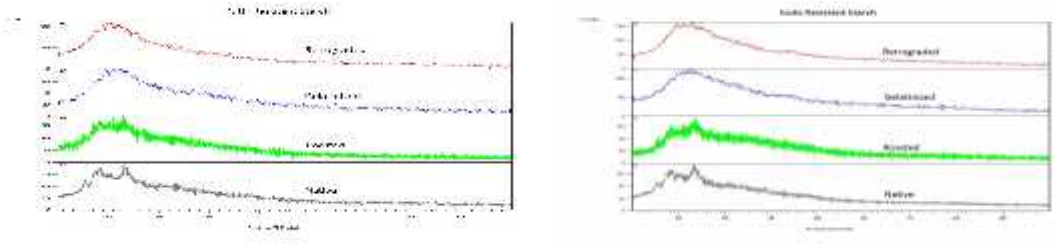


Figure 2 X-ray diffractogram of enzyme resistant starches isolated from Kodo and Kutki after different processing such as Native, Roasted, Gelatinized and Retrograded.

Conclusion: Starch digestibility is determined by starch structure which can be modified by different processing. Resistant starch content of foods can be increased by storage at low temperatures. X-ray diffraction and Fourier transform Infrared spectroscopy are the useful techniques to study changes in starch structure after processing.

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