Supplementary Material

Esterified starches enhance short-term satiety in mice via the structural and physicochemical alterations

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Section A: Determination of substitution degree of acetylated starch

Deionized water (50 mL) was added to a 500-mg sample in a 250-mL conical flask, with three drops of phenolphthalein being used as an indicator. Thereafter, 25 mL of sodium hydroxide (0.5 M) was added for saponification by shaking the mixture for 2 h (25°C, 300 rpm). Subsequently, the excess of alkali was quantified by titration using hydrochloric acid solution (0.5 M). Native starch was used as a blank control. The acyl content (AC) and DS were computed using the following equations:

$$AC(\%) = (V_b - V_s) \times C \times M \times 10^{-3} \times 100\%/m$$

$$DS = (162 \times AC) / [43 - (43 - 1) \times AC]$$

where V_b and V_s are the titration volumes of a blank sample and acylated starches, respectively; 'C' is the molarity of hydrochloric acid solution; '43' is the molecular weight of the acetyl group; 'm' is the weight of sample; '162' is the molar mass of the anhydroglucose unit; '1' is the atomic mass of hydrogen

Section B: Determination of substitution degree of phosphorylated starch

The degree of phosphorylated starch were quantified by NexION 350D inductively coupled plasma mass spectrometry (ICP-MS, Thermo Fisher Scientific Inc., American). The measurements were conducted in duplicate and in accordance with the legal requirement by FDA. The percentage of phosphate (%) was determined using the following formula:

$$Phosphate(\%) = \frac{(Blank - Sample) \times Molarity of HCl \times 0.031 \times 100}{Sample weight}$$

The degree of substitution (DS) was determined according to the following equation:

$$DS = \frac{162 \times n_p}{100 - 102 \times n_p} = \frac{162 \times \Delta P}{3100 - 102 \times \Delta P}$$

where *np* is the number of moles of incorporated phosphorus in 100 g starch ($np = \Delta P/31$), the molar mass of anhydroglucose is 162 g/mol, the dry starch sample weight is 100 g, the molar mass of NaPO₃²⁻ is 102 g/mol.

Section C: Determination of substitution degree of OSA-modified starch

1.5 g of OSA-modified starch was added to a 2.5 M HCl-isopropanol solution (7.5 mL) and stirred for 30 min. Next, 90% (v/v) isopropanol in water (30 mL) was added and stirred for 10 min. After centrifugation, the pellet was washed with 90% isopropanol solution, and chloride ions were detected with a 0.1 M AgNO₃ solution. The residue was added to 90 mL of deionized water, heated at 100 °C for 20 min, and titrated with NaOH (0.1 M) solution using phenolphthalein as an indicator. The DS calculation formula was as follows:

$$DS = \frac{162 \times (A \times M)/W}{1000 - 210 \times (A \times M)/W}$$

where 'A' is the volume (mL) of the NaOH solution, 'M' is the molar concentration, and 'W' is the weight (g) of OSA-modified starch.