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# PHYSICO-CHEMICAL CHARACTERIZATION OF NATIVE AND ACID-ALCOHOL (NITRIC ACID-METHANOL) MODIFIED POTATO STARCHES AND THEIR COMBINATIONS IN DIFFERENT PROPORTIONS

**Navdeep Singh Sodhi*, Avneet Kaur, Bhavnita Dhillon**

*Department of Food Science and Technology, Guru Nanak Dev University, Amritsar, Punjab, India*

**Corresponding author: navdeep.food@gndu.ac.in*

## ABSTRACT

The potato starch was extracted and chemically modified using nitric acid in methanol at 35°C. Mixtures were formulated by adding chemically modified starch to the native starch at different levels viz. 0, 10, 30, 50, 70, 90 and 100%. The swelling power of the starches decreased as the level of incorporation of modified starch increased and ranged between 18.72 to 0.77 g/g, while the solubility increased and ranged between 7.03 to 88.77%, at 90°C. The amylose was observed to decrease significantly ($p < 0.05$) from 24.55 to 3.26% upon modification. The granular morphology of potato starches as observed by scanning electron microscopy revealed partial protuberances upon modification. Analysis of X-ray diffractograms showed B-type crystalline structure of potato starch, which did not change upon modification, however, the sharper peaks of modified starch indicated increase in the relative crystallinity. A significant ($p < 0.05$) increase in light transmittance upon acid-alcohol modification was observed which decreased on refrigerated storage. These mixtures of native and modified potato starches differ significantly ($p < 0.05$) in their physico-chemical properties, thus starches with required functionality can be produced by mixing native and modified starches in suitable proportions.

**Keywords:** Potato starch, Acid-methanol treatment, Physico-chemical, Morphology, Crystallinity, Transmittance

## 1. INTRODUCTION

Potato (*Solanum tuberosum* L.) is a vital *rabi* crop grown worldwide (www.agricoop.nic.in). It is rich in vitamins, minerals and dietary fiber [1]. Potatoes are an important staple food as they contain phytonutrients and health promoting compounds such as carotenoids, flavonoids and caffeic acid. Also blood pressure-lowering compounds called kukoamines have been identified by the UK scientists in potatoes [2]. India is the second largest producer with metric 48,529,000 tonnes of potatoes produced in 2018 [3].

Potatoes are the fourth largest starch source in the world and it is one of the most abundant crops [4]. Potatoes contain 9-20% starch, 75-80% water, 2.5 to 3.2% protein, 0.1 to 0.2% fat and 0.6% fiber [5]. Potato starch is known for its characteristics such as high phosphorus content, high viscosity, high swelling and high paste clarity. It has large granular size and low tendency to retrograde [6-8]. Amylose, the linear component, and amylopectin, the branched component, are the main constituents of the potato starch. Amylopectin is the major component of potato starch. It is extensively branched and has a short chain with an average length of 22-25 glycosyl residues [9].

Native potato starch is a polysaccharide which consists of number of glucose units linked together by glycosidic bonds. It is a white powder which is tasteless and odourless and is not soluble in alcohol and water [10-11]. It is mostly used in food processing industries and has other commercial applications because it is inexpensive, readily available and abundant [12, 13]. Native starch has many limitations which can be overcome by physical, chemical and enzymatic modifications [14].

Different modification procedures to improve the properties and applications of starches are undertaken [12]. Acid modification changes the physico-chemical properties of starch like it decreases the viscosity and increases the solubility of starch granules. It also decreases the swelling power of starch [15-17]. As compared to native starch acid thinned starch produces firmer gels [18]. The granular form of starch is not changed after acid modification [19].

Researchers have shown that the higher yields of acid-thinned starches can be obtained by modifying starch with acid in the presence of alcohol. Moreover, lesser quantity of acid is required for this treatment [20-22]. The average degree of polymerization (DP) of starch treated with acid-alcohol depends upon its botanical source, acid concentration, type and concentration of alcohol, and treatment temperature [23-26].

However, to get acid-alcohol modified starches with different functionalities for various industrial applications, the chemical reactions are to be carried out several times under different processing conditions, making the process laborious and workers vulnerable to health hazards. So the present study was undertaken to explore the process of mixing, a process much simpler and safer than carrying out chemical reactions, acid-alcohol modified starch with native starch in different proportions to produce starches with different functionalities.

## 2. MATERIAL AND METHODS

### 2.1. Materials

The potato sample was purchased locally from the vegetable market of Amritsar. Analytical grade reagents were used in the present study.

### 2.2. Starch isolation

The method described by Singh *et al.* [27], briefly explained below, was used to isolate starch from potatoes. After washing the potatoes were brushed, peeled and diced. A juicer was employed to extract juice from these cubes. $K_2S_2O_5$ (5 g per liter) was added to the juice to prevent its browning.The filtration was carried out of this juice and the residues were discarded, whereas the sediments were collected after settling the filtrate for overnight. The sediments were dried in an oven at 40°C for a day to obtain the potato starch.

### 2.3. Acid-alcohol modification

Potato starch was acid-alcohol modified with the method given by Chang *et al.* [28]. The methodology is briefly explained as follows. Methanol (100 ml) was used to disperse 25 g of native potato starch (25g) at 35°C. Then nitric acid (5 M, 70 ml) was added to the starch to start the reaction and the same was allowed to proceed for 96 h at 35°C. Later on NaOH (1 M) was added to neutralize the slurry and to stop the reaction. The slurry was cooled for 5 minutes in an ice bath. Subsequently, it was centrifuged (3000 × g) for another 5 minutes. After neutralizing the precipitates, with ethanol (50%), these were filtered and finally put in a dryer at 40°C for drying.

### 2.4. Swelling power (g/g) and solubility (%)

The methodology of Leach *et al.* [29] was used to evaluate swelling power and solubility of potato starches at different temperatures (70, 80, 90°C). The starch slurry was obtained by heating the starch suspension (2%) at 90°C for half an hour with constant stirring. After cooling the slurry, the samples were centrifuged (3000 × g) for another half an hour. The supernatant, and sediments dried at 110°C for 24 h were used to calculate swelling power and solubility of the potato starches, respectively by using the following equations:

Swelling power (g/g)= Wt. of sediment/Wt. of sample

Solubility (%)= {(Wt. of petridish before drying- Wt. of petridish after drying)/Wt. of sample}x100

### 2.5. Amylose content

The method suggested by Williams *et al.* [30] was adopted to estimate amylose content of the potato starches. The absorbance of blue colour, developed due to the addition of an iodine reagent to the potato starch solution under standardized conditions, was measured at 625 nm using UV-VIS spectrophotometer (Cecil Aquarius 7400). A standard curve was used to determine amylose content.

### 2.6. Scanning electron microscopy

Digital scanning electron microscope (JSM 6100, Jeol) was employed to study the morphological characteristics of the potato starches. One drop of starch sample suspension (1%) in ethanol was put on an aluminium stud. The micrographs (700X) were acquired at an acceleration potential of 10kV.

### 2.7. X-Ray diffraction

Analytical Diffractometer (Bruker axs DS focus machine) Cu-Ka radiation with a wavelength of 0.154 nm was used to conduct X-ray diffraction (XRD) of potato starch samples. The diffractograms were obtained over a 2θ range of 5° to 40°, at a temperature of 25°C,with a scan speed of 4° per minute and by acquiring 6 data points per minute.

### 2.8. Light transmittance

A modified method of Craig *et al.* [31] was adopted to measure light transmittance of starch slurry. The starch slurry was obtained by heating the starch suspension (2%) at 90°C for half an hour with constant stirring. The starch slurry was kept at 4°C to study the effect of storage on its light transmittance. UV-VIS spectrophotometer (Cecil Aquarius 7400) was employed to measure light transmittance of starch slurries at 640 nm using distilled water as blank. The light transmittance was measured regularly every day for duration of six days.

### 2.9. Statistical analysis

The analysis of variance (ANOVA) was carried out for the data acquired in triplicate by using Minitab Statistical Software (Minitab Inc., USA).

## 3. RESULTS AND DISCUSSION

### 3.1. Swelling power

Swelling power was determined using method of Leach *et al.* [29]. The hydrogen bonds which stabilize the structure of double helilces in crystallites get broken down by heating the starch in excess water [32]. By establishing hydrogen bonds with water, the starch granules swell with increase in volume.

The swelling power of native, acid-alcohol modified potato starch and their mixtures in different ratios at different temperature of 70°C, 80°C and 90°C differed significantly (Table 1). It was observed that with the increase in level of modified starch in the starch mixtures, there was decrease in swelling power, while the increase in the treatment temperature caused an increase in the swelling power. The swelling power of potato starch decreased upon acid-alcohol modification and it ranged from 12.82 to 0.36 (g/g) at 70ºC, 15.40 to 0.40 (g/g) at 80°C and 18.72 to 0.77 (g/g) at 90°C in various formulated mixtures.

**Table 1: Swelling power of native and acid-alcohol ($HNO_3$-Methanol) modified potato starches and their mixtures in different proportions**

| Sample | Swelling Power (g/g) | | |
|---|---|---|---|
| | **70°C** | **80°C** | **90°C** |
| Native starch | $12.82^a$ | $15.40^a$ | $18.72^a$ |
| 90% native + 10% modified | $10.67^b$ | $13.57^a$ | $18.59^a$ |
| 70% native + 30% modified | $8.61^c$ | $11.32^b$ | $13.94^b$ |
| 50% native + 50% modified | $6.46^d$ | $9.24^c$ | $9.54^c$ |
| 30% native + 70% modified | $4.70^e$ | $5.73^d$ | $5.96^d$ |
| 10% native + 90% modified | $2.46^f$ | $2.47^e$ | $2.17^e$ |
| Modified starch | $0.36^g$ | $0.40^f$ | $0.77^e$ |

*Values with similar superscript in column do not differ significantly ($p<0.05$).*

Tester and Morrison [33] proposed that the crystallites within the molecules of amylopectin and the whole amylopectin molecular shape and weight determine the onset of swelling and gelatinization. With HCl-methanol hydrolysis of maize and potato starches, the swelling power experiences decline [34].

### 3.2. Solubility

Solubility was also determined using method of Leach *et al.* [29]. The solubility of native, acid-alcohol modified potato starch and their mixtures in different ratios at different temperature of 70°C, 80°C and 90°C differed significantly (Table 2).

**Table 2: Solubility of native and acid-alcohol ($HNO_3$-Methanol) modified potato starches and their mixtures in different proportions.**

| Sample | Swelling Power (g/g) | | |
|---|---|---|---|
| | **70°C** | **80°C** | **90°C** |
| Native starch | $4.47^a$ | $6.62^a$ | $7.03^a$ |
| 90% native + 10% modified | $9.93^a$ | $13.47^b$ | $13.63^b$ |
| 70% native + 30% modified | $22.99^b$ | $25.41^c$ | $25.75^c$ |
| 50% native + 50% modified | $39.15^c$ | $40.82^d$ | $40.08^d$ |
| 30% native + 70% modified | $52.04^d$ | $54.45^e$ | $56.92^e$ |
| 10% native + 90% modified | $75.28^e$ | $76.01^f$ | $78.02^f$ |
| Modified starch | $82.54^f$ | $85.43^g$ | $88.77^g$ |

*Values with similar superscript in column do not differ significantly ($p<0.05$).*

It was observed that with the increase in level of modified starch, there was increase in the solubility. The amylopectin degradation could cause disruption of granular structure of starch and increase in leaching with the heating of starch in water resulting in high starch solubility [34]. The solubility was also observed to increase with increase in temperature. The solubility of native and modified potato starch at 70°C ranged from 4.47-82.54 (%). While solubility at 80°C ranged from 6.62-85.43 (%) and at 90°C it was observed in the range from 7.03-88.77 (%). Similar results have been reported by Chang *et al.* [28] for acid methanol treated rice starches. The solubility increased at 90°C because this temperature was well above the gelatinization temperature [35]. Dutta et al. [36] also reported that with acid-alcohol treatment, the solubility of jack fruit seed starch increased.

### 3.3. Amylose content

The amylose content of native and acid-alcohol treated potato starches were evaluated using rapid calorimetric method as described by Williams *et al.* [30]. The amylose content of native and acid-alcohol modified starches differed significantly. The amylose content of native, modified potato starch and their mixtures in different proportions is shown in Table 3. It was observed that with the increased level of modified starch, there was decrease in the amylose content. Amylose content of native starch was 24.55%, which decreased to 16.23% for 50:50 ratio of native and modified potato starch mixture, further amylose content decreased to 3.26% for the modified potato starch.

Ferrini *et al.* [37] suggested that as the treatment time increased the acid methanol action on the cassava and maize starches caused a drastic reduction of amylose content. The results suggested that the molecules of amylose distributed in the amorphorus areas of the granules were preferentially attacked by acid-methanol. Atichokudomchai *et al.* [38] also indicated that during acid hydrolysis the amylose molecules are separated more easily than amylopectin molecules, which suggest that amylose innate preferentially in the amorphous region.

**Table 3: Amylose content of native and acid-alcohol ($HNO_3$-Methanol) modified potato starches and their mixtures in different proportions**

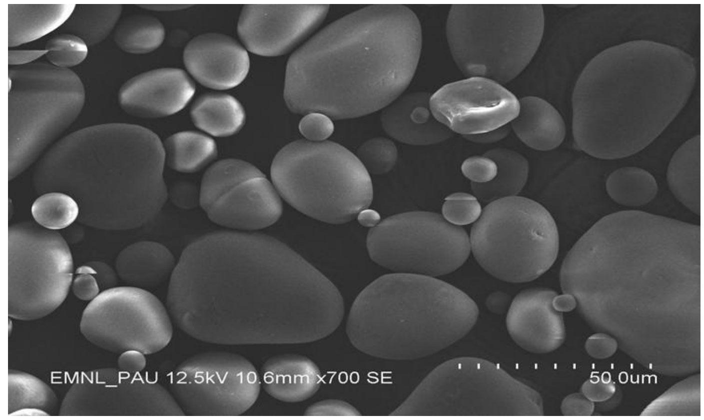
| Sample | Amylose content (%) |
|---|---|
| Native starch | $24.55^{a}$ |
| 90% native + 10% modified | $22.11^{ab}$ |
| 70% native + 30% modified | $20.21^{b}$ |
| 50% native + 50% modified | $16.23^{c}$ |
| 30% native + 70% modified | $11.04^{d}$ |
| 10% native + 90% modified | $7.01^{e}$ |
| Modified starch | $3.26^{f}$ |

*Values with similar superscript in column do not differ significantly ($p<0.05$).*

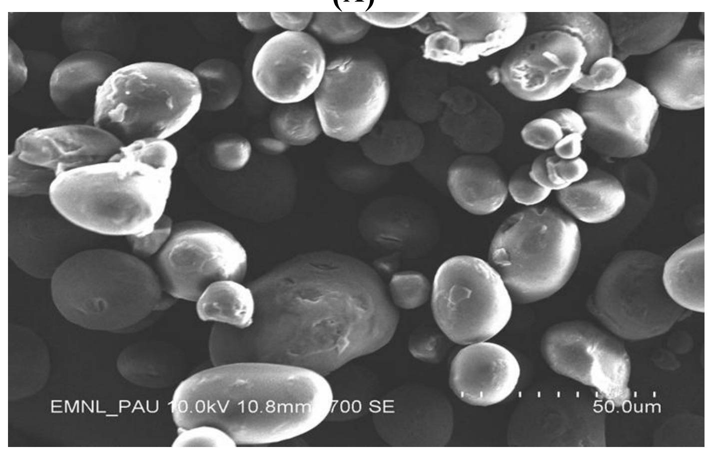
### 3.4. Scanning electron microscopy

SEM is used to observe the morphology of starch granules from different sources. Fannon *et al.* [39] indicated that SEM has been used to relate the morphology of the granule of starch genotype. Starch granules morphology depends on the physiology of the plant and biochemistry of amyloplast and the chloroplast [40]. The variation in shape and size may be due to the biological origin of starch granules [41]. Modification of starch involves biochemical, physical and chemical development on the surface of contacting phases. So SEM has been used to determine the structural changes caused by chemical modification [42, 43].

Granule morphology of native and acid-alcohol modified starch is presented in **Fig. 1.** Potato starch presented a smooth surface with shapes like oval, irregular and cuboidal. After acid-alcohol treatment the granular surface of potato starch changed to rough with partial protuberances. Similar observations have been reported earlier upon acid-alcohol modification of starches from lentil [21], chickpea [22], sorghum [16] and rice [15], maize and cassava [37].




**(A)**

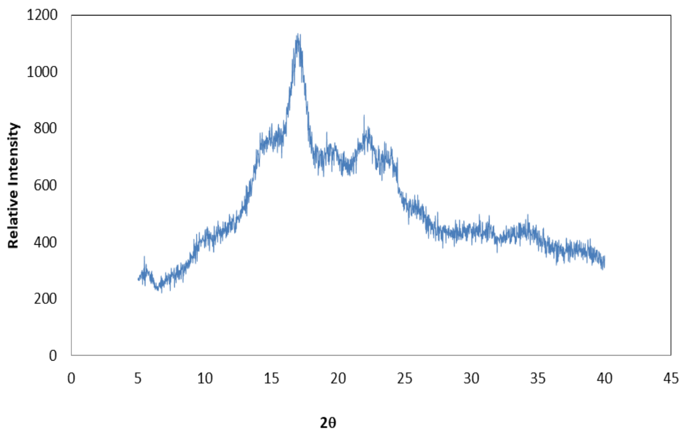



**(B)**

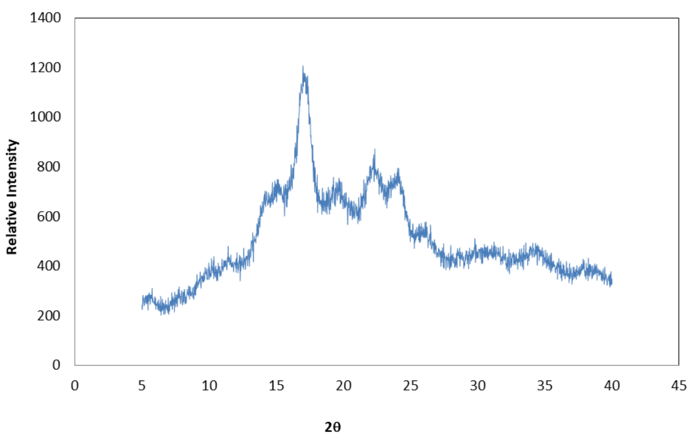
**Fig. 1: Scanning electron micrographs of (A) native potato starch and (B) acid-alcohol ($HNO_3$-methanol) modified potato starch**

### 3.5. X-ray diffraction

X-ray diffraction is an analytical technique used for phase identification of crystalline material. In general starch can be identified as of three types: A- type, B- type and C-type. The X-ray diffractograms of native and modified potato starches have been shown in **Fig. 2**. The strongest diffraction peaks for native and modified starches were found to be centered at 5.5°, 15°, 17°, 19.7°, 22.2° and 24° $2\theta$ angles, which indicated that both native and modified starches were having B-type crystalline structure [44].




**(A)**




**(B)**

**Fig. 2: X-ray diffractograms of (A) native potato starch and (B) acid-alcohol ($HNO_3$-methanol) modified potato starch**

X-ray diffractograms of acid-alcohol modified potato starches showed sharper peaks, indicating increase in crystallinity level, in comparison to those of native starches. Atichokudomchai *et al.* [45] during acid hydrolysis of tapioca starch also reported increase in the relative crystallinity levels and attributed the same to the removal of amorphous areas upon acid treatment.

### 3.6. Light transmittance

Light transmittance provides information on the starch paste behavior [46]. It also depends on the granule size, non-swollen granules, swelling capabilities, amylose content, amylose/amylopectin ratio and swollen granules remnants [8, 47-49]. In case of potato starch, light transmittance decreased with time of storage as shown in Table 4. It is reported that light transmittance of native as well as acid modified corn starch pastes decreased with increase in storage period [17]. Perera and Hoover [50] attributed increased retrogradation upon storage to the formation of functional regions due to the enhanced leaching of amylose and amylopectin chains.

Upon modification, the light transmittance increased. The increase in light transmittance of acid thinned starch is attributed to the decrease in retrogradation tendency and leaching of amorphorus region which enhances interactive bond between the amylopectin molecules [51]. Similar results have been reported by Sandhu *et al.* [17] for acid-thinned corn starch pastes.

**Table 4: Light transmittance of native and acid-alcohol ($HNO_3$-Methanol) modified potato starches and their mixtures in different proportions**

| Sample | Light transmittance (%) | | | | | |
|---|---|---|---|---|---|---|
| | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
| Native starch | $3.80^a$ | $2.57^a$ | $2.10^a$ | $1.83^a$ | $0.97^a$ | $0.47^a$ |
| 90% native + 10% modified | $4.67^b$ | $3.60^b$ | $2.77^b$ | $2.20^a$ | $1.67^b$ | $1.10^b$ |
| 70% native + 30% modified | $5.30^b$ | $4.57^c$ | $3.60^c$ | $2.97^b$ | $2.30^c$ | $1.80^c$ |
| 50% native + 50% modified | $7.40^c$ | $5.70^d$ | $4.57^d$ | $4.07^c$ | $3.40^d$ | $2.53^d$ |
| 30% native + 70% modified | $8.10^{cd}$ | $6.47^e$ | $5.40^e$ | $5.07^d$ | $4.53^e$ | $3.53^e$ |
| 10% native + 90% modified | $8.70^d$ | $7.30^f$ | $6.57^f$ | $6.03^e$ | $5.37^f$ | $4.30^f$ |
| Modified starch | $9.73^e$ | $8.83^g$ | $7.93^g$ | $7.00^f$ | $6.03^g$ | $5.60^g$ |

*Values with similar superscript in column do not differ significantly ($p<0.05$).*

## 4. CONCLUSION

In conclusion, the acid-alcohol modified potato starch and its combination mixtures in different proportions with native starch showed significant changes in their various physico-chemical properties in comparison to native potato starch. The combination mixtures showed decrease in swelling power and increase in solubility. The SEM of native starch granule showed smooth surface and after acid-alcohol modification the granular surface of potato starch changed with partial protuberances. The X-ray diffraction pattern of native potato starch was observed to be of B-type, which remained the same after acid-alcohol modification. The percentage of light transmittance increased after acid-alcohol modification. The present study suggests that the mixtures of native and acid-alcohol modified potato starches in different proportions can replace the acid-alcohol modified starches, produced by carrying out chemical reactions several times under different processing conditions, as these represent characteristics similar to them. This process can emerge as a cost effective, safer and quicker method for obtaining tailor made starches with desirable functional properties for various industrial applications. However, further studies need to be conducted to develop a perfect mixing technique so that the process may be scaled-up.

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