Effects of partial precipitation and freeze-drying on morphology and physicochemical properties of rice starch hydrolysates

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ABSTRACT

Agricultural bio-catalysis is of immense scientific interest due to its increasing importance in the efforts for more sustainable agriculture while optimizing environmental impacts. In our studies, native rice starch was hydrolyzed with various alpha-amylase concentrations (0, 0.1, 0.2, and 0.3% w/w of starch) at 50°C for 20 min; then purified by partial precipitation (PP) with organic solvents, or freeze-drying (FD) without further purification. The rice starch hydrolysates (RSH) produced by different methods (PP or FD) were determined for dextrose equivalent (DE), morphology, and some physicochemical properties including bulk density, moisture content, hygroscopicity, and water solubility. The results showed that at the same alpha-amylase treatment conditions, the RSH obtained by the PP method had lower DE values and production yields than those of RSH obtained by FD method. The FD-RSH had higher DE values, lower bulk densities and moisture contents, higher hygroscopicity and water solubility. In morphology, the PP-RSH (DE 10.2) had a larger particle size and more condensed microstructure compared to the FD-RSH of almost similar DE 13.5. These findings showed that the PP method resulted in lower-DE RSH with different morphological and physicochemical properties compared to those obtained by the FD method.

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1. Introduction

Maltodextrins and glucose syrups are starch hydrolysis products (SHP) have found wide applications in the food, cosmetic and pharmaceutical industries (Castro et al., 2016). They are commercially produced from native starch through partial hydrolysis, purification, and spray-drying (Takeiti et al., 2010). They are usually classified by their values of Dextrose Equivalent (DE), a quantity that indicates the number of dextrose molecules released from the hydrolysis of starch and expressed as a percentage of the dextrose on a dry-weight basis (Dokic et al., 2004; Yunianta et al., 2015). Starch has a DE value of zero, while glucose has a DE value of 100. Maltodextrins are low convert starch products with DE values lower than 20, while high convert starch products with values equal and higher than 20 are known as corn syrup solids and glucose syrups (Klinkesorn et al., 2004; Saavedra-Leos et al., 2015; Balto et al., 2016). Their physicochemical and functional properties are influenced and controlled by the type (acid or enzymatic) and extend of hydrolysis, amylose to amylopectin ratio, source of starch, etc. (Dokic et al., 2004).

Starches from various botanical sources such as corn, cassava, manioc, wheat, oatmeal, sago, canna, maize, potato, and rice can be used for production of SHP (Klinkesorn et al., 2004; Moore et al., 2005; Takeiti et al., 2010). Traditional methods of acid hydrolysis resulted in products which are not completely soluble, colored and have a starchy taste, while enzymatic methods have been used to prepare soluble, non-hazy low DE-value maltodextrins (Dokic et al., 1998). Besides, the purification process such as partial precipitation of hydrolysates with polar organic solvents or drying methods (spray-drying/freeze-drying) could affect the hydrolysates' molecular mass, DE values, degree of polymerization (DP) range, and physicochemical properties (Kalac et al., 1984; Balto et al., 2016; Wang et al., 2020). This paper aims to study the effects of partial precipitation (PP) and freeze-drying (FD) methods on DE values, morphological and physicochemical properties of rice starch hydrolysates to have a better understanding of the properties of SHP produced from different botanical source, different purification and drying methods.

2. Materials and Methods

2.1. Materials and chemicals

Native normal rice starch (NRS) (Tai Ky Food Co., Vietnam) was purchased from a supermarket in Ho Chi Minh City, Vietnam. *Aspergillus oryzae* alpha-amylase (AAM) and commercial dextrin GLUCIDEX-12 were purchased from HiMedia Laboratories (India) and Roquette Freres (France). The organic solvents, acetone 100% and ethanol 96°, and chemical reagents, glucose ($C_6H_{12}O_6$), iodine (I_2), potassium iodine (KI), hydrochloride (HCl 37%), sodium chloride (NaCl), sodium hydroxide (NaOH), sodium dihydrogen phosphate dihydrate ($NaH_2PO_4.2H_2O$), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4.12H_2O$), 3,5-dinitrosalicylic acid (DNS) ($C_7H_4N_2O_7$), and sodium potassium tartrate tetrahydrate ($KNaC_4H_4O_6.4H_2O$) were provided by Xilong Scientific (China).

2.2. Alpha-amylase hydrolysis of rice starch

The NRS was hydrolyzed with a fungal alpha-amylase following the method of Do et al. (2023) with minor modifications. NRS slurries containing 20% of NRS (w/v) in 0.1 M sodium phosphate

buffer of pH 6.0 were gelatinized at 95°C for 30 min and hydrolyzed with various concentrations of AAM (0, 0.1, 0.2, and 0.3% w/w of NRS) at 50°C for 20 min. The reactions were terminated by heating the mixtures at 95°C for 30 min.

2.3. Rice starch hydrolysates (RSH) purification and drying

After enzyme termination, each hydrolyzed mixture was cooled to room temperature and divided into two equal weight portions: the rice starch hydrolysates (RSH) in the first portion was purified by PP with organic solvents: hydrolysate precipitation using 3-fold volume of ethanol 96° following purification using ethanol 96° and acetone, then oven-drying at 45°C for 24 h; while the RSH in the remaining portion was obtained by FD method without purification. Yield of RSH was calculated as the percent weight of hydrolyzed starches to the initial weight of rice starch used for hydrolysis (Gunawan et al., 2023).

2.4. Dextrose Equivalent (DE) determination

DE values of NRS, commercial dextrin, and RSH were determined according to the method of Yunianta et al. (2015) with some modifications. Approximately 35 mg of sample was dissolved in 5 mL of distilled water, mixed with 15 mL of DNS solution, made up to 50 mL with distilled water, boiled for 45 min, cooled to room temperature, and then measured for absorbance at a wavelength of 540 nm. The dextrose or reducing sugar content in the sample was compared with glucose standard and the DE value of the sample was calculated according to the following formula, where C is reducing sugar content (mg/mL), V is volume of sample solution (mL), and m is sample weight (mg).

$$DE = \frac{C \times V}{m} \times 100\%$$

2.5. Scanning electron microscopy (SEM) observation

Morphological characteristics of the starch samples were observed using a Scanning Electron Microscope (S-4800, Hitachi, Japan) according to Do et al. (2023).

2.6. Physicochemical properties

Bulk density was obtained by gravimetric method according to Takeiti et al. (2010), weighing a sample powder poured into a 25 mL graduated cylinder. Bulk density was calculated as the material weight divided by the bulk volume.

Moisture content of the samples was determined by gravimetric method using an oven, measured as the percent moisture loss after drying to the initial wet weight of the sample (Duong et al., 2024).

Hygroscopicity was analyzed using 1 g of sample that was put in an aluminum cup and dried with the oven over the past 24 h, then dried sample was conditioned at relative humidity 96% in a closed saturated K_2SO_4 solution-containing chamber and weight was performed daily until equilibrium reached according the method of Hartiningsih et al. (2020). Percent moisture reabsorption of the sample was calculated concerning its initial dried weight.

Solubility of samples was analyzed according to Hartiningsih et al. (2020). Weighed 0.5 g of sample, dissolved into 50 mL of distilled water and stirred at 4000 rpm for 2 min using a homogenizer (T25, IKA, Germany). The suspension was centrifuged at 4000 rpm for 15 min, and 25 mL of the supernatant was taken and dried in the oven at 105°C for 48 h and obtained dry weight. Solubility was calculated as percent dry weight dissolved in the supernatant to the sample weight.

2.7. Statistical analysis

All measurements were performed in triplicate and results were expressed as means ± standard deviation. The analysis of variance (ANOVA) and the least significant difference (LSD) were performed at a value of $P < 0.05$.

3. Results and Discussion

3.1. Effects of hydrolysis degree, recovery and purification method on DE values

Table 1 showed measured DE values and yields of RSH obtained by PP and FD method. All the results were statistically significant differences. The PP method produced RSH with lower DE values (1.3 - 10.2) and yields (31.2 - 66.2%) compared to those (1.1 - 37.6 and 82.5 - 89.2%) obtained by the FD method. These results showed that the RSH produced by PP method were more purified with higher DP and higher molecular mass molecules, while those obtained by the FD method had higher yields of lower DP and lower molecular mass molecules. Fractional precipitation of aqueous solutions of partly hydrolyzed starches using organic solvents was performed to obtain different molecular mass fractions (Kalac et al., 1984). Ethanol was used for precipitation of starch and SHP in aqueous solutions and high ratio of ethanol (70% ethanol) narrowed ranges of maltooligosaccharides and preferentially removed glucose and maltose from SHP (Balto et al., 2016; Gunawan et al., 2023). Therefore, the PP method used in this study could narrow ranges of SHP to higher DP range and remove glucose and maltose which resulted in lower yields of RSH. The FD method without purification resulted in RSH with higher DE values and higher yields. Based on DE values, RSH could be classified into maltodextrins (DE 2-20) including PP-2001 (DE 3.9), PP-2002 (DE 7.2), PP-2003 (DE 10.2), and FD-2001 (DE 13.4), and glucose syrups were FD-2002 (DE 20.7) and FD-2003 (DE 37.6).

Table 1. Measured dextrose equivalent (DE) values of different types of rice starch hydrolysates (RSH)

Sample	C_{AAM} (%)	Yield (%)	DE
PP-control[1]	0	66.2 ± 1.4[d]	1.30 ± 0.06[b]
FD-control[2]	0	88.8 ± 1.3[g]	1.09 ± 0.02[a]
PP-2001[3]	0.1	51.2 ± 3.3[c]	3.88 ± 0.04[c]
FD-2001[4]	0.1	86.5 ± 1.1[f]	13.45 ± 0.06[f]
PP-2002[5]	0.2	31.2 ± 3.9[a]	7.15 ± 0.01[d]
FD-2002[6]	0.2	82.5 ± 0.4[e]	20.72 ± 0.10[g]
PP-2003[7]	0.3	39.7 ± 5.1[b]	10.22 ± 0.02[e]
FD-2003[8]	0.3	89.2 ± 2.1[h]	37.64 ± 0.06[h]

The data within a column followed by the different superscript letter are statistically significant difference ($P < 0.05$). C_{AAM}: alpha-amylase concentration; DE: dextrose equivalent; PP: partial precipitation; FD: freeze-drying.

[1]RSH (rice starch hydrolysates) obtained by PP of AAM-untreated rice starch.

[2]RSH obtained by FD of AAM-untreated rice starch.

[3]RSH obtained by PP of 0.1% AAM-treated rice starch.

[4]RSH obtained by FD of 0.1% AAM-treated rice starch.

[5]RSH obtained by PP of 0.2% AAM-treated rice starch.

[6]RSH obtained by FD of 0.2% AAM-treated rice starch.

[7]RSH obtained by PP of 0.3% AAM-treated rice starch.

[8]RSH obtained by FD of 0.3% AAM-treated rice starch.

3.2. Morphological characteristics

The NRS, PP-2003 (DE 10.2), FD-2001 (DE 13.5), and commercial maltodextrin GLUCIDEX-12 (DE 12) powders were chosen for SEM observations in Figure 1 to see the influence of alpha-amylase treatments, purification and drying method on the morphologies of RSH when compare to those of NRS and commercial maltodextrin. SEM micrographs showed that granules of the NRS had irregular cubic shapes with size less than 10 mm (Figure 1A), the PP-2003 has non-granular condense body shape with particle size larger than 50 mm (Figure 1B), while the FD-2001 (Figure 1C) and the commercial maltodextrin GLUCIDEX-12 (Figure 1D) have fragment structures with various sizes. The morphological differences found could be attributed to the PP and FD method of RSH which led to their different physicochemical properties mentioned below.

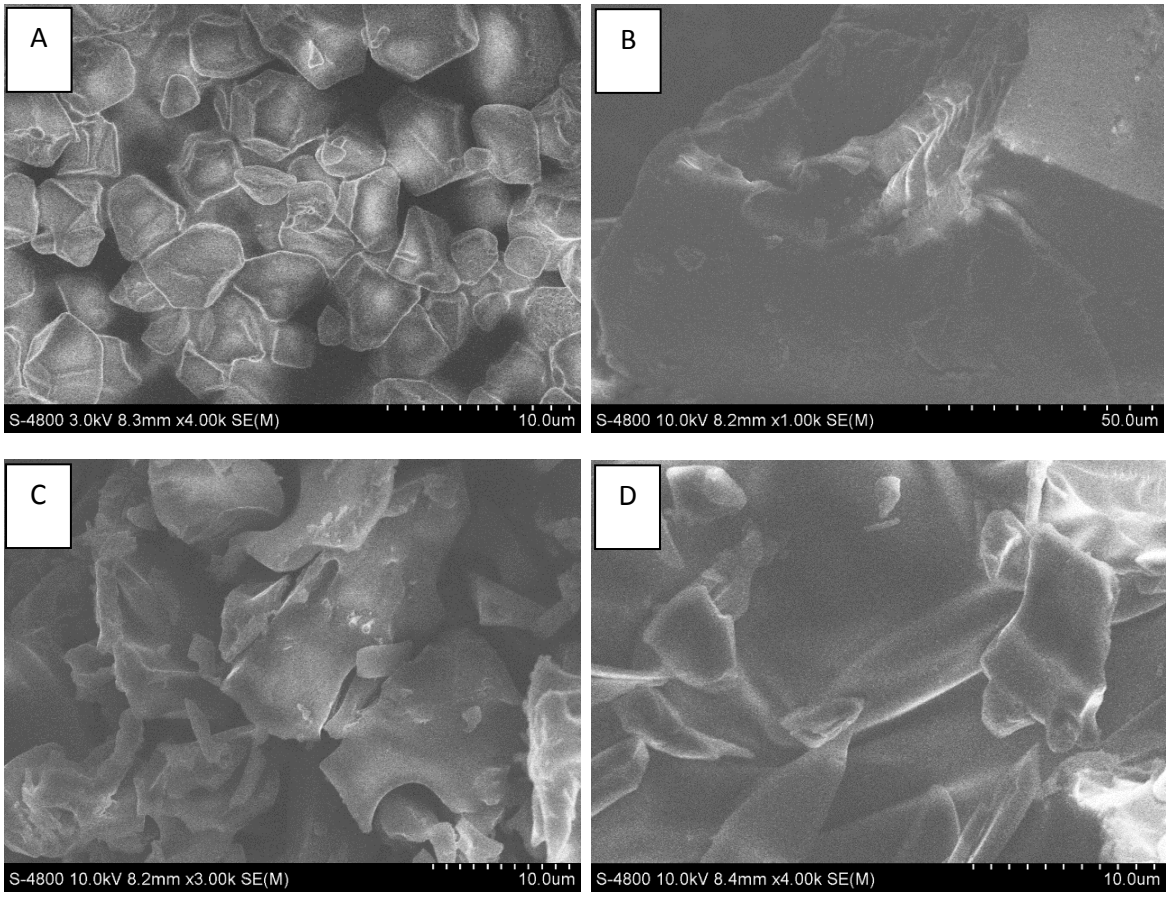

Figure 1. SEM micrographs of NRS (A), PP-2003 (B), FD-2001 (C), and GLUCIDEX-12 (D). NRS: normal rice starch; PP: partial precipitation; FD: freeze-drying.

3.3. Physicochemical properties

Table 2 showed the physicochemical properties of native normal rice starch (NRS), commercial maltodextrin GLUCIDEX-12, and RSH obtained by PP and FD method. The RSH produced by PP method (PP-RSH) have higher bulk densities (0.416 - 0.647 g/mL) than those of RSH produced by FD method (FD-RSH) (0.377 - 0.479 g/mL) at same enzyme treatment conditions, however their lower bulk densities were lower than that of commercial maltodextrin (0.746 g/mL). Higher bulk densities of PP-RSH and commercial maltodextrin could be explained from their morphological characteristics since they have larger particle sizes and more condense microstructures which have been observed in Figure 1B and 1D. In terms of moisture content and hygroscopicity, the PP method produces RSH with higher moisture content (9.3 - 11.9%) but lower hygroscopicity (20.4 - 48.9%) at same enzyme treatment conditions when compared to those obtained by FD method (4.4 - 6.0% and 22.0 - 66.7%), respectively. In terms of solubility, the PP method produced RSH with lower solubility (5.8 - 34.8%) compared to that obtained by FD method (9.8 - 76.4%). The higher moisture content, lower hygroscopicity and solubility of PP-RSH can result from the large size and condense microstructure of product particles, narrow higher DP and lower DE values; whereas the lower moisture content, higher hygroscopicity and solubility of FD-RSH can result from the smaller particle sizes, less condense microstructures, and higher DE values. From these obtained results, the PP and FD method produced RSH with different morphological and physicochemical properties that might be beneficial or unbeneficial for technological applications. For instance, the FD-RSH have dramatic high hygroscopicity would not be suitable for keeping the powder state for a long time, while the PP-RSH have dramatic low solubility would not be suitable for product formulation without heating.

Table 2. Physicochemical properties of normal rice starch, commercial dextrin, and rice starch hydrolysis products

Sample	Bulk density (g/mL)	Moisture content (%)	Hygroscopicity (%)	Solubility (%)
NRS (DE 0)	0.573 ± 0.035^{e}	11.5 ± 0.2^{h}	8.2 ± 1.9^{a}	0.6 ± 0.0^{a}
PP-control[1] (DE 1.3)	0.416 ± 0.003^{b}	9.3 ± 0.1^{f}	20.4 ± 0.5^{b}	5.8 ± 0.3^{b}
FD-control[2] (DE 1.1)	0.446 ± 0.011^{c}	5.7 ± 0.1^{d}	22.0 ± 0.8^{c}	9.8 ± 0.3^{c}
PP-2001[3] (DE 3.9)	0.563 ± 0.018^{e}	10.9 ± 0.0^{g}	32.1 ± 0.2^{d}	34.8 ± 0.6^{f}
FD-2001[4] (DE 13.5)	0.472 ± 0.006^{d}	6.0 ± 0.0^{e}	46.0 ± 2.5^{f}	76.4 ± 0.0^{i}
PP-2002[5] (DE 7.2)	0.647 ± 0.013^{f}	11.9 ± 0.0^{i}	39.2 ± 1.7^{e}	20.2 ± 0.3^{d}
FD-2002[6] (DE 20.7)	0.479 ± 0.009^{d}	5.1 ± 0.2^{b}	64.9 ± 1.3^{h}	74.4 ± 0.6^{h}
PP-2003[7] (DE 10.2)	0.642 ± 0.003^{f}	11.9 ± 0.1^{i}	48.9 ± 0.1^{g}	26.2 ± 0.3^{e}
FD-2003[8] (DE 37.6)	0.377 ± 0.019^{a}	4.4 ± 0.0^{a}	66.7 ± 0.7^{h}	73.0 ± 0.8^{g}
GLUCIDEX-12 (DE 12)	0.746 ± 0.021^{g}	5.4 ± 0.3^{c}	34.5 ± 0.7^{d}	100.0 ± 0.0^{j}

The data within a column followed by the same superscript letter are not statistically significant difference ($P > 0.05$). NRS: normal rice starch; DE: dextrose equivalent; PP: partial precipitation; FD: freeze-drying.

[1]RSH (rice starch hydrolysates) obtained by PP of AAM-untreated rice starch.

[2]RSH obtained by FD of AAM-untreated rice starch.

[3]RSH obtained by PP of 0.1% AAM-treated rice starch.

[4]RSH obtained by FD of 0.1% AAM-treated rice starch.

[5]RSH obtained by PP of 0.2% AAM-treated rice starch.

[6]RSH obtained by FD of 0.2% AAM-treated rice starch.

[7]RSH obtained by PP of 0.3% AAM-treated rice starch.

[8]RSH obtained by FD of 0.3% AAM-treated rice starch.

4. Conclusions

The differences in physicochemical properties of starch hydrolysis products from the same botanical source and enzyme treatment conditions can be explained from the differences in morphological characteristics, DE values and the purification and drying method. The partial precipitation method produces starch hydrolysis products with large condense particles, lower DE values and yields, higher bulk densities and moisture contents, lower hygroscopicity and solubility; while the freeze-drying method produces starch hydrolysis products with less condense fragments, higher DE values and yields, lower bulk densities and moisture contents, higher hygroscopicity and solubility. Thus, based on these findings, suitable purification and drying method can be applied for production of starch hydrolysates with desired morphological and physicochemical properties.

Conflict of interest

The authors declare no conflict of interest.

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