

Starch Structures and Physicochemical Properties of a Novel β -glucan-enriched Oat Hydrocolloid Product with and without Supercritical Carbon Dioxide Extraction*

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Abstract: Starch structures and physicochemical properties of C-trim30, a β -glucan-enriched oat product (32% β -glucan), with or without supercritical carbon dioxide extraction (SCD) were studied to evaluate suitability for commercial applications and potential to degrade starch to increase β -glucan concentration. Scanning electron micrographs showed C-trim30 was composed of 200-300 μ m long, porous particles. HPSEC equipped with MALLS and RI detectors showed C-trim30 had three peaks, corresponding to amylopectin with weight-average molecular weight (M_w) of 1.0×10^8 , breakdown amylopectin product (M_w 1.1×10^7) and amylose (M_w 1.7×10^6). β -glucans were not observed due to HPSEC column absorption. C-trim30 amylopectin M_w and gyration radii increased after SCD suggesting aggregation of molecules occurred. No thermal transitions were observed for C-trim30 heated 0-150°C. C-trim30 pasting properties, measured using Rapid ViscoAnalyser, showed high peak viscosity (291 RVU) at 30°C, high breakdown (200 RVU), final (273 RVU) and setback (183 RVU) viscosity after heated to 95°C while stirred. SCD increased peak (423 RVU) and breakdown (318 RVU) viscosity. C-trim30 heated from 15 to 110°C showed higher water-holding capacity occurred without SCD. SCD oil fatty acid composition of 82% unsaturated was apposite for health-food applications. Study suggests C-trim30 with and without SCD could function as fat substitutes.

Key words: Oat bran, starch, β -glucan, physicochemical, lipid extraction, hydrocolloid

INTRODUCTION

The β -glucans in oat bran provide many human health benefits such as serum cholesterol lowering (Anderson and Chen, 1986; Mälkki *et al.*, 1992; Uusitupa *et al.*, 1992; Behall *et al.*, 1997; Pomeroy *et al.*, 2001), reduced coronary heart disease (Berg *et al.*, 2003), reduced diabetic symptoms (Wood *et al.*, 1994; Pick *et al.*, 1996; Tappy *et al.*, 1996), lowered blood pressure (Saltzman *et al.*, 2001) and cancer prevention (Adom and Liu, 2002). Concentrated forms of β -glucans, such as Oatrim (Inglett, 1993) and Nutrim-OB (Carrière and Inglett, 2000) have been developed to assist food manufacturers deliver health-beneficial oat hydrocolloids to consumers.

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A higher concentrated β -glucan ingredient is desired by food manufacturers enabling greater amounts of health-promoting compounds to be added to food formulations. To satisfy this demand, oat hydrocolloid products with even greater concentrations of β -glucans, known as C-trim, have been developed. Rheological and textural properties of C-trim20 (containing 20% β -glucan, dry weight basis (dwb) and its evaluation in cake formulations have been studied (Lee *et al.*, 2005). Additional knowledge about the physicochemical properties of C-trim oat hydrocolloids could provide useful information for their incorporation into foods that will appeal to consumers.

There has been little research investigating the physicochemical properties of oat bran products. Zhou *et al.* (2000) reported three thermal transitions for wholemeal oats that were heated from 25 to 125°C. A significant positive correlation has been reported in oats between the contents of both total and extractable mixed-linkage β -glucan and suspension viscosity held at 37°C for 100 min (Luhalo *et al.*, 1998) and Zhou *et al.* (2000) found similar results heating wholemeal oats up to 90°C.

In this study, starch structures and thermal and pasting properties of C-trim30 with and without supercritical carbon dioxide (SCD) extraction were studied. C-trim30 is an oat hydrocolloid with 30% β -glucan (dwb) produced by jet-cooking oat bran slurry, followed by centrifugation and drum-drying. The thermal and pasting properties will provide insight into potential applications of C-trim30. In addition to β -glucan enrichment achieved by SCD extraction, we were interested in changes in starch properties during C-trim30 production and SCD extraction to explore opportunities to further enhance β -glucan content by starch degradation. Additionally, fatty acid composition of extracted oil was studied to assess its potential industrial uses to improve the overall economics of β -glucan enrichment processing.

MATERIALS AND METHODS

Production of C-trim30 Oat β -glucan Hydrocolloid

Oat bran concentrate (β -glucan, 10.5%; protein, 18.6%; total lipid, 7.4% starch 59.6% and ash, 3.9% on a dry basis) was obtained from Quaker Oats Company (Chicago, IL, Lot No. 18608408, Item No. 26629). Oat bran concentrate (100 g) was mixed with 1.9 L of water and slurry was adjusted to pH 6.55 after mixing with spatula to suspend, then vigorously mixed at 4000 rpm for 1 h using a colloid mill (Polythron PT6000 with Aggregat PT-DA-6060/2WEC, Brinkmann Instruments Inc., Westbury, NY). Wet solids were separated and saved by a 400 mesh sieve (38 μ m pore opening). The sieve liquid was centrifuged at 3500 g for 15 min to separate out dense starch particles and the supernatant was mixed with the saved wet solids. The reconstituted slurry was subjected to jet-cooking (65 psi, 141°C, 1.2 L/min flow rate), followed by filtering the jet-cooked slurry using a 200 mesh sieve (75 μ m pore opening). The separated liquid was centrifuged at 3500 g for 15 min and then drum-dried at 140°C, producing C-trim30 product and its composition (β -glucan, 32.0%; protein, 14.4%; total lipid, 2.3%; ash, 4.8% and starch, 46.5%, on a dry basis) was analyzed (AACC, 1995; AOAC, 2003).

Supercritical Carbon Dioxide Extraction

C-trim30 had lipids removed by supercritical carbon dioxide (SCD) extraction, which used 50 g of C-trim30 powder placed in a stainless steel high-pressure cell (9.5 cm long with 1.6 cm i.d.) and extracted using SCD at 85°C and 68.9 MPa. Five-hundred liters expanded carbon dioxide was used for extraction at a flow rate of 2.8 L/min. Operating conditions have been described in greater detail previously (King *et al.*, 1989).

Oil Fatty Acid Composition

Fatty acid composition of oil extracted from C-trim30 was analyzed by injecting fatty acid methyl esters (House *et al.*, 1994) into a Hewlett Packard 5890 Series II gas chromatogram (Hewlett Packard, Santa Clarita, CA) equipped with a flame ionization detector and an SP-2380 column (60 m × 0.25 mm i.d., 0.20 µm film thickness, Supelco, Bellefonte, PA) using helium as the carrier gas at a linear flow velocity of 18 cm/sec (Eller and King, 2000).

Scanning Electron Microscopy

Scanning electron microscope (JOEL model 6400V, Tokyo, Japan) was used to study morphology of C-trim30 with and without SCD at 500x, 1500x and 5000x magnification. C-trim30 powder, spread on silver tape and mounted on a brass disk, was coated with gold/palladium (60/40).

Molecular Weight Distribution of Amylopectin and Amylose

Weight-average molecular weight and z-average gyration radius of amylopectin and amylose from the starch fraction of C-trim30 with and without SCD was determined using high-performance size-exclusion chromatography equipped with multi-angle laser-light scattering and refractive index detectors (HPSEC-MALLS-RI). C-trim30 samples for injection were prepared as described by Yoo and Jane (2002a) in which oat samples were dispersed in 90% dimethyl sulfoxide at 100°C with stirring for 1 h, followed by stirring at room temperature for further 24 h, then precipitated in 5 × volume of absolute ethanol and redispersed for 30 min in boiling water with stirring. HPSEC system consisted of a HP 1050 series isocratic pump (Hewlett Packard, Valley Forge, PA), a multi-angle laser-light scattering detector (Dawn DSP-F, Wyatt Tech. Co., Santa Barbara, CA) and a HP 1047A refractive index detector (Hewlett Packard, Valley Forge, PA). To separate amylopectin from amylose, Shodex OH pak KB-G guard column and KB-806 and KB-804 analytical columns (Showa Denko K.K., JM Science, Grand Island, NY) were used. Operating conditions and data analysis are described by Yoo and Jane (2002b) except that sample injection concentration was 0.3 mg mL⁻¹.

Thermal Properties

Thermal properties of C-trim30 with and without SCD were determined using a differential scanning calorimeter (DSC 2920 modulated, TA Instruments, New Castle, DE). Approximately 10 mg of C-trim30 powder was weighed in a stainless steel pan, mixed with 30 mg of deionized water and sealed. Sample was allowed to equilibrate for 2 h and scanned at a rate of 10°C/min over a temperature range of 10-150°C. An empty pan was used as reference. All thermal properties were carried out in triplicate for each C-trim sample.

Pasting Properties

C-trim30 pasting properties, with and without SCD, were analyzed using a Rapid Visco Analyser (RVA-4, Foss North America, Eden Prairie, MN) using a similar method for starch suspensions (Jane *et al.*, 1999). C-trim30 suspensions (8% w/w), measured in quintuplicate for both with and without SCD, were prepared by weighing C-trim30 powder (2.24 g, dry oat product basis) into a RVA canister and making up the total weight to 28 g with deionized water. C-trim30 suspension was equilibrated at 30°C for 1 min, heated at a rate of 6.0°C/min to 95°C, maintained at 95°C for 5.5 min and then cooled to 50°C at a rate of 6.0°C/min and maintained at 50°C for 5 min. Constant paddle rotating speed (160 rpm) was used throughout entire analysis except for a speed of 960 rpm for the first 10 sec to disperse sample.

Water-holding Capacity

Water-holding capacity of C-trim30 with and without SCD was analyzed using a thermogravimetric analyzer (TGA 2050, TA Instruments, New Castle, DE). Approximately 20 mg of oat product was adjusted to 75% moisture using deionized water and samples were heated from 15 to 110°C at a rate of 10°C/min and then maintained at 110°C for 15 min.

Results and Discussion

C-trim30 Morphology

Scanning electron micrographs of C-trim30 with and without SCD are shown in Fig. 1. C-trim30 consisted of large particles ($\approx 200\text{-}300\ \mu\text{m}$ length, Fig. 1A) that have smooth and rough exterior regions with some large pores. At 5000x magnification, a lumpy surface with small pores in the smooth regions is observed for C-trim30 particles (Fig. 1B). SCD did not appear to change the morphology of bran particles (Fig. 1C).

Molecular Weight Distribution of Amylopectin and Amylose

HPSEC analysis showed C-trim30 with and without SCD extraction had three high molecular weight starch components present (Table 1). The largest molecules detected were amylopectins, that had their weight-average molecular weight (M_w) reduced eight times relative to pure oat starch (unpublished data). β -glucans were measured in extract injected into HPSEC system but were not

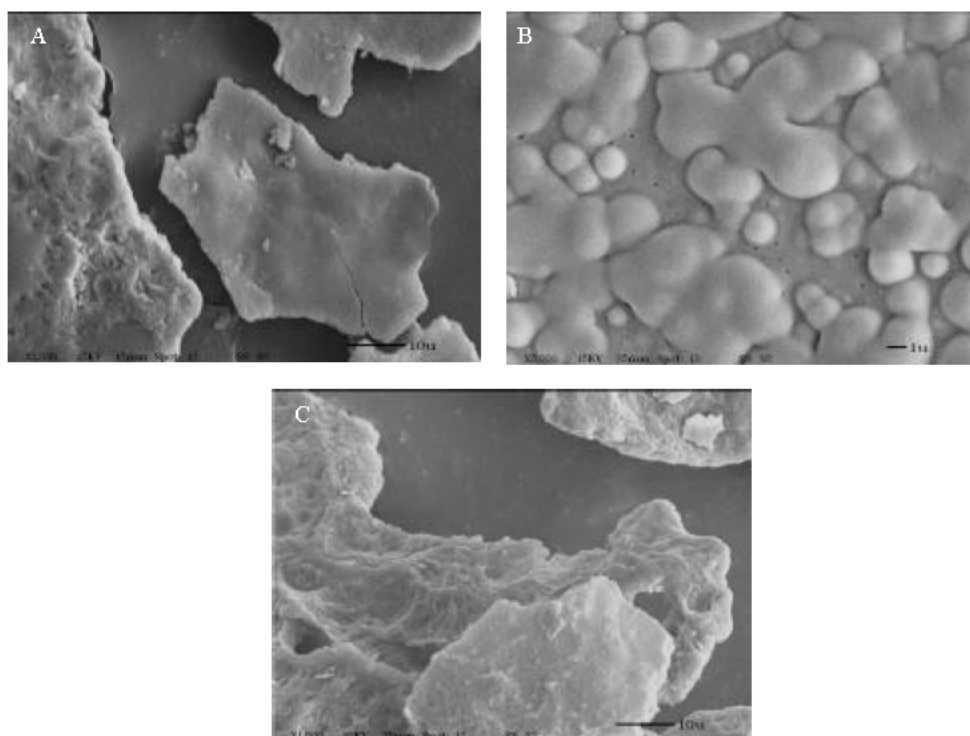


Fig. 1: Scanning electron micrographs of C-trim30 at 500x (A) and 5000x (B) magnification without supercritical carbon dioxide extraction (SCD) and 1500x magnification (C) with SCD extraction

Table 1: Average amylopectin molecular weight, polydispersity and gyration radius of C-trim30 with and without supercritical carbon dioxide (SCD) extraction of lipids^{ab}. Values after \pm represent the standard deviation

Peak		C-trim30	C-trim30+SCD
Amylopectin	Molecular weight ($M_w \times 10^6$, g/mol) ^f	1.02 \pm 0.09	3.15 \pm 0.01
	Polydispersity (M_w/M_n)	1.47 \pm 0.26	2.79 \pm 0.76
	Gyration radii (nm) ^d	270.0 \pm 56	482 \pm 64
	Density (g/mol/nm ³) ^e	7.0 \pm 4.3	2.7 \pm 0.1
Amylopectin breakdown product	Molecular weight ($M_w \times 10^7$, g/mol) ^f	1.13 \pm 0.49	2.36 \pm 0.03
	Polydispersity (M_w/M_n)	2.39 \pm 0.21	2.98 \pm 0.29
	Gyration radii (nm) ^d	206 \pm 4	374 \pm 2
	Density (g/mol/nm ³) ^e	1.3 \pm 0.5	0.5 \pm 0.0
Amylose	Molecular weight ($M_w \times 10^6$, g/mol) ^f	1.66 \pm 0.01	1.14 \pm 0.20
	Polydispersity (M_w/M_n)	1.84 \pm 0.07	1.57 \pm 0.01
	Gyration radii (nm) ^d	190 \pm 13	209 \pm 31
	Density (g/mol/nm ³) ^e	0.3 \pm 0.1	0.1 \pm 0.0

^a Data were obtained from two injections, ^b All samples were dissolved in 90% DMSO solution and precipitated with 5 vol. ethanol; Freshly prepared oat product aqueous solution (100 μ L; 0.8 mg/mL) was injected to HPSEC system, ^c weight-average molecular weight, ^d z-average radius of gyration, ^e Density is equal to M_w/R_z^3 . Values for density may not correspond directly to data in table due to rounding of M_w and R_z .

observed in the chromatogram because of absorption by column packing material. C-trim30 extracted with SCD had amylopectins with higher M_w , lower uniformity (higher polydispersity (polyd)), wider gyration radius (R_z) and lower density compared with C-trim30 without SCD extraction. This suggests that during SCD extraction, amylopectin molecules in C-trim30 are aggregating together or are complexing with amylose or β -glucans.

C-trim30 amylopectins had comparable M_w and polyd, but higher R_z and considerably lower density compared to Nutrim-OB, an enriched oat hydrocolloid prepared by jet-cooking that has about 12% β -glucan content (Stevenson *et al.*, 2006). SCD extraction of C-trim30 resulted in amylopectins with two times higher M_w , polyd and R_z and seven times lower density than SCD-extracted Nutrim-OB (Stevenson *et al.*, 2006). Centrifugation to remove dense starch particles prior to jet-cooking in the production of C-trim30 is the primary processing step that differs from Nutrim-OB, explaining the observed differences in density.

The observed peak with M_w of 1.13×10^7 in C-trim30 is most likely a breakdown product of amylopectin during jet-cooking or drum-drying and is not likely to be intermediate starch fraction as this was not observed on analysis of pure oat starches (unpublished data). Changes in M_w , polyd, R_z and density of this amylopectin breakdown product after SCD extraction showed similar trends to amylopectin molecules, indicating aggregation or complexing with other molecules, or less degradation was also occurring.

The observed peak with M_w of 1.66×10^6 was broader than the amylopectin peak and was characteristic of amylose molecular weight distribution. Oat β -glucans are unlikely to be contributing to this peak since their maximum M_w has been reported to be 2×10^5 (Roubroeks *et al.*, 2001; Tosh *et al.*, 2004). The lower M_w , polyd and unaffected R_z of amylose from SCD extracted C-trim30 suggests that amylose was partially degraded to more uniform molecules that maintained their spatial dimensions.

The starch molecules present after production of C-trim30 and SCD extraction are still very large and indicates there is opportunity to further enrich β -glucan content by starch degradation. The heat-stability demonstrated by starch molecules subjected to jet-cooking and SCD extraction suggests heat-resistance due to interactions among starch molecules or possibly with β -glucans. Interference between starch and β -glucans in barley has previously been observed (Knuckles *et al.*, 1997) and other gums, such as guar galactomannan have been reported to acts as physical barriers to enzymatic degradation of starch (Brennan *et al.*, 1996).

Thermal and Pasting Properties

No thermal transitions were observed for C-trim30 between 10 and 150°C. Therefore, the higher β -glucan-enriched product, C-trim30, had no melting of amorphous amylose-lipid thermal transitions that were observed for Nutrim-OB both with and without SCD extraction despite both oat bran products experiencing similar thermal processing steps (Stevenson *et al.*, 2006).

Pasting properties of C-trim30 with and without SCD extraction are shown in Table 2. C-trim30 had high peak, breakdown, final and setback viscosity. C-trim30 formed a paste instantly at initial test temperature of 30°C (Fig. 2). SCD extraction of C-trim30 resulted in higher paste viscosity and setback viscosity, with the latter indicating that lipids were complexing with other molecules, restricting their mobility during cooking. The high, instantaneous viscosity formed at room temperature for C-trim30 with SCD extraction could make it suitable for smoothies, yogurts and other refrigerated foods, whereas its high setback and final viscosity are well suited for foods requiring strong gel formation such as pie fillings. The high viscosity could function as a fat substitute in foods and be a useful ingredient in developing foods that prevent obesity (Davidson and Swithers, 2005). Relative to Nutrim-OB (Stevenson *et al.*, 2006), C-trim30 had similar peak viscosity, less breakdown viscosity, slower shear-thinning rate, higher final and setback viscosity and instantaneous pasting at room temperature, which can all be attributed to the higher β -glucan content of C-trim30.

Water Retention

Water-holding capacity of C-trim30 with and without SCD extraction is shown in Table 3. C-trim30 with SCD extraction had lower water-holding capacity than C-trim30 without SCD extraction throughout the entire test range from 15 to 110°C. Although C-trim30 with SCD

Table 2: Pasting properties of C-trim30 with and without supercritical carbon dioxide (SCD) extraction of lipids measured by Rapid Visco-Analyser^a. Values after \pm represent the standard deviation

Pasting parameter	C-trim30	C-trim30+SCD
Peak viscosity ^b	290.7 \pm 45.7	422.8 \pm 27.5
Trough ^b	90.3 \pm 10.8	104.6 \pm 5.00
Breakdown ^b	200.4 \pm 38.3	318.1 \pm 22.9
Final viscosity ^b	272.9 \pm 33.7	328.1 \pm 15.9
Setback ^b	182.6 \pm 23.0	223.4 \pm 10.9

^a 8% (w/w) C-trim30 suspension, with and without SCC, measured five times, ^b Viscosity measured in Rapid Visco-Analyser units (RVU), 1 RVU = 12 centipoise

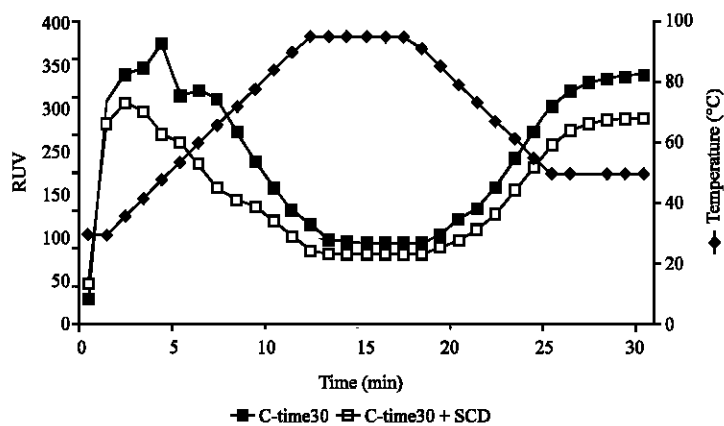


Fig. 2: Rapid Visco-Analyser pasting profiles of C-trim30 with and without supercritical Carbon Dioxide Extraction (SCD) of lipids. Note peak viscosity of C-trim30 after SCD extraction is lower than listed in Table 2 because pasting profiles are average viscosity at each given time for all replicates and there was wide variation in peak time among the replicates

Table 3: Thermogravimetric analysis of 25% (w/w) C-trim30 in deionized water, with and without supercritical carbon dioxide (SCD) extraction of lipids. Percentage water loss for each oat product is shown at selected temperatures during heating. Values after ± represent the standard deviation

Temperature	C-trim30	C-trim30+SCD
55°C	3.2±0.0	5.1±0.4
80°C	10.6±0.1	12.4±0.2
105°C	24.8±0.2	27.9±0.5
110°C	29.3±0.2	33.0±0.7
110°C for 2.5 min	58.2±2.9	61.5±3.7
110°C for 5 min	75.6±3.2	78.9±3.5
110°C for 10 min	96.7±1.9	98.4±0.8

Table 4: Mean fatty acid composition (relative percent) of oil extracted by supercritical carbon dioxide from C-trim30. Values after ± represent the standard deviation*

Fatty acid	C-trim30
14:0	0.22±0.000
16:0	15.68±0.008
16:1	0.21±0.019
18:0	1.90±0.003
18:1	45.72±0.012
18:2	34.06±0.007
18:3	1.04±0.013
20:0	0.20±0.026
20:1	0.98±0.000

* n = 3 analyses

extraction had lower water-holding capacity when maintained at 110°C for 15 min, the variation was too high to infer any significant differences. Enhanced water removal from plant materials during dehydration operations after SCD extraction has been previously reported (Knorr, 2003). C-trim30 had considerably higher water-holding capacity compared to that reported for Nutrim-OB (29.3 versus 60.7% water loss heated to 110°C) (Stevenson *et al.*, 2006), which would be due to C-trim30 having 2.5 times greater β -glucan content. Scanning electron micrograph of C-trim30 (Fig. 1A) show that it has less large pores relative to Nutrim-OB (Stevenson *et al.*, 2006), demonstrating that the pores may not function in a sponge-like, water-retaining manner. The high water-holding capacity and viscosity of C-trim30 would make it an attractive ingredient to substitute fat in food formulations. The slight decrease in water retention of SCD extracted C-trim30 is most likely an undesirable feature, but as a fat-substitute, the increase in viscosity would outweigh a small moisture loss.

Oil Extracted from C-trim30

Fatty acid composition of oil extracted by SCD from C-trim30 is shown in Table 4. The oil consisted of 82% unsaturated fatty acids, predominantly oleic and linoleic acid, with palmitic acid the main saturated lipid present. Fatty acid composition was in good agreement with other studies on oat oil (Saastamoinen *et al.*, 1989; Welch and Leggett, 1997). The high unsaturation and low linolenic acid levels of the SCD extracted oil makes it suitable for incorporating in healthy foods and long-term storage without high levels of oxidation. Therefore, oil extracted from oat bran by SCD, has valuable properties that can improve the overall economics of β -glucan enrichment processes.

CONCLUSIONS

This study investigated starch structures and physicochemical properties of the β -glucan-enriched oat bran product, C-trim30 (32% β -glucan content, dry weight basis), with and without supercritical carbon dioxide (SCD) extraction. Although substantial decreases in molecular weight were observed relative to pure unmodified oat starch, amylopectins in C-trim30 were still large ($>1 \times 10^6$) and there is potential to further enhance β -glucan concentration by starch degradation. C-trim30 had considerably

high peak, breakdown, final and setback paste viscosity compared to other β -glucan-enriched oat products. C-trim30 also exhibited high viscosity at room temperature and had very high water-holding capacity. The high viscosity and moisture retention of C-trim30 make it very suitable as a fat replacement in foods. SCD extracted C-trim30 had slightly less water retention, but considerably higher paste viscosity that makes it an excellent fat substitute.

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