

## Effect of soaking and autoclaving treatments on structure, properties and resistant starch (RS3) content of edible tapioca pearls

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### Abstract

In the present study, the effect of different soaking and autoclaving treatments on structure, properties and resistant starch (RS3) content of edible tapioca pearls were investigated. Before and after treatments, the structural changes in the tapioca samples were observed using Fourier transform infrared (FT-IR), near infrared (NIR) and Raman spectroscopy, and pasting properties were investigated with the help of rapid visco analyser (RVA). The results showed that all soaking and autoclaving treatments increased RS3 content of the tapioca pearls. Autoclaving treatments showed highest increase in the RS3 content of tapioca pearls than soaking treatments. Findings of FT-IR studies showed that elevated RS3 content of tapioca pearls after soaking and autoclaving treatments may be due to decrease in intra and intermolecular hydrogen bonds, and/or increase in the crystallinity of tapioca pearls starch. In addition, FT-IR investigations confirmed that bands at 2928 and 2856  $\text{cm}^{-1}$  were characteristically sensitive to soaking and autoclaving treatments. Results of NIR spectral analysis demonstrated that soaking and autoclaving treatments may increase structural changes in the skeletal mode of tapioca starch. Raman spectroscopic studies further confirmed that increase in the RS3 content of tapioca pearls samples after soaking treatments might be due to the increase in amylose content. In addition, Raman spectroscopic studies further highlighted that the autoclaving treatments brought complete structural modification in the tapioca starch. Pasting properties of untreated tapioca pearls samples were found to decline with treatments. Studied Tapioca pearls sample lost almost all pasting property parameters after autoclaving treatments. In conclusion, autoclaving treatments were good to increase RS3 content of tapioca pearls than soaking treatment, but they had adverse impact on pasting property and structural integrity of tapioca pearls starch. On contrary to this, soaking treatments preserve pasting property parameters as well as moderately increased the RS3 content of tapioca pearls.

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### Introduction

Starch is the major source of carbohydrate in the food and its products, and is also receiving industrial importance due to its thickening, stabilizing, gelling and binding properties. The functional characteristics of starches are basically depends on their physicochemical properties such as mean granular shape, size and amylose/amylopectin ratio. (Singh *et al.*, 2003; Singh *et al.*, 2006). Native starches are often modified by physical, chemical, and enzymatic processes in order to extend their food and industrial applications (Jobling, 2004; Zavareze and Dias, 2011). These days, physical modification of starches through heat-moisture and annealing treatments are receiving much interest in order to increase their functional properties (Zavareze and Dias, 2011). Resistant starch (RS) is one of the important functional starches, which functions as a

prebiotic, and also has several applications including in weight and blood glucose management (Li and Gao, 2010; Li *et al.*, 2011; Fuentes-Zaragoza *et al.*, 2011; Kasote *et al.*, 2014). RS has been classified into four subtypes, RS1 to RS4 (Sajilata *et al.*, 2006). RS1 represents physically inaccessible form of starch which is locked within cell walls and food matrixes, and consequently prevents amylolysis. RS2 is in granular form and resistant to enzyme digestion. RS3 refers to retrograded or crystalline non-granular starch generally formed during cooling of gelatinized starch. RS4 includes chemically modified or re-polymerized starches (Sajilata *et al.*, 2006; Fuentes-Zaragoza *et al.*, 2011). Generally, RS content of the food material is influenced by food processing methods. Interaction between starch polymers during food processing treatments leads to formation of RS (Garcia-Rosas *et al.*, 2009). The formation of RS (mainly RS3) is affected by many factors such as

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water content, heating temperature and time, number of heating and cooling cycles, as well as freezing and drying (Englyst and Cumming, 1987).

Tapioca or cassava pearls is a staple starchy food obtained from the root of cassava (*Manihot esculenta*), locally termed as Sabudana (Hindi and Marathi). In India, tapioca pearls are routinely used in different food preparations. In addition, it has also been used as a thickening agent. Thus far, numerous research reports are available, which showed that tapioca starch could be modified by various physical, enzymatic and chemical methods in order to obtain desirable functional property (Onyango *et al.*, 2006; Mutungi *et al.*, 2009; Kouadio *et al.*, 2013). Amongst them, physical and enzymatic methods have been commonly used so as to increase the nutritionally important RS3 content in the tapioca starch, because raw tapioca starch had comparatively higher content of RS2 than RS3. Mutungi *et al.* (2009) showed that heat-moisture treatment increases RS3 content of debranched tapioca starch. Furthermore, formation of RS3 in tapioca starch was found to be depends on autoclaving incubation, time and temperature (Onyango *et al.*, 2006). However, there is little information available on effect of soaking, pre-soaking and autoclaving, as well as sun drying treatments on structure, properties and RS3 content of edible tapioca pearls. Therefore, present study is aimed at to investigate the effect of different soaking and autoclaving treatments on structure, properties and RS3 content of tapioca pearls.

## Material and Methods

### Materials

Tapioca pearls were purchased from local market of Pune, India, and stored in a cool and dry place until used. Enzymes were procured from Sigma–Aldrich. All reagents, chemicals used were of analytical grade, obtained from Sigma–Aldrich and Merck.

### Processing methods

Fine, uniform powder of tapioca pearls was prepared by using a homemade grinder. Powdered samples (1 g) were soaked overnight in distilled water (1:4 and 1:5, w/v) at room temperature and in refrigerator (TS1 to TS4). The remaining water was then drained and samples were dried at 50°C in an oven for 24 h. In another set of experiments, both pre-soaked and raw tapioca pearls powder were dispersed in water (1:4 and 1:5, w/v) and autoclaved at 120°C for 20 min. Cooled samples were kept at room temperature and refrigerator temperature for 12 h, and then dried in oven at 50°C (12 h) and sunlight

(10 h) (TS5 to TS14). All processed dried tapioca pearls samples were finally grinded into a fine powder and used for RS3 content analysis.

### Determination of RS3

RS3 content was estimated according to the modified AOAC official method described by Tribess *et al.* 2009. Briefly, 100 mg samples were washed twice with 8 ml ethanol (8%, v/v), centrifuged at 1500 X g for 10 min. The residues were treated with 4 mL Tris-maleate/NaOH buffer (0.1M, pH 6) containing amyloglucosidase (4 U/ml),  $\alpha$ -amylase (300 U/ml) and pepsin (500 U/ml). Then, the tubes were tightly capped, mixed and incubated at 37°C for 16 h with continuous shaking. Caps were removed and contents were treated with 8 ml ethanol, mixed and centrifuged at 1500 X g for 10 min and residue separated from supernatant. This suspension and centrifugation step was repeated two times. The tubes were placed in an ice water bath with shaking. Later on, 3 ml KOH (2 M) was added to each tube and the slurry was kept on shaker, for 20 min. Then, each tube was treated with 10 ml sodium acetate buffer (1.2 M, pH 3.8). Immediately, 0.1 ml amyloglucosidase was added (3200 U/ml, sodium acetate buffer, pH 4.75), with vigorous mixing. Tubes were capped and incubated in shaker bath at 50°C for 30 min. After incubation, the tube volume was adjusted to 20 ml with distilled water and centrifuged at 1500 X g for 10 min. Finally, glucose was quantified from the resultant supernatants with glucose oxidase/peroxidase (GOD/POD) reagent assay kit (ACCUREX, Biomedical Pvt., Ltd., India) against reagent blank. RS3 was calculated as  $\Delta E \times F/W \times 9.27$ . Where,  $\Delta E$  = absorbance of reaction against reagent blank, F = conversion from absorbance to mg (100  $\mu$ g of D-glucose divided by its GOD/POD absorbance), W = weight of the sample.

### Analysis of structural changes by FT-IR, NIR and Raman spectroscopy

Effects of different soaking and autoclaving treatments on the structural changes of tapioca pearls starch were analyzed by FT-IR, NIR and Raman spectroscopy. Infrared spectra were recorded with a Spectrum One, PerkinElmer FT-IR spectrometer using dispersions of the samples in potassium bromide. Spectra were scanned in the range 4000–450  $\text{cm}^{-1}$ .

Near-infrared spectra were recorded in the range 848-1048 nm with a Infratec™ 1241 Grain Analyzer instrument (Foss Company, Hamburg, Germany Transmittance mode). The samples were placed in a cup of 3.8 cm diameter and 1 cm height and the NIR scans (average of 10 scans at one position) were

registered.

The Raman spectra were measured with a JobinYvon (Horiba group) LABRAM HR spectrometer supplied with an OLYMPUS BX40 microscope accessory. The excitation line was a red radiation from a He-Ne laser emitting at 632.8 nm. The sample powders were put on glass slides and analyzed through a  $\times 100$  U.L.W.D. microscope objective (numerical aperture: 0.80) and an irradiation power of a few mW at the sample was used. The spectra were recorded in the  $100\text{--}3500\text{ cm}^{-1}$  spectral range with an integration time of 1 s.

### Pasting properties analysis

The pasting properties of untreated and treated tapioca pearl samples were evaluated using Rapid Visco Analyser (RVA-4) (Perten Instruments, Newport Scientific, Warriewood, Australia). Viscosity profiles of tapioca pearls samples were recorded using their water suspensions (3:25, w/v). A programmed heating and cooling cycle was used, where the samples were held at  $50^\circ\text{C}$  for 1 min, heated to  $95^\circ\text{C}$  at  $6^\circ\text{C}/\text{min}$ , held at  $95^\circ\text{C}$  for 2.7 min, before cooling from  $95\text{--}50^\circ\text{C}$  at  $6^\circ\text{C}/\text{min}$ , and holding at  $50^\circ\text{C}$  for 2 min. The trough viscosity, breakdown, setback and final viscosity [all expressed in centipoises (cP)] of 10.0% dm suspensions were determined. The pasting temperature ( $^\circ\text{C}$ ), that is, the temperature at which the derivative  $[d(\text{viscosity})/d(\text{time in min})]$  increased, was also determined along with peak time. Viscosity profile measurements were carried out in duplicate.

### Statistical analysis

All the sample analyses were performed in duplicate/triplicate. Experimental data was subjected to analysis of variance (ANOVA) using Microsoft Excel-2007, and expressed as mean. Significance was tested using least significant difference (LSD) test.

## Results and discussion

### Effect of different processing methods on RS3 content

It has been found that soaking and autoclaving treatments had a significant impact on the resistant starch content of most of the starches. Niba and Hoffman (2003) reported that soaking and autoclaving processing conditions influenced on the resistant starch content of the sorghum. The RS3 content of the untreated and processed tapioca pearls samples obtained from the experimental design are shown in Figure 1. Results showed that all processing treatments significantly increased RS3 content of

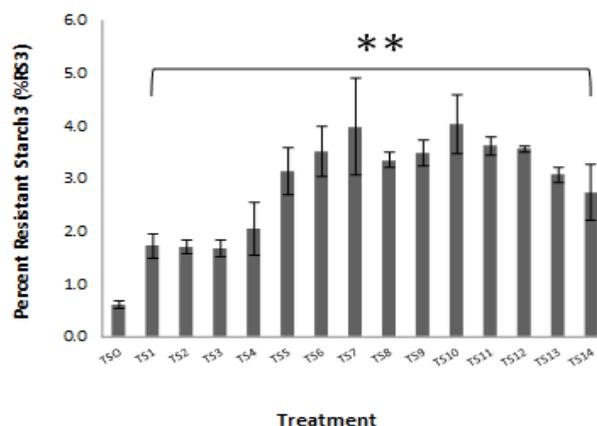


Figure 1. Effect of different soaking and autoclaving treatments on RS3 content of tapioca pearls:

[TS0, untreated tapioca starch sample; TS1 and TS2, overnight soaked tapioca samples in distil water (1:4 and 1:5, w/v respectively) at room temperature and dried at  $50^\circ\text{C}$  for 24 h; TS3 and TS4, overnight soaked tapioca pearls samples in distil water (1:4 and 1:5, w/v respectively) at refrigerator temperature and dried at  $50^\circ\text{C}$  for 24 h; TS5, autoclaved tapioca pearls samples having water content (1:4, w/v) and dried at  $50^\circ\text{C}$  for 24 h; TS6, autoclaved tapioca pearls samples having water content (1:5, w/v) and dried at  $50^\circ\text{C}$  for 24 h; TS7 and TS8, overnight pre-soaked tapioca pearls samples at room temperature and autoclaved in distil water (1:4 and 1:5, w/v respectively), stored at room temperature for 12 h and dried  $50^\circ\text{C}$  for 24 h; TS9 and TS10, overnight pre-soaked tapioca pearls samples at room temperature and autoclaved in distil water (1:4 and 1:5, w/v respectively), stored at refrigerator temperature for 12 h and dried at  $50^\circ\text{C}$  for 24 h; TS11 and TS12, overnight pre-soaked tapioca pearls samples at room temperature and autoclaved in distil water (1:4 and 1:5, w/v respectively), stored at room temperature for 12 h and sun dried for 10 h; TS13 and TS14, overnight pre-soaked soaked tapioca pearls samples at room temperature and autoclaved tapioca pearls samples in distil water (1:4 and 1:5, w/v respectively) stored at refrigerator temperature for 12 h and sun dried for 10 h]. Values are means of three determinations  $\pm$  SD. \*\* $P < 0.001$ .

tapioca pearls. Autoclaving treatments showed comparatively highest increase in RS3 content than soaking treatments. This high RS3 levels after autoclaving might be because of the increase in the crystallinity. It has been reported that crystallinity of starches increases due to the reorientation of starch granule molecules or chains, which makes starch less susceptible to hydrolysis with amylolytic enzymes (Buleon *et al.*, 1998; Sun *et al.*, 2014). Maximum RS3 was observed in sample TS10 (overnight pre-soaked and autoclaved tapioca pearls sample in distilled water (1:5, w/v) kept overnight at refrigerator temperature and dried at  $50^\circ\text{C}$  for 24 h). This could be due to the retrogradation of starch, which is greatly affected by storage temperature, and storage of starch gels at lower temperatures generally increases retrogradation (Eerlingen *et al.*, 1993; Farhat *et al.*, 2000; Alsaffar, 2011). These findings help to establish processed tapioca pearls as a prebiotic, and/or low glycemic food product.

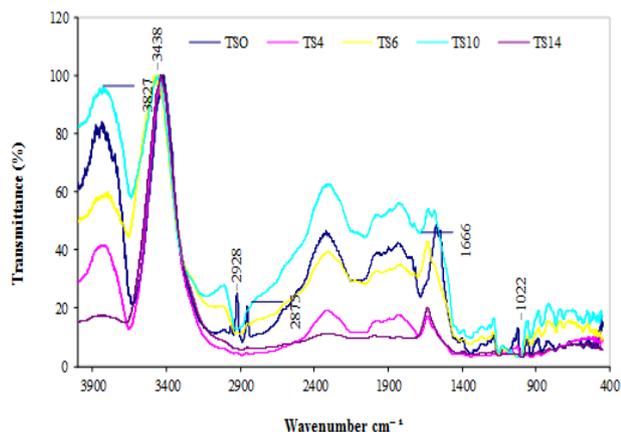


Figure 2. FT-IR spectroscopic analysis of tapioca pearls samples:

TS0- untreated tapioca pearls sample; TS4- overnight soaked tapioca pearls sample in distilled water (1:5 w/v) at refrigerator temperature and dried at 50°C for 24 h; TS6- autoclaved in tapioca pearls sample distilled water (1:5, w/v), and dried at 50°C for 24 h; TS10- Overnight pre-soaked tapioca pearls at room temperature and autoclaved in distilled water, stored at refrigerator for 12h (1:5, w/v), and dried at 50°C for 24 h; TS14- overnight pre-soaked tapioca pearls and autoclaved in distilled water (1:4, w/v), stored at refrigerator temperature for 12 h and sun dried for 10 h.

#### Analysis of structural changes by FT-IR, NIR and Raman spectroscopy

FT-IR, NIR and Raman spectroscopic studies were undertaken in order to analyze the effect of soaking and autoclaving treatments on the structural changes of tapioca pearl starch. It has been well established that the FT-IR spectrum of starch helps to understand the changes in its structure on a molecular level (short-range order), such as starch chain conformation, crystallinity, and retrogradation (Van Soest *et al.*, 1995). The IR transmittance bands at 1047 and 1022  $\text{cm}^{-1}$  are sensitive to ordered or crystalline structures, and amorphous structures in the starch, respectively. Furthermore, the ratio of 1047  $\text{cm}^{-1}$ /1022  $\text{cm}^{-1}$  has been used to express the amount of crystalline to amorphous domains in the starches (Van Soest *et al.*, 1995; Capron *et al.*, 2007). The resultant ratios of 1047  $\text{cm}^{-1}$ /1022  $\text{cm}^{-1}$  for untreated (TS0), various soaked (TS2 to TS4) and autoclaved (TS5 to TS14) tapioca pearls samples were in the order: untreated < soaked < autoclaved. This increase in ratios after soaking and autoclaving treatments could be due to their soaring in crystalline perfection (Chung *et al.*, 2009). Amongst all autoclave treatment samples, TS10 had comparatively highest 1047  $\text{cm}^{-1}$ /1022  $\text{cm}^{-1}$  ratio (Figure 2). High 1047  $\text{cm}^{-1}$ /1022  $\text{cm}^{-1}$  ratio was congruent with high RS3 content of sample. The untreated tapioca pearls samples showed characteristic bands at 2928 and 2826  $\text{cm}^{-1}$  ( $\text{CH}_2$  vibrations), which were found to be disappeared with soaking and autoclaving treatments,

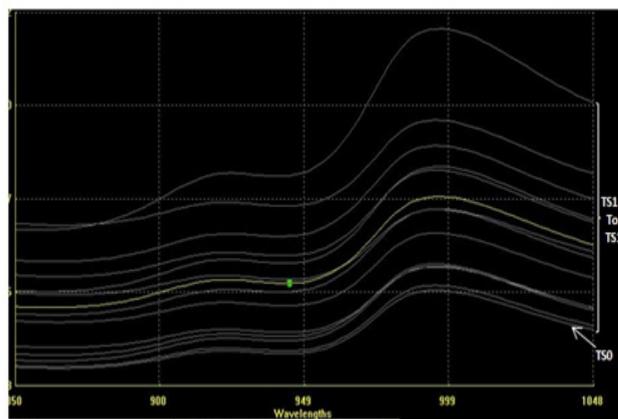


Figure 3. NIR spectroscopic analysis of untreated (TS0) and different soaking and autoclaving treatment (TS1 to TS14) tapioca pearls samples.

this confirmed that bands at 2928 and 2826  $\text{cm}^{-1}$  are characteristically sensitive to these treatments. An extreme band at 3438  $\text{cm}^{-1}$  was attributed to hydrogen bonded O–H stretching vibration. The intensity of this band was observed to be decreased with soaking and autoclaving treatments. This finding confirmed that soaking and autoclaving treatments could be decreases the intra and intermolecular hydrogen bonds of native tapioca pearls starch.

In addition to this, NIR spectroscopic analysis was also carried out to study structural changes of tapioca starch before and after different soaking and autoclaving treatments, because NIR has a higher precision compared to FT-IR. Figure 3 shows the NIR spectrum of untreated and different soaked and autoclaved tapioca pearls samples. The NIR spectra of soaked and autoclaved tapioca starch samples characteristically showed changes in peak shapes and increasing intensities within range 900 to 1048  $\text{cm}$ . These observed changes could be due to the modifications on the skeletal modes of the pyranose ring (García-Rosas *et al.*, 2009). The observed peak intensities within range 900 to 1048  $\text{cm}$  for untreated (TS0), various soaked (TS1 to TS5) and autoclaved (TS4 to TS14) tapioca pearls samples were in the order: untreated < soaked < autoclaved. Among all, the sample TS10 had comparatively highest peak intensity within range 900 to 1048  $\text{cm}$ , which could be linked to its high amount of structural changes in the skeletal mode (Figure 3), and can also be correlated with its highest RS3 content. Besides structural change analysis, findings of both FT-IR and NIR studies can also be used to develop quantitative analysis method for estimation of RS3 content of tapioca pearls using these techniques.

Raman spectroscopic analysis has been found to be valuable in understanding the structural changes in the starches occurred during gelatinization and

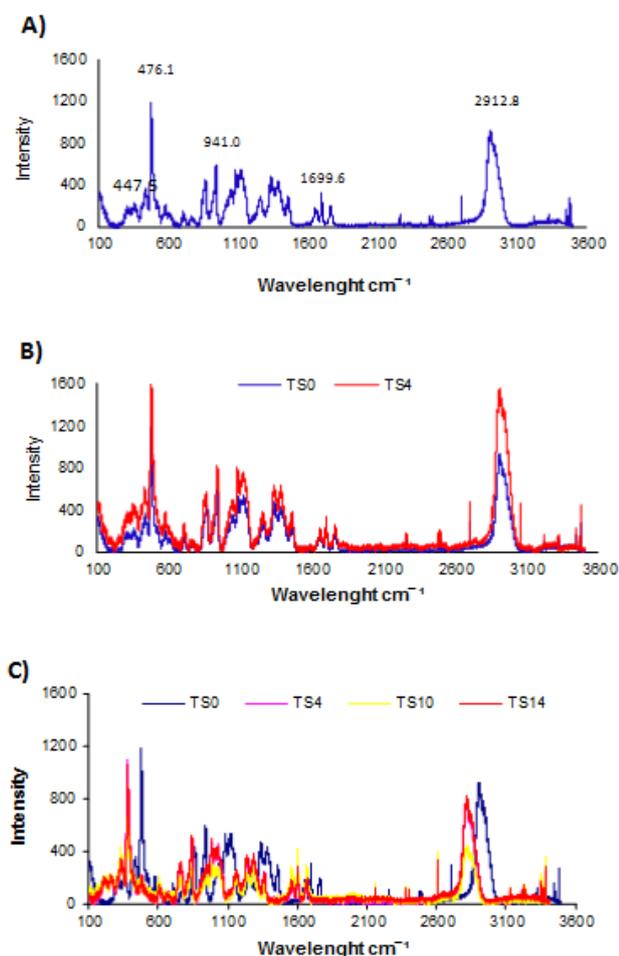


Figure 4. Raman spectroscopic analysis of tapioca pearls sample.

A) Raman spectra of untreated tapioca pearls sample (TS0); B) Raman spectra of untreated tapioca pearls sample (TS0) and overnight soaked tapioca pearls sample in distilled water (1:5 w/v) at refrigerator temperature and dried at 50°C for 24 h (TS4); C) Raman spectra of untreated tapioca pearls sample (TS0), overnight soaked tapioca pearls sample in distilled water (1:5 w/v) at refrigerator temperature and dried at 50°C for 24 h (TS4); TS10- Overnight pre-soaked tapioca pearls at room temperature and autoclaved in distilled water, stored at refrigerator for 12h (1:5, w/v), and dried at 50°C for 24 h; TS14- overnight pre-soaked tapioca pearls and autoclaved in distilled water (1:4, w/v), stored at refrigerator temperature for 12 h and sun dried for 10 h.

retrogradation (Bulkin *et al.*, 1987; Kim *et al.*, 1989; Kizil and Irudayaraj, 2006). Therefore, in the present work Raman spectroscopic studies were undertaken in order to analyze the effect of soaking and autoclaving on molecular structural changes of tapioca pearls starch.

In Raman spectra, electrically symmetrical bonds (i.e. having no dipole moment) are active. It has been observed that physical and chemical modifications of starches cause typical change in Raman peak position and intensity (Fechner *et al.*, 2005). The intensities of C–H bonds (2800–3050  $\text{cm}^{-1}$ ) and skeletal peaks (450–750  $\text{cm}^{-1}$ ) are extremely sensitive to changes induced by the processes of gelatinization and retrogradation

(Manno *et al.*, 2009). In addition, Phillips *et al.* (1999) correlated the varying intensities of 1657  $\text{cm}^{-1}$  Raman band, with the amount of amylose present in maize starches. The Raman spectrum of untreated tapioca pearls sample had characteristic intense peaks at 2912 and 476  $\text{cm}^{-1}$  (Figure 4A). The Raman spectra of untreated tapioca pearls sample TS0, and 1:5 (w/v) water soaked tapioca pearls sample at refrigerator temperature (TS4) are shown in Figure 4B. Untreated samples exhibited a typical pattern of Raman spectral peaks. TS0 and TS4 showed very strong skeletal mode peaks at around 480  $\text{cm}^{-1}$  and 2912  $\text{cm}^{-1}$ , which are associated with the  $\alpha$ -1,4-glycosidic linkage and symmetrical and anti-symmetrical CH stretching (Almeida *et al.*, 2010; Flores-Morales *et al.*, 2012). Raman spectra of TS0 and TS4 showed slight positional peak intensity changes at wavelength 480  $\text{cm}^{-1}$  and 2900  $\text{cm}^{-1}$ . The observed peak intensity changes at these wavelengths could be due to the variations in the amount of amylose and amylopectin (Almeida *et al.*, 2010). The resultant peak intensities of soaked sample TS4 at wavelengths 480  $\text{cm}^{-1}$  and 2900  $\text{cm}^{-1}$  were comparatively higher than those of TS0, which confirmed that soaking conditions affect amylose and amylopectin content of tapioca starch. The intensity of Raman peak at 1657  $\text{cm}^{-1}$  of TS4 was also comparatively higher than that of TS0, which further confirmed that soaking condition could increase the amylose content of tapioca starch without much structural change. TS4 showed slight broadening of O-H stretch region (3000–3600  $\text{cm}^{-1}$ ) due to the uptake of water by the starch (Kizil and Irudayaraj, 2006). TS0 and TS4 exhibited no peak within the region 1500–1600  $\text{cm}^{-1}$  confirming absence of protein or interaction of starch with protein (Thygesen *et al.*, 2003). These results clearly showed that the increase in the resistant starch content of tapioca starch after soaking might be due to increase in the amylose content.

Retrogradation, the characteristic behavior of starch–water systems, is the reorganization of starch molecules after heat treatment (Fechner *et al.*, 2005). All autoclaving treatments showed significant increase ( $p < 0.001$ ) in the RS3 content. The Raman spectroscopic studies of autoclaved samples demonstrated that the intense shifting of whole Raman spectra towards the lower wavelength (Figure 4C). The observed shifting in Raman peak position could be linked with the complete structural modification of the starch due to molecular order loss. These results confirmed that increase in resistant starch content of tapioca pearls after autoclaving might be due to their complete structural modification.

Table 1. Pasting properties analysis of Tapioca pearls samples before and after different treatments (soaking and autoclaving) using Rapid visco analyser (RVA) [R.T.- room temperature; D.W.- Distil water]

Sample id.	Tapioca pearls sample conditions	treatments	Pasting temp. °C	Peak time (min)	Trough viscosity (RVU)	Breakdown (RVU)	Setback (RVU)	Final viscosity (RVU)
TS0	Untreated tapioca sample (control)	pearls	59.2	6.6	1002	2046	717	1688
TS1	Overnight soaked tapioca pearls sample in D.W. (1:4 w/v) at R.T.		60.8	7.0	1185**	1496	644	1829
TS2	Overnight soaked tapioca pearls sample in D.W. (1:5 w/v) at R.T.		60.4	6.8	1196**	1658	607	1803
TS3	Overnight soaked tapioca pearls sample in D.W. (1:4 w/v) at refrigerator temperature		60.6	6.7	1216**	2017	698	1914**
TS4	Overnight soaked tapioca pearls sample in D.W. (1:5 w/v) at refrigerator temperature		59.5	6.7	1226**	1835	707	1933**
TS5	Autoclaved in tapioca sample D.W. (1:4, w/v), and dried at 50°C for 24 h	pearls	70.1**	8.9	188	31	139	325
TS6	Autoclaved in tapioca sample D.W. (1:5, w/v), and dried at 50°C for 24 h	pearls	79.0**	9.0	84	29	165	248
TS7	Overnight pre-soaked at R.T. and autoclaved tapioca pearls in D.W., stored at R.T. for 12h (1:4, w/v), and dried at 50°C for 24 h		76.0**	9.0	108	25	170	274
TS8	Overnight pre-soaked tapioca pearls at R.T. and autoclaved in D.W., stored at R.T. for 12h (1:5, w/v), and dried at 50°C for 24 h		70.8**	9.0	178	37	190	359
TS9	Overnight pre-soaked tapioca pearls at R.T. and autoclaved in D.W., stored at refrigerator for 12h (1:4, w/v), and dried 50°C for 24 h		72.0**	9.0	58	18	138	196
TS10	Overnight pre-soaked tapioca pearls at R.T. and autoclaved in D.W., stored at refrigerator for 12h (1:5, w/v), and dried at 50°C for 24 h		69.1**	9.0	203	33	204	404
TS11	Overnight pre-soaked tapioca pearls and autoclaved in D.W. (1:4, w/v), stored at R.T. for 12 h and sun dried for 10 h		60.6	9.0	656	138	193	849
TS12	Overnight pre-soaked tapioca pearls and autoclaved in D.W. (1:5, w/v), stored at R.T. for 12 h and sun dried for 10 h		60.6	8.5	523	261	168	691
TS13	Overnight pre-soaked tapioca pearls and autoclaved in D.W. (1:4, w/v), stored at refrigerator temperature for 12 h and sun dried for 10 h		56.7	8.6	1095	291	294	1389
TS14	Overnight pre-soaked tapioca pearls and autoclaved in D.W. (1:4, w/v), stored at refrigerator temperature for 12 h and sun dried for 10 h		52.2	8.0	1388**	686	521	1909**
<b>LSD (0.05)</b>			1.6	0.69	111	304	109	157
<b>LSD (0.01)</b>			2.1	0.96	154	420	150	217

All the significance was tested against mean value of control tapioca pearls sample, \*\*P<0.001

D.W. means distilled water

R.T. means room temperature

### *Effect of different processing methods on pasting properties of tapioca starch*

The RVA pasting profiles of untreated and various processed tapioca pearls samples are summarized in Table 1. It has been reported that processing treatments such as heat moisture treatment influence on the pasting properties of starches. The pasting temperature (the temperature where viscosity first increases by at least 25 cP over a 20 s period), peak time (the time at which peak viscosity occurred), trough viscosity (hot

paste viscosity), breakdown (peak viscosity - holding strength or trough viscosity), setback (final viscosity - holding strength) and final viscosity (the viscosity at the end of test after cooling to 50°C and holding at this temperature) for untreated tapioca pearls starch sample (TS0) was 59.2°C, 6.6 min., 1002 cP, 2046 cP, 717 cP and 1688 cP, respectively. Soaking at room temperature with distilled water content 1:4 and 1:5, w/v (TS1 and TS2) did not significantly modify the RVA properties of tapioca pearls samples, except

the holding strength i.e. through viscosity (Table 1), while soaking at lower temperature with distilled water content 1:4 and 1:5, w/v (TS3 and TS4), tapioca starch samples showed higher holding strength and final viscosity. All the presoaked autoclaved samples with different water, storage temperature and drying treatments (TS5 to TS14) showed highest reduction in the all RVA pasting parameters as compared to the untreated sample (TS0). The lowering of RVA pasting parameters indicated reduced starch gelatinization after cooling. Autoclaving treatments found to be increased crystallization; this lowers the rate of water absorption and swelling of starch granules. In addition, it has also been observed that most of the RVA properties were negatively correlated with RS3 content. Results of pasting properties of soaked tapioca samples were somewhat comparable to that of untreated tapioca pearls sample, whereas, autoclaved tapioca pearls samples were exhibiting complete lose all the functional pasting property parameters. These findings confirmed that soaking treatments helps to increase the RS content as well as to preserve pasting property parameters of tapioca pearls which are essential to maintain its food processing quality.

## Conclusion

In this study, we have demonstrated for the first time that how different soaking and autoclaving treatments affects on the structure, properties and RS3 content of edible tapioca pearls. All the soaking and autoclaving treatments increased RS3 content of tapioca pearls. FT-IR, NIR and Raman spectroscopy studies confirms these increase in RS3 content of tapioca pearls after soaking and autoclaving treatments was due to change in amylose to amylopectin ratio and increase in crystallinity, respectively. Pasting properties of untreated tapioca pearls samples were found to decline with treatments. Studied Tapioca pearls sample lost almost all pasting property parameters after autoclaving treatments. Altogether, these finding confirmed that autoclaving treatments are good to achieve highest RS3 content of the tapioca pearls, but fails to preserve its pasting property. On contrary to this, soaking treatments preserve pasting property parameters as well as moderately increased the RS3 content of tapioca pearls.

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## References

- Almeida, M.R., Alves, R.S., Nascimbem, L.B.L.R., Stephani, R., Poppi, R.J. and De Oliveira, L.C. 2010. Determination of amylose content in starch using Raman spectroscopy and multivariate calibration analysis. *Analytical and Bioanalytical Chemistry* 397: 2693–2701.
- Alsaffar, A.A. 2011. Effect of food processing on the resistant starch content of cereals and cereal products – a review. *International Journal of Food Science* 46: 455–462.
- Buleon, A., Colonna, P., Planchot, P. and Ball, S. 1998. Starch granules: Structure and biosynthesis. *International Journal of Biological Macromolecules* 23: 85–112.
- Bulkin, B.J., Kwak, Y. and Dea Iaian, C.M. 1987. Retrogradation kinetics of waxy-corn and potato starches a rapid Raman spectroscopic study. *Carbohydrate Research* 160: 95–112.
- Capron, I., Robert, P., Colonna, P., Brogly, M. and Planchot, V. 2007. Starch in rubbery and glassy states by FTIR spectroscopy. *Carbohydrate Polymers* 68: 249–259.
- Chung, H.J., Liu, Q. and Hoover, R. 2009. Impact of annealing and heat-moisture treatment on rapidly digestible, slowly digestible and resistant starch levels in untreated and gelatinized corn, pea and lentil starches. *Carbohydrate Polymers* 75: 436–447.
- Eerlingen, R.C., Crombez, M. and Delcour, J.A. 1993. Enzyme resistant starch I. Quantitative and qualitative influence on incubation time and temperature of autoclaved starch on resistant starch formation. *Cereal Chemistry* 70: 339–344.
- Englyst, H.N. and Cummings, J.H. 1987. Resistant starch: A new food component: A classification of starch for nutritional purpose. In *Cereals in European Context*, p. 221–223. England: Ellis Horwood, Chichester.
- Farhat, I.A., Blanshard, J.M.V. and Mitchell, J.R. 2000. The retrogradation of waxy maize starch extrudates: effects of storage temperature and water content. *Biopolymers* 53: 411–422.
- Fechner, P.M., Wartewig, A.K., Kiesow, A., Heilmann, A., Kleinebudde, P. and Neubert, R.H.H. 2005. Influence of water on molecular and morphological structure of various starches and starch derivatives. *Starch/Stärke* 57: 605–615.
- Flores-Morales, A., Jiménez-Estrada, M. and Mora-Escobedo, R. 2012. Determination of the structural changes by FT-IR, Raman, and CP/MAS <sup>13</sup>C NMR spectroscopy on retrograded starch of maize tortillas. *Carbohydrate Polymers* 87: 61–68
- Fuentes-Zaragoza, E., Sa´ Nchez-Zapata, E., Sendra, E., Sayas, E., Navarro, C., Ndez-Lo´ Pez, J. and Pe´ Rez-Alvarez, J.A. 2011. Resistant starch as prebiotic: A

- review. *Starch/Stärke* 63: 406–415
- Garcia-Rosas, M., Bello-Perez, A., Romas, G., Flores-Morales, A. and Mora-Escobedo, R. 2009. Resistant starch content and structural changes in maize (*Zea mays*) tortillas during storage. *Starch/Stärke* 61: 414–421.
- Jobling, S. 2004. Improving starch for food and industrial applications. *Current Opinion in Plant Biology* 7: 210–218.
- Kasote, D.M., Nilegaonkar, S.S. and Agte, V.V. 2014. Effect of different processing methods on resistant starch content and in vitro starch digestibility of some common Indian pulses. *Journal of Scientific and Industrial Research* 73: 541–546.
- Kim, I.H., Yeh, A.I., Zhao, B.L. and Wang, S.S. 1989. Gelatinization kinetics of starch by using Raman spectroscopy. *Biotechnology Progress* 5: 172–174.
- Kizil, R. and Irudayaraj, J. 2006. Discrimination of irradiated starch gels using FT-Raman spectroscopy and chemometrics. *Journal of Agricultural and Food Chemistry* 54: 13–18.
- Kouadio, O.K., N'dri, D.Y., Nindjin, C., Marti, A., Casiraghi, M.C., Faoro, F., Erba, D., Bonfoh, B. and Amani, N.G. 2013. Effect of resistant starch on the cooking quality of yam (*Dioscorea* spp.) and cassava (*Manihot esculenta*) based paste products. *Journal of Food Composition and Analysis* 64: 484–493.
- Li, S., Ward, R. and Gao, O. 2011. Effect of heat-moisture treatment on the formation and physicochemical properties of resistant starch from mung bean (*Phaseolus radiatus*) starch. *Food Hydrocolloids* 25: 1702–1709.
- Li, S.L. and Gao, O.Y. 2010. Effect of heat-moisture treatment on the formation and properties of resistant starches from mung bean (*Phaseolus radiatus*) starches. *World Academy of Science, Engineering and Technology* 48: 812–819.
- Manno, D., Filippo, E., Serra, A., Negro, C., De Bellis, L. and Miceli, A. 2009. The influence of inulin addition on the morphological and structural properties of durum wheat pasta. *International Journal of Food Science and Technology* 44: 2218–2224.
- Mutungi, C., Rost, F., Onyango, C., Jaros, D. and Rohm, H. 2009. Crystallinity, thermal and morphological characteristics of resistant starch type III produced by hydrothermal treatment of debranched cassava starch. *Starch/Stärke* 61: 634–645.
- Niba, L.L. and Hoffman, J. 2003. Resistant starch and  $\beta$ -glucan levels in grain sorghum (*Sorghum bicolor* M.) are influenced by soaking and autoclaving. *Food Chemistry* 81: 113–118.
- Onyango, C., Bley, T., Jacob, A., Henle, T. and Rohm, H. 2006. Influence of incubation temperature and time on resistant starch type III formation from autoclaved and acid-hydrolysed cassava starch. *Carbohydrate Polymers* 66: 494–499.
- Phillips, D.L., Xing, J., Liu, H.J., Pan, D.H. and Corke, H. 1999. Potential use of Raman spectroscopy for determination of amylose content in maize starch. *Cereal Chemistry* 76: 821–823.
- Sajilata, M.G., Singhal, R.S. and Kulkarni, P.R. 2006. Resistant starch- a review. *Comprehensive Reviews in Food Science and Food Safety* 5: 1–17.
- Singh, N., Kaur, L., Sandhu, K.J., Kaur, J. and Nishinar, K. 2006. Relationships between physicochemical, morphological, thermal, rheological properties of rice starches. *Food Hydrocolloids* 20: 532–542.
- Singh, N., Singh, J., Kaur, L., Sodhi, N.S. and Gill, B.S. 2003. Morphological, thermal and rheological properties of starches from different botanical sources. *Food Chemistry* 81: 219–231.
- Sun, Q., Dai, L., Nan, C. and Xiong, L. 2014. Effect of heat moisture treatment on physicochemical and morphological properties of wheat starch and xylitol mixture. *Food Chemistry* 143: 54–59.
- Thygesen, L.G., Løkkey, M.M., Micklander, E. and Engelsen, S.B. 2003. Vibrational microspectroscopy of food. Raman vs. FT-IR. *Trends Food Science and Technology* 14: 50–57.
- Tribess, T.B., Herná'Ndez-Uribe, J.P., Me'Ndez-Montealvo, M.G.C., Menezes, E.W., Bello-Perez, L.A. and Tadini, C.C. 2009. Thermal properties and resistant starch content of green banana flour (*Musa cavendishii*) produced at different drying conditions. *LWT - Food Science and Technology* 42: 1022–1025.
- Van Soest, J.J.G., Tournois, H., De Wit, D. and Vliegthart, J.F.G. 1995. Short-range structure in (partially) crystalline potato starch determined with attenuated total reflectance Fourier-transform IR spectroscopy. *Carbohydrate Research* 279: 201–214.
- Zavareze, E.D.R. and Dias A.R.G. 2011. Impact of heat-moisture treatment and annealing in starches: A review. *Carbohydrate Polymers* 83: 317–328.