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Physicochemical, rheological and structural characterization of acetylated oat starches

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ABSTRACT

Starches isolated from three different varieties of oat were modified with acetic anhydride to make starches with high contents of type 4 resistant starch (RS4). The acetyl group percentage (Ac) and degree of substitution values (DS) of oat starches ranged from 0.64 to 1.50 and 0.02-0.05, respectively. The absorption peaks at 1737 cm⁻¹, 1337 cm⁻¹ and 1243 cm⁻¹ in the FTIR spectrum confirms the presence of acetyl groups in the starch molecule. SEM micrographs revealed significant changes in external morphology of acetylated starches due to the formation of small pores. XRD analysis reflects the formation of amylose-lipid complex with decreased crystallinity in acetylated starches. The viscosity of starches decreased on acetylation, exhibited shear-thinning behavior as reflected from the convex shaped graph with viscosities of acetylated starches following the order; SKO90 > SKO20 > Sabzaar.

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1. Introduction

Oat is receiving increased scientific and public interest because of nutritional and neutraceutical health benefits associated with its consumption (Chu et al., 2013; Butt, Nadeem, Khan, Shabir, & Butt, 2008). Mostly oat is consumed as whole grain products like breakfast cereals, infant foods, porridge, and specialty breads (Tester & Karkalas, 1996). Besides it oat can be an important starch source which is also the major component of grain and could be usually found at levels between 60 and 65 g/100 g. But native starch has very narrow range of food applications due to their less stability to retrogradation, high gelatinization temperatures and high gel turbidity. Also to achieve the target of food as a neutraceutical there is need to modify the structural architecture of starch in order to develop appreciable quantity of resistant starch (RS). Different modifications of starch have been employed for improving the functionality and increase the resistance to enzymatic hydrolysis (Ashwar, Gani, Shah, Wani, & Masoodi, 2015). Acetylation is one such chemical modification in which some of the hydroxyl groups of starch chain are replaced by acetyl group, thereby altering its molecular structure and hence its properties. The maximum limit of acetyl content for food starches permitted by the FDA is 2.5 g/ 100 g. In India the demand of modified starch is growing day by day

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and is approximately 27,500 tons per year with 8-9 percent of growth rate per annum (Anon, 2006, pp. 1–17). Therefore modified oat starch could be a better alternative to meet the demands of food industries which has been otherwise ignored. So far very little work reporting properties of acetylated oat starch are available (Mirmoghtadaie, Kadivar, & Shahedi, 2009; Galdeano, Mali, Grossmann, Yamashita, & Garcia, 2009; Berski et al., 2011). The present work was undertaken to investigate the effect of acetylation on the RS4 (resistant starch type 4) content inclusive of physicochemical, morphological, structural and rheological properties of oat starch from three varieties. This comprehensive information on the properties of acetylated oat starch can be helpful to expand its use as an ingredient in food industry which otherwise utilizes primarily potato, corn, wheat, and rice starches as raw material.

2. Materials and methods

2.1. Materials

Three oat varieties were procured form Sher-e-Kashmir University of Agricultural Sciences and Technology (SKAUST), Shalimar, Srinagar, J&K, India. Amyloglucosidase, pancreatin, and sodium acetate buffer (3 mol/L) were obtained from Sigma- Aldrich, St. Louis, USA. GOPOD Assay Kit was procured from Megazyme International Ldt, Bray, Ireland. All the other chemicals used were of analytical grade and purchased from HIMEDIA, Mumbai- India.





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2.2. Starch extraction

Starch was isolated according to the method described by Shah, Masoodi, Gani, and Ashwar (2016). Briefly aqueous oat flour slurry (flour: water, 1:10) was maintained at pH 9, mixed intermittently for 1 h and filtered through muslin cloth. This was followed by centrifugation of filtrate at $3000 \times g$ for 15 min and then scraping off the upper layer containing impurities while as lower starch layer was washed three times with distilled water and then dried at 40° C.

2.3. Acetylation of oat starchwith acetic acid

Oat starches were acetylated according to the method described by Otto, Baik, and Czuchajowska (1997) with some modifications. Starch dispersion (100 g/500 mL of H₂O) was stirred for 20 min on magnetic stirrer. The pH of starch slurry was adjusted to pH 8.0 (1 mol/L NaOH) followed by addition of 7.65 g of acetic anhydride drop wise with continuous stirring while maintaining the pH between 8.0 and 8.5. The reaction was allowed to proceed for 5 min and then stopped by adjusting the pH to 4.5 with HCl (0.5 mol/L). The final suspension was centrifuged for 3 min at 1000 \times g and the recovered starch was washed three times with distilled water, then dried at 30°C.

2.4. Determination of acetyl percentage and degree of substitution

Percent of acetyl groups (Ac) and degree of substitution (DS) were determined by the method of Wurburg (1964). Starch (1.0 g) was dispersed in a 250 mL flask containing 50 mL of ethanol (75 mL/L). The flask was covered properly with aluminum foil before heating at 50° C for 30 min in a water bath. After cooling the suspension, potassium hydroxide (KOH) (40 mL, 0.5 mol/L) was added and titrated out with HCl (0.5 mol equi/L). The solution was allowed to stand for 2 h, and again titrated to remove any additional alkali, which may have leached from the sample. Native starch sample was used as a blank.

$$\begin{array}{l} \text{Ac } (g/100g) = [(V_B - V_S) \times \text{Molarity of HCl} \times 0.043 \times 100] \\ \times / \text{Weight of sample} \end{array}$$

 V_B and V_S represent titration volume of blank and sample expressed in mL, and sample weight was expressed in grams.

Degree of substitution (DS) is defined as the average number of sites per glucose unit that possesses asubstituent group (Whistler & Daniel, 1995).

 $DS = 162 \times Ac/[4300 - (42 \times Ac)].$

Where Ac is the acetyl group percent.

2.5. ATR-fourier transforms infrared (FTIR) spectroscopy

The infrared spectra of starch samples were obtained using ATR-FTIR spectrophotometer (CARY 630, Agilent Technologies, USA) within the range of 600-4000 cm⁻¹ at a resolution of 4 cm⁻¹.

2.6. XRD analysis

XRD patterns of starch samples were recorded with a wavelength of 0.154 nm using X-ray diffractometer (X'Pert PRO, Panalytical, Netherlands). The relative percent crystallinity (RC) of starch was calculated as:

Relative percent crystallinity(RC) = $[A_c/(A_c + A_a)] \times 100$;

where A_c is the crystalline area; A_a is the amorphous area on the X-ray diffractograms.

2.7. Scanning electron microscopy

The fine dried powder of starch samples were placed on an adhesive tape attached to an aluminum stub and coated vertically with gold-palladium. The samples were examined by scanning electron microscope (Hitachi S-300H-Tokyo, Japan) to study its morphological characteristics.

2.8. Resistant starch content

Resistant starch (RS) content was determined using glucose oxidase/peroxidase (GOPOD) assay Kit (Megazyme International Ldt, Bray, Ireland), by AACC method (2000).

2.9. Rheological measurement

The flow properties of starch suspension were measured using rotational rheometer (MCR 102, Anton Paar) according to the method mentioned in Shah, Masoodi, Gani, and Ashwar (2016). The flow behavior index, yield stress and consistency coefficient were determined from the flow curve of shear rate (r) versus shear stress (τ) using Herschel-Bulkley model

$$\tau = \tau_0 + K\gamma^n$$

 τ (Pa) is the shear stress, τ_0 (Pa) is the yield stress, K is the consistency index (Pa sⁿ), and n is the flow behavior index.

To determine the zone of linear viscoelasticity, amplitude sweep test at constant frequency of 1 Hz and shear stress ranging between 0.01–100 Pa was carried for each starch gel separately. After choosing the appropriate shear stress (1 Pa) the frequency sweep test using a frequency range of 0.1–100 Hz.

2.10. Water and oil absorption capacity

2.5 g starch on dry weight basis (db) was mixed with 20 mL distilled water or mustard oil and then stirred for 30 min at 25°C. The slurry was then centrifuged at $3000 \times g$ for 10 min and the supernatant was decanted. The gain in weight was expressed as water/oil absorption capacity (Gani et al., 2014).

2.11. Freeze thaw stability

Aqueous starch suspension (6 g/100 g) was heated in a water bath at a temperature of 90°C, for 30 min. To measure freeze thaw stability, the gels were frozen at -16 °C for 24 h, thawed at 25°C for 6 h and then refrozen at -16°C. Five cycles of freeze thaw were performed. The tubes were centrifuged at 1000 × g for 20 min at 10°C and the released water was measured as freeze thaw stability (Shah et al., 2016).

2.12. Statistical analysis

Mean values, standard deviation, two-way analysis of variance (ANOVA) were computed using a commercial statistical package SPSS (IBM statistics 22). These data were then compared using Duncan's multiple range tests at 5% significance level.

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