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*Research Article*

# Study of Modified *Trapa Bispinosa* Roxb. Starch as a Natural Filler in Compact Face Powders

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## ABSTRACT

This study aimed to prepare chemically modified starch from *Trapa bispinosa* Roxb. kernel for use as a filler in color cosmetic products to reduce the use of talcum in formulations due to concerns about potential contamination and safety issues. The starch was chemically modified using a 0.2 M sodium hydroxide solution, yielding 36.60 of modified starch that appeared as a fine, non-agglomerated powder free of foreign matter. Colorimetric analysis indicated a slightly yellowish-white appearance, with L*, a*, and b* values of 89.28±0.69, -1.71±0.04, and 7.19±0.06, respectively, and a whiteness index of 83.18±1.74. SEM analysis revealed oval starch granules with smooth surfaces, occasional small protrusions, and particle sizes ranging from 16.25 to 25.5 μm. The water and oil absorption capacities were 0.40±0.15 and 0.35±0.20 mL/g, respectively. Cytotoxicity evaluation using human dermal fibroblast cells demonstrated no cellular toxicity, indicating biocompatibility and suitability for cosmetic applications. Flowability assessment based on the angle of repose, Carr's index, and Hausner ratio indicated good flow properties. Compact face powder formulations containing modified *T. bispinosa* Roxb. starch at concentrations ranging from 12.5% to 50% were developed, and the results showed that the modified starch could replace talcum at levels up to 25% without adversely affecting the physical properties of the formulation.

**Keywords:** *Trapa bispinosa* Roxb., Modified starch, Color cosmetic products, Compact face powder

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## Introduction

Color cosmetic products intended for facial application such as face powder, highlighter, bronzer, blush, and eye shadow are designed to enhance skin coloration, providing a more vibrant appearance compared with the natural skin tone. These products contribute to a youthful and healthy-looking complexion and help conceal facial imperfections. The primary constituents of this category of products are fillers, such as talcum, perlite, magnesium stearate, and bismuth oxychloride. As each filler possesses specific advantages as well as inherent limitations, a combination of different fillers is typically employed to optimize product performance and enhance sensory characteristics [1]. Among the most widely used fillers is talcum, a naturally occurring mineral. Talcum is favored for its white, translucent appearance, softness, smooth skin feel, and water-repellent properties. However, naturally occurring talcum may be contaminated with asbestos fibers. Inhalation of asbestos-containing particles can lead to their accumulation in the lungs, potentially causing respiratory system disorders and increasing the risk of lung cancer [2, 3]. Although talcum used in cosmetic products is required to undergo purification processes to remove such contaminants, complete purification cannot be fully achieved. As a result, some countries, such as the United States, have imposed restrictions or bans on the use of talcum in certain categories of cosmetic products [4, 5]. Moreover, with the current trend of consumers increasingly favoring products formulated with natural ingredients due to their perceived safety and skin-beneficial properties, a growing number of studies have focused on the development of modified natural starches for cosmetic applications as alternatives to talcum-based fillers.

For example, starch isolated from *Myrosma cannifolia* (Guapo) has been investigated as a potential alternative to talcum. The results demonstrated that the starch exhibited favorable flow properties and high water absorption capacity, and its incorporation into a face powder formulation resulted in acceptable and effective product performance [6]. Similarly, sago starch derived from *Metroxylon sagu* Rottb. has been investigated for use in the development of a cooling body powder. The study demonstrated that sago starch exhibited favorable properties and could function as a primary filler to replace talcum in the formulation. Moreover, the developed cooling body powder showed satisfactory characteristics comparable to those of commercially available products, while volunteer testing confirmed the absence of skin irritation and a high level of user satisfaction [7]. In addition, modified starches prepared from unripe Namwa banana (*Musa acuminata*) and unripe green Cavendish banana (*Musa sapientum* L.) have been studied for cosmetic applications, demonstrating their potential suitability as functional cosmetic ingredients [8]. The results showed that the prepared starches exhibited suitable properties for use as fillers in powder formulations. When incorporated at concentrations of up to 15% w/w, the starches did not adversely affect the sensory attributes or texture of the final products. In addition, jasmine rice–derived modified flour, modified with polyethylene glycol-50 shea butter, has been investigated for use in the development of a compact face powder. The findings demonstrated that this modified jasmine rice flour could effectively reduce the talcum content in the formulation. The resulting compact face powder exhibited favorable physical properties, including smoothness, a soft skin feel, and adequate impact resistance in accordance with established evaluation criteria [9].

*Trapa bispinosa* Roxb., commonly known as water chestnut, is an aquatic crop widely cultivated in many Asian countries, including Thailand, particularly in the central region [10]. The plant produces distinctive fruits characterized by a horn-like appearance. The edible portion of the fruit is commonly consumed and contains a high proportion of starch. In addition, *T. bispinosa* flesh contains sugars, proteins, and minerals [11, 12]. Owing to its natural sweetness and pleasant taste, water chestnut is widely consumed as food and used as an ingredient in various culinary preparations [13]. From an industrial perspective, starch extracted from *T. bispinosa* has been utilized as a binder in pharmaceutical products [14]. The starch content in dried *T. bispinosa* kernel has been reported to be approximately 40.89% [15, 16]. Starch granules obtained from water chestnut flesh are oval or spherical in shape, with smooth surfaces and no visible fissures [17, 18]. These characteristics make *T. bispinosa* starch particularly suitable for further development as a filler in color cosmetic products, as it can impart a soft and non-abrasive skin feel. Accordingly, this study aimed to investigate the properties of modified starch derived from *T. bispinosa* kernel, including its appearance, morphological characteristics, water and oil absorption capacities, toxicity, and flow properties, in order to develop a natural filler as a potential substitute for talcum in pressed face powder formulations.

## Materials and Methods

*Preparation of T. bispinosa starch*

The ripe *T. bispinosa* fruits were collected between October and November 2021. The plant species was confirmed by the National Park, Wildlife Conservation Department, Ministry of Natural Resources and Environment, Thailand. The fruits were washed thoroughly in tap water to eliminate the impurity, the fruit's peel was removed and the kernels of the fruit were collected. The fruit's kernels were then sliced into thin layer and dried under sunlight until completely dried. Afterward, they were ground into a fine powder and sieved through a 100-mesh sieve to obtain a uniform particle size. According to the United States Pharmacopeia (USP) powder fineness classification, powders passing through a 100-mesh sieve are considered fine powders suitable for topical applications [19]. The *T. bispinosa* flesh powder was then washed with distilled water and left to settle, after which the supernatant was decanted. This process was repeated until the supernatant was clear and colorless. The remaining sediment was filtered and dried at 45°C until completely dried. Finally, the sediment was ground into a fine powder and sieved through a 100-mesh sieve to obtain the *T. bispinosa* starch.

*Preparation of modified T. bispinosa starch*

Modified *T. bispinosa* starch was prepared according to methods described in previous studies, with slight modifications [17]. Briefly, 10 g of *T. bispinosa* starch were mixed with 20 mL distilled water and treated with 0.2 M Sodium hydroxide solution for 30 minutes. Later, the mixture was then washed with distilled water and centrifuged at 3000×g for 5 minutes. This washing and centrifugation process was repeated until the supernatant became clear, colorless, and reached neutral pH. The remaining sediment was filtered and dried at 45 °C until completely dried. The remaining sediment was

then filtered and dried at 45 °C until completely dry. The dried material was ground into a fine powder and passed through a 100-mesh sieve to obtain the modified *T. bispinosa* starch. The organoleptic properties of the modified starch, including appearance and color were evaluated and compared with those of native *T. bispinosa* starch and talcum. Color parameters were determined using a chromameter (CR 400, Konika Minolta, Japan) and expressed as L*, a*, and b* values where L* represents lightness (ranging from 0 for black to 100 for white), +a* indicates redness and -a* indicates greenness, and +b* indicates yellowness while -b* indicates blueness. The whiteness index (WI) was subsequently calculated using the following equation:

$$WI = 100 - \sqrt{(100\text{-}L^*)^2+a^{*2}+b^{*2}}$$

where L* is the lightness, a* is redness or greenness, and b* is yellowness or blueness of the sample.

In addition, the yield of the modified *T. bispinosa* starch was calculated using the following equation:

$$\text{Yield } (\%) = (W_1 \times 100)/(W_2)$$

where $W_1$ is the weight of modified *T. bispinosa* starch, and $W_2$ is the weight of the weight of *T. bispinosa.*

*Moisture content*

The moisture content of the modified *T. bispinosa* starch was determined according to the Association of Official Analytical Chemists (AOAC) method [20]. Weighing dishes were dried in a hot-air oven at 105 °C for 1 hour, cooled in a desiccator for approximately 30 minutes, and weighed. Subsequently, 2 g of the modified *T. bispinosa* starch was accurately weighed, placed into the pre-dried dishes, and evenly distributed to obtain a uniform layer. The samples were then dried in the oven at 105 °C for 5 hours, cooled in a desiccator for approximately 30 minutes, and reweighed. All measurements were performed in triplicate, and the moisture content was calculated using the following equation:

$$\text{Moisture } (\%) = (W_2 - W_3) \times 100 \,/\, (W_2 - W_1)$$

where $W_1$ is the weight of the empty weighing dish, $W_2$ is the weight of the dish with the initial sample and $W_3$ is the weight of the dish with the dried sample.

*Morphology*

The morphologies of the modified *T. bispinosa* starch was determined by using a scanning electron microscopy (SEM). The modified starch samples were placed on double-side adhesive carbon tape and coated with a gold layer. The starch sample granules were measured at 1000× magnification.

*Fourier transform infrared (FTIR) spectroscopy*

The functional groups of the modified *T. bispinosa* starch were analyzed by Fourier transform infrared (FTIR) spectroscopy following the method described by previous studies [21]. The modified starch was dried to a constant weight, and approximately 2–5 mg of the dried sample was thoroughly mixed with 400 mg of FTIR-grade potassium bromide (KBr). The mixture was then compressed into a pellet using a manual press. The prepared pellet was scanned using a Fourier transform infrared spectrometer (Spectrum 400, PerkinElmer, USA) equipped with attenuated total reflectance (ATR) in transmittance mode. Spectra were collected at room temperature with 100 scans per sample over a wavenumber range of 4000–400 $cm^{-1}$.

*Water and Oil Absorption Capacities*

The water and oil absorption capacities of the modified *T. bispinosa* starch was studied following the methodology outlined in prior studies [22]. In brief, Water or soybean oil (10 mL) was added to 1 g of the modified starch sample. The mixture was allowed to stand at room temperature for 30 minutes before being centrifuged at 3500 rpm for 30 minutes. Later, the clear supernatant was measured for its volume in a 10 ml graduated cylinder. The volume of water and oil after centrifugation was calculated and compared to the volume before centrifugation. The results were expressed in g/mL of water or oil absorbed.

*Cytotoxicity test*

The toxicity of the modified *T. bispinosa* starch was evaluated in human dermal fibroblast cells using the sulforhodamine B assay adapted from the method described by previous studies [23]. The modified starch sample was prepared by weighing 100 mg of starch powder, which was then dissolved in 1 mL of dimethyl sulfoxide (DMSO). The solution was mixed thoroughly using a vortex mixture and then sonicated for 15 minutes. Since the starch was not completely dissolved, the mixture was then centrifuged at 10,000 rpm for 10 minutes to remove the undissolved particles. Only the clear supernatant was collected to obtain the modified starch solution for further study with the fibroblast cells. Human fibroblast cell at passage 35 were seeded into 96 well plate ($4\times10^3$ cell per well) and maintained in the using Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum and 1% Penicillin/Streptomycin in 5% $CO_2$ at 37 °C for 24 hours. Cells were then treated with various concentration of starch solution (1.325, 2.65, 5.30, 10.60, 21.20, and 53.0 μg/mL) for 72 hours. DMEM was used as the negative control and 0.5%DMSO was used as the solvent control. Later, the supernatant was removed and cells were washed repeatedly with phosphate buffer saline (PBS). After the incubation period, the cells were washed with 200 μL of phosphate-buffered saline, and the samples were fixed with 40% trichloroacetic acid and incubated at 4 °C for 45 minutes. After washing with PBS, the cells were stained with sulforhodamine B for 30 minutes. The staining solution was then discarded, and 100 μL of 10 mM Tris base solution was added. Finally, the absorbance was measured on a microplate reader at 510 nm was measured using a microplate reader. The experiments

were done in triplicate. The percentage of cell viability was calculated according to the following equation:

$$\text{Cell viability (\%)} = (A/B) \times 100$$

where A was the absorbance of the treated with sample and B was the absorbance of the control (untreated with sample).

*Flow ability*

1) Angle of repose

Angle of repose of modified *T. bispinosa* starch was studied using fixed funnel method described in previous published studies with slightly modification [24]. In brief, the funnel was fixed to the burette at the height of 10 cm and a graph paper was place below the funnel on the table. 10 g. of modified *T. bispinosa* starch was carefully poured through the funnel. The height and radius of the pile were measured. The angle of repose of the modified *T. bispinosa* starch was calculated using the following equation:

$$\text{Angle of repose } (\theta) = \tan^{-1} (h/r)$$

where h is the height of the pile and r is the radius of the pile. The test was carried out in triplicate.

2) Carr's Index and Hausner ratio

Bulk density (Bd) and tapped density (Td) of the modified *T. bispinosa* starch was measured according to previous report [25]. 50 g of modified *T. bispinosa* starch samples (Wp) were gently poured through a glass funnel into a 100 mL cylinder. The volume occupied by the sample was recorded as bulk volume (Vp). The powders were tapped on a wooden surface at a height of 7 inches until o further change in volume was detected, at which point the volume was recorded as tapped volume (VpT). The bulk density and tapped density of the modified starch was calculated using the following equation:

$$Bd = Vp / Wp$$

$$Td = Vpt / Wp$$

where Bd is the bulk density, Td is the tapped density, Wp is the weight of the starch sample, Vp is the bulk volume and VpT is the tapped volume. The test was carried out in triplicate.

The bulk density and tapped density values obtained from the experiment were used to calculate the Carr's Index and Hausner ratio using the following equations. The test was carried out in triplicate.

$$\text{Carr's Index} = (Td - Bd) \times 100 / Td$$

$$\text{Hausner ration} = Td / Bd$$

*Compact face powder formulation*

Five compact face powder formulations containing talcum and modified *T. bispinosa* starch as a filler at various concentrations ranging from 12.5-50% were formulated to determine the highest concentration of the modified starch appropriate for the formulations as shown in Table 1. The

formulations were prepared by separating the ingredients into part A and part B. For part A, talcum or modified *T. bispinosa* starch was placed in a mortar and ground with silica, kaolin, mica, magnesium stearate, and titanium dioxide until a homogeneous mixture was obtained. Iron oxide yellow, red, and black pigments were then added and further ground in the mortar until uniformly blended. For part B, triisostearin, diisostearyl malate, isopropyl myristate, capric/caprylic triglyceride, tocopheryl acetate and phenoxyethanol (and) chlorphenesin (and) glycerin (and) aqua were mixed until a uniform mixture was obtained. Subsequently, part B was incorporated into part A and ground until thoroughly mixed. The resulting mixture was then passed through an 80-mesh sieve to obtain a uniform powder. The mixture was subsequently compressed using a semi-automatic powder press at a pressure of 1500 psi. The characteristics of the compact face powder formula including appearance, color and flow ability were further determined.

**Table 1** Compact face powder formula.

| Part | Ingredient | Function | (%w/w) | | | | |
|---|---|---|---|---|---|---|---|
| | | | F1 | F2 | F3 | F4 | F5 |
| A | Talcum | Filler | 50.00 | 37.50 | 25.00 | 12.50 | - |
| | *T. bispinosa* modified starch | Filler | - | 12.50 | 25.00 | 37.50 | 50.00 |
| | Silica | Filler | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 |
| | Nylon-12 | Lubricant | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| | Kaolin | Filler | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 |
| | Mica | Filler | 9.00 | 9.00 | 9.00 | 9.00 | 9.00 |
| | Magnesium stearate | Lubricant | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 |
| | Titanium dioxide | Colorant | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 |
| | CI77491 (Iron oxide red) | Colorant | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 |
| | CI77492 (Iron oxide yellow) | Colorant | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 |
| | CI77499 (Iron oxide black) | Colorant | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| B | Triisostearin | Binder | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| | Diisostearyl malate | Binder | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| | Isopropyl myristate | Binder | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| | Capric/Caprylic triglyceride | Binder | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 |
| | Phenoxyethanol (and) Chlorphenesin (and) Glycerin (and) Aqua | Preservative | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | Tocopheryl acetate | Antioxidant | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |

*Pay-off test*

The adhesion of five compact face powder formulations containing talcum and modified *T. bispinosa* starch at different concentrations to the puff was evaluated using a pay-off test based on a previously described method [1].

*Breakage test*

The breakage resistance of five compact face powder formulations containing talcum and varying concentrations of modified *T. bispinosa* starch was evaluated by dropping each compact vertically from a height of 8 inches onto a wooden surface three times. After the test, the compacts were visually examined for evidence of breakage or structural damage [1].

*Statistical analysis*

Data were expressed as mean values±standard deviation (SD). Statistical analysis of the data was analyzed by performing a one-way analysis of variance (ANOVA) and Duncan post-hoc tests using SPSS program (SPSS ver. 22.0 for Windows, SPSS Inc., Chicago, IL, USA) and the statistical significance was $p < 0.05$.

## Results and Discussion

*Trapa bispinosa modified starch*

The plant sample was taxonomically authenticated at the Suebnakhasathien Building, Department of National Parks, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment. The specimen was identified as *Trapa bispinosa* Roxb. belonging to the family Lythraceae. After peeling, the inner flesh of the fruit was observed to be white in color. The morphology of *T. bispinosa* fruits and the peeled kernel are shown in Figure 1. The fruit kernel were subsequently dried, ground into a fine powder, and used for the preparation of modified starch by alkaline treatment with a 0.2 M sodium hydroxide solution. The resulting modified starch was obtained as a fine white powder, with no evidence of agglomeration or foreign matter as shown in Figure 2. The percentage yield of the modified starch was 36.60%, calculated based on the dry weight of the plant material. Color measurements were performed using a colorimeter in the CIELAB color space, and the results revealed distinct differences between native and modified *T. bispinosa* starch. The native starch exhibited a yellowish-reddish coloration, whereas the modified starch displayed a whitish-yellow appearance. After modification with a 0.2 M sodium hydroxide solution, an increase in lightness (L*) was observed, accompanied by reductions in yellowness (a*) and redness (b*), resulting in a higher whiteness index. These results were consistent with previous reports which demonstrated that alkaline modification using sodium hydroxide disrupts hydrogen bonding within starch granules and removes non-starch impurities, resulting in increased whiteness, altered granule structure, and improved functional properties of the starch [26, 27]. When the color parameters of the modified starch were compared with those of talcum, comparable lightness (L*) and green color intensity were observed (a*), whereas a significantly higher blue color intensity (b*) was detected for the modified starch. In addition, the whiteness index of the modified starch was found to be significantly lower than that of talcum ($p < 0.05$). The Mean of color values (color's lightness (L*), red/green intensity (a*), yellow/blue intensity (b*) and white index (WI) of talcum, native and modified *T. bispinosa* starch are shown in Table 2.

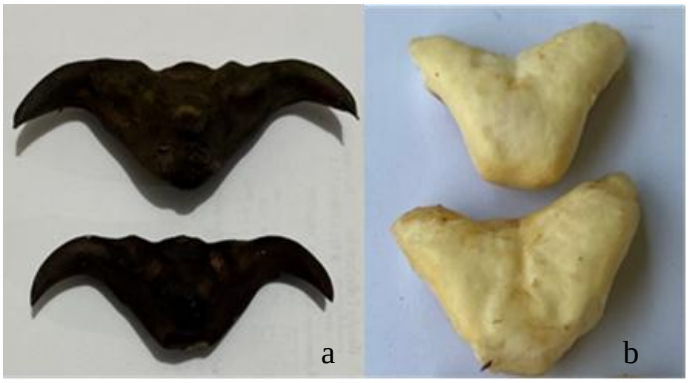


**Figure 1** The morphology of (a) *T. bispinosa* fruits and (b) *T. bispinosa* peeled kernel.

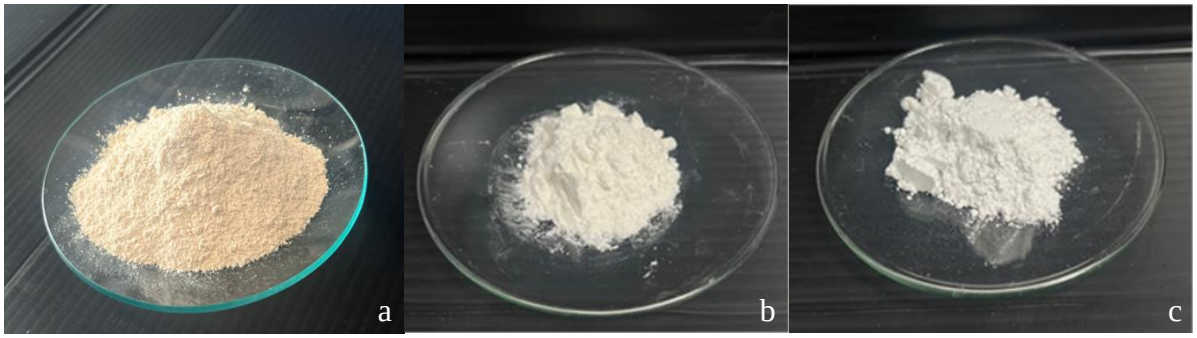


**Figure 2** Appearance of (a) native *T. bispinosa* starch (b) modified *T. bispinosa* starch and (c) talcum.

**Table 2** Mean of color values (color's lightness (L*), red/green intensity (a*), yellow/blue intensity (b*) and white index (WI) of talcum, native and modified *T. bispinosa* starch.

| Sample | Color | | | |
|---|---|---|---|---|
| | L* | a* | b* | WI |
| Talcum | $89.78 \pm 2.80^{a}$ | $-1.92 \pm 0.16^{b}$ | $1.70 \pm 0.08^{c}$ | $90.64 \pm 1.79^{a}$ |
| *T. bispinosa* starch | $70.87 \pm 0.35^{b}$ | $2.94 \pm 0.03^{a}$ | $19.12 \pm 0.06^{a}$ | $65.03 \pm 1.86^{c}$ |
| Modified *T. bispinosa* starch | $89.28 \pm 0.69^{a}$ | $-1.71 \pm 0.04^{b}$ | $7.19 \pm 0.06^{b}$ | $83.18 \pm 1.74^{b}$ |

Values are expressed as mean ± standard deviation (SD) of triplicate experiments (n = 3). The different superscript letter in the same column represents significant differences ($p<0.05$).

*Moisture content*

Moisture content analysis of the prepared modified *T. bispinosa* starch revealed a value of $9.58 \pm 0.10\%$ which comparable to previous studies [17, 28]. Moisture content directly influences the flow behavior of starch powders; elevated moisture levels promote particle agglomeration, which can impair powder flow and increase the risk of microbial contamination [29, 30].

*Morphology*

The morphological characteristics of the modified *T. bispinosa* starch granules were examined using scanning electron microscopy (SEM). The results indicated that the modified *T. bispinosa* starch

granules were oval in shape with occasional surface protrusions, smooth, intact surfaces and no observable cracks, with particle sizes ranging from 16.25 to 25.5 μm as shown in Figure 3. These results were consistent with previous reports [13, 17, 18, 28]. Such rounded granule morphology has been associated with a smooth and soft skin feel upon application [31]. Differences in starch granule morphology among plant sources are therefore critical determinants of physicochemical behavior and cosmetic performance. However, when applied in face powder formulations, the softness and smoothness of the product may be further enhanced by incorporating appropriate amounts of talcum, kaolin, or zinc oxide to improve skin slip during application [1, 7].

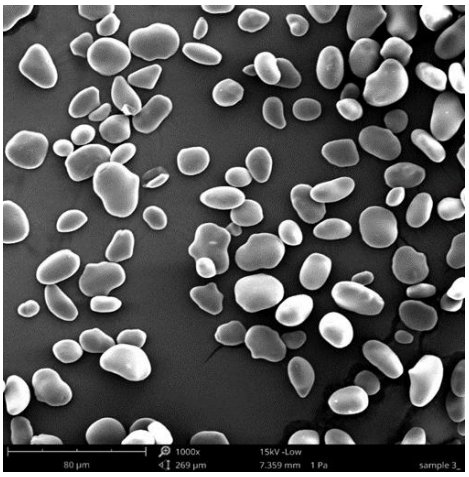


**Figure 3** The morphology of modified *T. bispinosa* starch ×1,000.

*Fourier transform infrared (FTIR) analyzer*

The FTIR spectra of the modified *T. bispinosa* starch is displayed in Figure 4. The modified *T. bispinosa* starch exhibited a band in the infrared spectral region at 3280.50 $cm^{-1}$, which indicates the presence of stretching vibrations of hydroxyl functional groups (O-H) involved in inter- and intra-molecular interactions, a hallmark of starch structure. Concurrently, a diminished band in the infrared spectral region at 2931.27 $cm^{-1}$ corresponds to the stretching vibrations attributed to C-H groups, indicative of $CH_2OH$ groups present in amylose and amylopectin molecules. Furthermore, the modified starch demonstrated absorption at a wave number of 1638.21 $cm^{-1}$, signifying the presence of a C=O group (carbonyl group). This is due to the fact that starch is a glucose homopolymer containing a native carbonyl group and a hydroxyl group within its structure. At 1542.16 $cm^{-1}$, C-H bending vibrations were observed. The peaks at 1406.20 $cm^{-1}$, 1371.80 $cm^{-1}$, and 1336.64 $cm^{-1}$ represented O-H bending vibrations. A weak band at 1239.6 $cm^{-1}$ corresponded to the C-O stretching vibration of the acetyl group. The peak noted at 1148.24 $cm^{-1}$ indicated C-O-C asymmetric stretching of the glycosidic bond, while at 1075.89 $cm^{-1}$, C-O, C-C, and O-H bond stretching was observed, and 994.57 $cm^{-1}$ corresponded to C-O-H bending. Additionally, weak bands at 927.39 $cm^{-1}$, 858.34 $cm^{-1}$, 761.23 $cm^{-1}$, and 703.08 $cm^{-1}$ were attributed to C-O-C ring vibrations of carbohydrates and also demonstrate α-1,4-glycosidic linkages in starch.

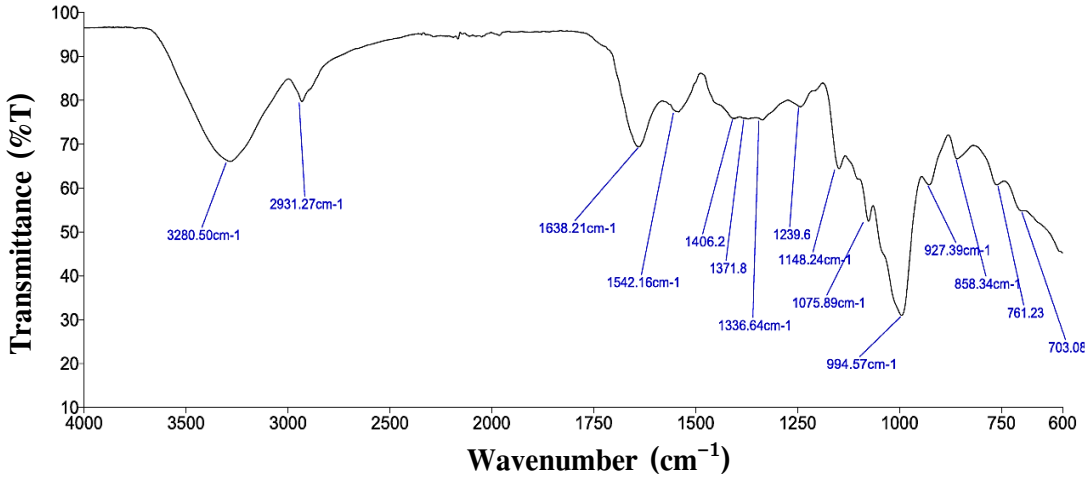


**Figure 4** FT-IR spectroscopy of modified *T. bispinosa* starch.

*Water and Oil Absorption Capacities*

The water and oil absorption capacities of the modified starch derived from *T. bispinosa* were evaluated, and values of 0.40±0.15 and 0.35±0.20 mL/g, respectively, were obtained, which are comparable to those reported in previous studies [28, 32]. However, the water absorption of the modified starch was found to be lower than that reported for other starches commonly used in facial powder formulations, such as rice and corn starch [33, 34]. For facial powder applications, powder materials should exhibit appropriate water and oil absorption capacities to enhance skin comfort and reduce shine by absorbing perspiration and excess sebum, without adversely affecting the smoothness or the visual appearance of the powder on the skin [1].

*Cytotoxicity test*

Cytotoxicity tests are valuable methods for assessing the potential toxic and harmful effects of substances on human health that may arise during use. The cytotoxicity of modified *T. bispinosa* starch was evaluated in human dermal fibroblast cells and shown in Figure 5. The results demonstrated that the viability of human dermal fibroblast was ranging from 85.90-95.19% which indicated that the tested substance is non-cytotoxic toward fibroblast cell [35].

*Flow ability*

The flow properties of the prepared modified starch were evaluated (Table 3), and it was found that treatment with a sodium hydroxide solution resulted in a reduced angle of repose of 27.0±1.73°, indicating excellent flowability. According to the United States Pharmacopeial Convention [36] an angle of repose in the range of 25–30° corresponds to excellent flow properties. The angle of repose represents the maximum slope angle at which a pile of material remains stable without collapsing or sliding, reflecting the interparticle friction and resistance to movement among powder particles [37]. The Carr's index and Hausner ratio of the modified *T. bispinosa* starch were determined to be 13.46±1.70 and

1.15±0.02, respectively. These parameters indirectly reflect powder density, particle size and shape, surface area, moisture content, interparticle cohesion, and compressibility. Based on the United States Pharmacopeia [36] the results indicate that the modified starch exhibits good flow properties. Flowability is a critical parameter in powder characterization as it affects the uniformity of powder handling during the development of cosmetic products [38]. The favorable flow properties of the modified starch suggest minimal particle aggregation and caking, which is advantageous for industrial-scale production.

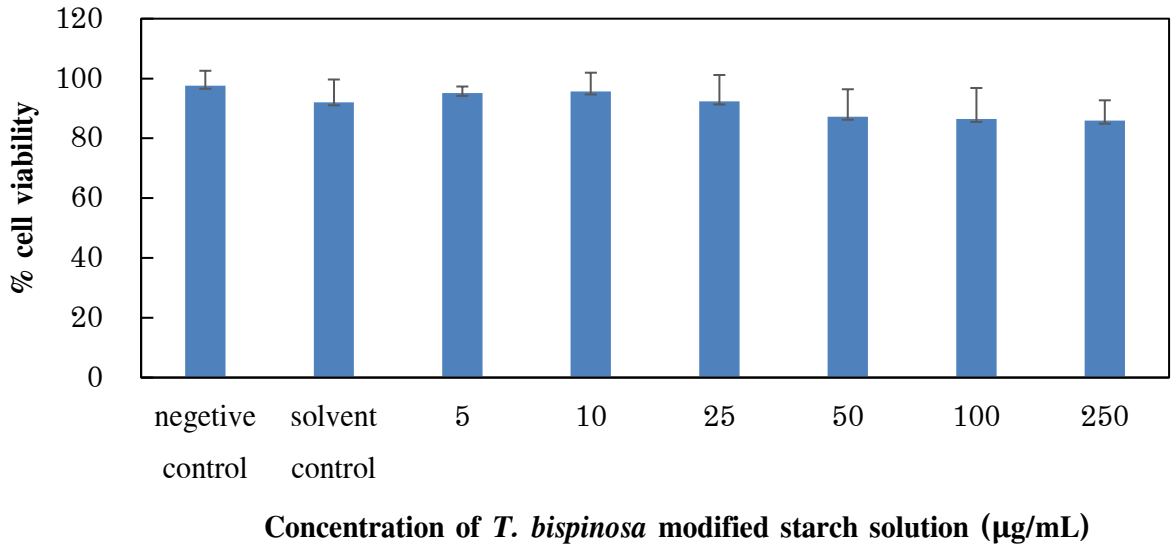


**Figure 5** Cytotoxicity test of modified *T. bispinosa* starch extract in human dermal fibroblast. Data presented as mean±SD.

**Table 3** Comparison of flowability between native *T. bispinosa* starch, modified *T. bispinosa* starch and talcum.

| Characteristic | Native *T. bispinosa* starch | modified *T. bispinosa* starch | Talcum |
|---|---|---|---|
| angle of Repose | 34.33±2.61$^{a}$ | 27.0±1.73$^{b}$ | 31.0±2.64$^{ab}$ |
| Carr's index | 20.36±3.85$^{ab}$ | 13.46±1.70$^{b}$ | 25.03±5.56$^{a}$ |
| Hausner ratio | 1.26±0.04$^{ab}$ | 1.15±0.02$^{b}$ | 1.34±0.11$^{a}$ |

Values are expressed as mean ± standard deviation (SD) of triplicate experiments (n = 3). The different superscript letter in the same column represents significant differences ($p < 0.05$).

*Compact face powder formulation*

Compact face powder formulations containing modified *T. bispinosa* starch at concentrations ranging from 12.50% to 50% were prepared and shown in Figure 6. All formulations were homogeneous and exhibited a beige coloration due to the incorporated pigments. An increase in the proportion of modified *T. bispinosa* starch in the formulations resulted in a decrease in lightness (L*), accompanied by increases in redness (a*) and yellowness (b*) (Table 4). It was found that face powder formulations containing starch at

levels of 12.5–25% provided a smooth and soft tactile sensation comparable to that of the base formulation containing talcum alone (F1) indicating that the modified starch could serve as a potential substitute for talcum in face powder formulations. However, when incorporated at levels exceeding 25%, a reduction in smoothness and softness was observed.

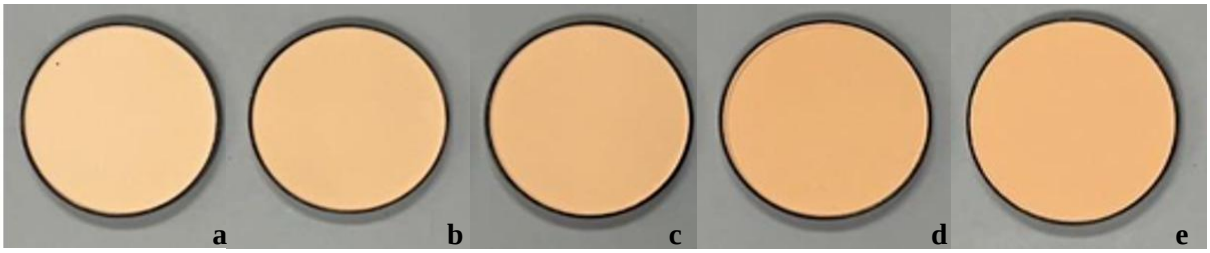


**Figure 6** Appearance of compact face powder formulation (a) base compact face powder, (b) compact face powder containing 12.50%, (c) 25% (d) 37.5% and (e) 50% of modified *T. bispinosa* starch.

**Table 4** Mean of color values (color's lightness (L*), red/green intensity (a*), yellow/blue intensity (b*) and white index (WI) of Compact face powder formula.

| Formula | L* | a* | b* | WI |
|---|---|---|---|---|
| **F1** | 78.35±0.05$^{a}$ | 7.03±0.07$^{e}$ | 22.63±0.15$^{e}$ | 67.90±0.10$^{a}$ |
| **F2** | 76.91±0.29$^{b}$ | 8.00±0.22$^{d}$ | 24.28±0.86$^{d}$ | 65.54±0.46$^{b}$ |
| **F3** | 75.17±0.36$^{c}$ | 8.96±0.09$^{c}$ | 25.30±0.05$^{c}$ | 63.44±0.29$^{c}$ |
| **F4** | 72.80±0.44$^{d}$ | 9.99±0.18$^{b}$ | 26.33±0.05$^{b}$ | 60.85±0.42$^{d}$ |
| **F5** | 71.98±0.07$^{e}$ | 10.59±0.08$^{a}$ | 27.38±0.14$^{a}$ | 59.42±0.09$^{e}$ |

Values are expressed as mean ± standard deviation (SD) of triplicate experiments (n = 3). The different superscript letter in the same column represents significant differences ($p<0.05$).

The flow properties of the compact face powder formulations were evaluated and shown in Table 5. No significant differences in tapped density, Carr's index, or Hausner ratio were observed among the five formulations. Bulk density is the ratio of a powder's given mass to its bulk volume while tapped density describes the bulk density of a powder following a prescribed consolidation or compression process and significantly influences packaging efficiency, transport behavior, and commercial handling of powders. The bulk density and tapped density values were further used to calculate Carr's index and Hausner ratio, which are indicators of powder compressibility and interparticle cohesiveness, respectively [39].

**Table 5** Bulk density, tapped density, Carr's index and Hausner ratio of Compact face powder formula.

| Formula | Bulk density (g/ml) | Tapped density (g/ml) | Carr's Index (%) | Hausner ratio |
|---|---|---|---|---|
| **F1** | $0.44\pm0.01^{b}$ | $0.65\pm0.01^{a}$ | $31.92\pm1.31^{a}$ | $1.47\pm0.03^{a}$ |
| **F2** | $0.47\pm0.01^{a}$ | $0.66\pm0.05^{a}$ | $29.48\pm5.85^{a}$ | $1.42\pm0.12^{a}$ |
| **F3** | $0.48\pm0.03^{a}$ | $0.66\pm0.03^{a}$ | $27.83\pm3.65^{a}$ | $1.39\pm0.07^{a}$ |
| **F4** | $0.47\pm0.01^{a}$ | $0.68\pm0.04^{a}$ | $31.80\pm3.55^{a}$ | $1.44\pm0.07^{a}$ |
| **F5** | $0.48\pm0.02^{a}$ | $0.66\pm0.01^{a}$ | $27.82\pm1.41^{a}$ | $1.39\pm0.03^{a}$ |

Values are expressed as mean ± standard deviation (SD) of triplicate experiments (n = 3). The different superscript letter in the same column represents significant differences ($p<0.05$).

*Pay-off test*

The pay-off test was conducted to evaluate the adhesion of the powders to the puff [1]. Pay-off is a critical performance parameter for compact powders, as it reflects the amount of product transferred from the pan to the applicator during use. The results demonstrated that all compact face powder formulations exhibited high adhesive properties (Figure 7).

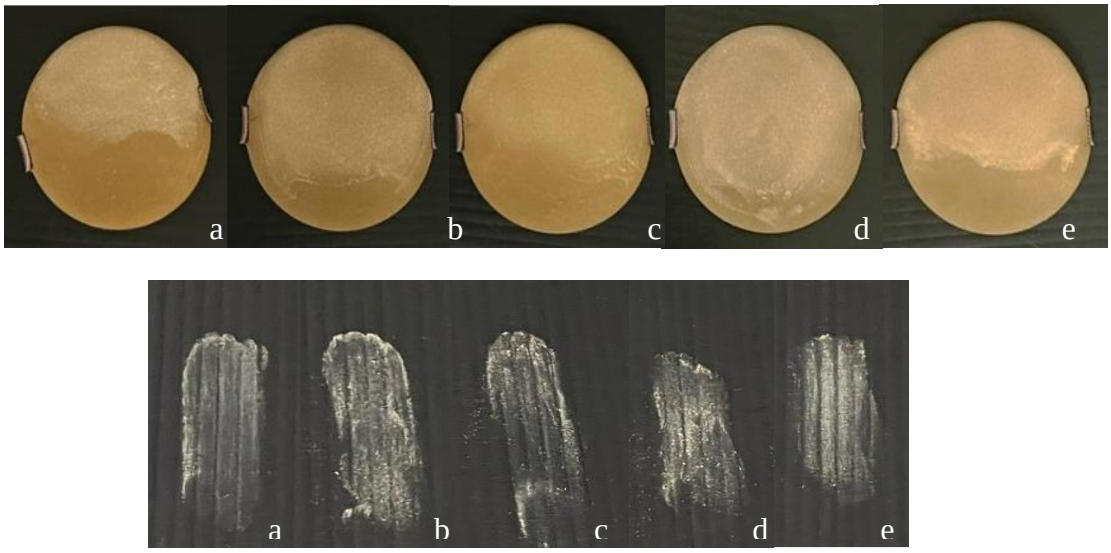


**Figure 7** The appearance of the puffs for the pay-off test formulation (a) base compact face powder, (b) compact face powder containing 12.50%, (c) 25% (d) 37.5% and (e) 50% of modified *T. bispinosa* starch.

*Breakage test*

The breaking test was conducted to assess the ability of the compact powder to remain intact under conditions simulating transportation and routine handling. The integrity of the compact powder reflects its mechanical stability and resistance to damage during normal use. The results revealed that no cracking of the powder cake was observed in the base compact face powder formulation containing

talcum alone (F1) or in the formulations containing 12.5–25% modified *T. bispinosa* starch (F2 and F3). In contrast, cracking of the compact was observed in F3 and F4 which containing more than 25% modified *T. bispinosa* starch after the breakage test (Figure 8). Based on these findings, the maximum allowable content of modified *T. bispinosa* starch in the formulation was determined to be 25%.

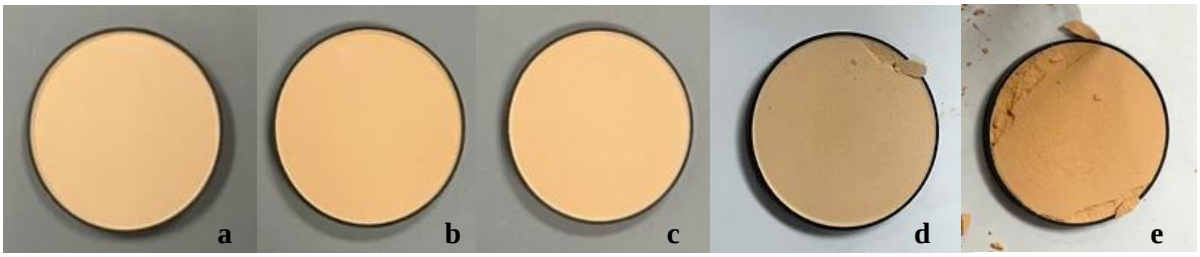


**Figure 8** The breaking test of compact face powder (a) base compact face powder, (b) compact face powder containing 12.50%, (c) 25% (d) 37.5% and (e) 50% of modified *T. bispinosa* starch.

## Conclusions

This study is the first to demonstrate the use of chemically modified *T. bispinosa* starch as a talcum substitute in compact face powder formulations for color cosmetic applications. The results showed that the modified starch prepared using a 0.2 M sodium hydroxide solution exhibited favorable physical properties and good flowability. Cytotoxicity evaluation confirmed that the modified starch was safe and non-cytotoxic, indicating its suitability for cosmetic applications. The modified starch could be effectively used as a filler in compact face powder formulations, thereby reducing the amount of talcum required. It could be incorporated at levels of up to 25% without adversely affecting the physical properties of the formulations. Nevertheless, further investigations, including skin irritation assessments and consumer satisfaction studies in human volunteers, are necessary to fully confirm its safety and performance in cosmetic applications.

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