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## Investigation of freezing temperature and time to improve resistant starch content and quality of pure mung bean starch vermicelli

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#### Abstract

Mung bean vermicelli is one of the commonly consumed styles of noodles because of its convenience in Asian countries as well as being a good source of resistant starch (RS). It was hypothesized that suitable freezing conditions could produce mung bean vermicelli with a greater RS content and good cooking quality compared to the commercially available product. Therefore, this study investigated the optimum freezing conditions to increase the RS content in mung bean vermicelli. After extrusion, boiling, and cooling, the drained vermicelli was incubated at 4 °C for 1 h 30 min before using different freezing conditions by varying the processing temperature (-5, -10, -15, or -20 °C) and time (6, 12, 18, or 20 h for each temperature). The highest RS content was observed in dried mung bean vermicelli (12.83% at -10 °C for 18 h; V1018), whereas the commercial vermicelli had the lowest RS content (9.73%). The cooking time, water absorption, volume increase, and resistant starch content of cooked V1018 were higher with values of 12.69 min, 502.97%, 19.13% and 7.32%, respectively, than those of the commercial vermicelli (8.56 min, 347.34%, 15.19% and 5.58%, respectively). Furthermore, the cooking loss, tensile strength, and elasticity of V1018 were 1.08%, 0.0101 N, and 11.96 mm, respectively, which were lower than for the commercial vermicelli (1.96%, 1.16-folds, and 2.16-folds, respectively). The color of dried V1018 vermicelli had more lightness, redness, and yellowness, while the cooked V1018 vermicelli had more lightness, greenness, and blueness than the respective commercial products. Hence, incubating at 4 °C for 1 h 30 min, followed by freezing at -10 °C for 18 h was recommended to produce pure mung bean starch vermicelli with a high resistant starch content and good quality.

Keywords: Mung bean, Vermicelli, Resistant starch, Freezing, Retrogradation.

**Abbreviations:** RS\_resistant starch; RS1\_resistant starch type 1; RS2\_resistant starch type 2; RS3\_resistant starch type 3; RS4\_resistant starch type 4; V1018\_mung bean vermicelli incubated at 4 °C for 1 h 30 min, followed by freezing at -10 °C for 18 h.

#### Introduction

Resistant starch (RS) cannot be converted into glucose in the human digestion system; consequently, it passes through the small intestine to the colon where it is fermented by the colon's microbiota (BeMiller, 2020). RS is a form of dietary fiber and can be found in many starch products (Raigond et al., 2015). The health benefits of RS in carbohydrate products with a low glycemic index include helping to control obesity and diabetes and reducing the risk of cardiovascular disease (Htoon et al., 2010). Basically, RS is a natural food component; thus, RS can be neutral to the organism and add only a little calorific content to food. As such a type of dietary fiber, RS plays a function in promoting the benefits of physiological effects, including laxation and lower blood sugar and cholesterol levels (Raigond et al., 2015). Therefore, consuming more RS might have a great health benefit and encourage a healthier food lifestyle.

Naturally, RS presents in four types: RS1, RS2, RS3, and RS4. RS1 is the starch fraction that is surrounded by cell wall

materials and a protein matrix or is trapped in a food matrix, resulting in granules, non-granular particles, or a physically accessible structure (BeMiller, 2020). RS2 is known as native, uncooked granular rice; its structure is based on its genetic background that affects the granule and pore sizes (BeMiller, 2020). Basically, RS1 and RS2 are found in small amounts in starchy food and are not stable during processing (Photinam et al., 2016). RS3 is a retrograded starch, mainly consisting of retrograded or recrystallized amylose, which is formed during the cooling process of gelatinized starch at low or room temperature in cooked starchy products (Raigond et al., 2015). RS4 is a chemically modified starch with it structure based on its genetic background, granule size, and channels or degree of substitution (BeMiller, 2020). Consumed RS3 is safer than RS4 due to its starch retrogradation form and it is more stable during food processing (Photinam et al., 2016).

Mung bean vermicelli is a legume product mostly made from mung bean starch that provides a good source of RS (10–20 %),

according to Photinam et al. (2016). The RS content of Thai mung bean vermicelli product was reported to be 11.3%, while that of Thai instant rice noodles and rice sheet noodles was 2.40% (Vatanasuchart et al., 2009). Basically, RS can be formed by the starch itself through retrogradation during the production of vermicelli, especially in a freeze-thaw cycle (Chung et al., 2006). The freezing process is important for the retrogradation of mung bean starch, which facilitates the separation of noodle strands and creates RS3. Therefore, the objective of the current study was to identify the suitable conditions based on freezing temperature and time to produce pure mung bean starch vermicelli that is high in RS content and quality.

#### **Results and discussion**

# Effect of freezing conditions on resistant starch content in dried mung bean vermicelli

RS in mung bean vermicelli is retrograded starch, which is formed when gelatinized starch is cooled or stored at low temperature or room temperature (Raigond et al., 2015). Amylose and amylopectin influence the formation of RS, with the retrogradation or recrystallization of amylose being faster than that of amylopectin and the amylose crystalline structure is more stable than the amylopectin crystalline structure (Wang et al., 2015). The RS contents of the dried mung bean vermicelli samples treated with different freezing conditions are presented in Table 1. The RS content in the commercial mung bean vermicelli was  $9.73 \pm 0.92\%$ , while the mung bean vermicelli incubated at 4 °C for 1.5 h had a higher RS value  $(10.04 \pm 0.92\%)$  due to starch retrogradation. The RS content of the mung bean vermicelli with the different freezing treatments was in the range 10.19-12.86%, which was higher than for the commercial product and for without freezing. In general, the results showed that the RS content increased as the freezing time increased gradually from 6 h to 18 h and then more slowly at 24 h. These results were similar to those reported by Photinam et al. (2016), who studied vermicelli with mixed bean starch as a component. They reported that the RS content in their uncooked vermicelli increased as the freezing time increased, with the highest content being in the sample frozen at -13 °C for 19 h and 24 h. This could be explained by the increase in the RS being mainly related to the recrystallization of amylose molecules with a linear chain, while amylopectin molecules would be crystallized later due to their short chain and branching structure (Chung et al., 2006; Zhou and Lim, 2012). Tortillas stored for 24, 48, and 72 h had increased levels of RS with 35.1, 40.5, and 44.2 g/kg, respectively, at 25 °C, while at 4 °C, the levels were 36.0, 41.8, and 45.3 g/kg, respectively (Islas-Hernandez et al., 2006). The RS content in wheat flour noodles was investigated by Tian et al. (2021), who reported increases following storage times of 4, 24, and 48 h at different temperatures. The RS content increased 2.84-3.81, 2.02-2.65, and 1.56-2.09-folds at storage temperatures of 25, 4, and -18 °C, respectively. However, in some cases, the RS content decreased following prolonged storage, due to the reverse retrogradation of amylopectin to form crystallite, caused by the short branch chains and branching structure (Chung et al., 2006; Zhou and Lim 2012). Niba (2003) found that the RS content in stored cornbread

increased up to 4 d and decreased after 7 d at -20  $^{\circ}$ C. Photinam et al. (2016) reported that the RS content in mixed bean starch vermicelli decreased after longer freezing times from 25 h to 52 h, while the highest content was measured after 21 h of freezing at -13  $^{\circ}$ C.

In the current study, the RS content in the mung bean vermicelli during 24 h of storage increased from 10.04% to 12.11% at -5 °C, from 11.18% to 12.86% at -10 °C, from 11.59% to 11.54% at -15 °C, and from 10.19% to 11.34% at -20 °C. Notably, at freezing temperatures of -5 °C and -10 °C, the formation of RS was higher than at -15 °C and -20 °C, due to lower temperatures having higher freezing rates (Yu et al., 2010). It was found that with rapid cooling, the degree of starch retrogradation declined, because the available time was restricted for starch molecule rearrangement. Furthermore, it was difficult for the starch chains to reassociate after being frozen at very low temperature (Chang et al., 2021). This was in agreement with the research of Yu et al. (2010) on the starch retrogradation of cooked rice, where the RS content decreased following freezing at -20, -30, -40, -60, and -100  $^{\circ}$ C with freezing rates of 0.09, 0.26, 0.33, 0.53, and 1.45 °C/min, respectively.

In the current study, the most suitable freezing temperature and storage time to increase the RS content were for the mung bean vermicelli frozen at -10  $^{\circ}$ C for 18 h and 24 h. Therefore, the conditions of freezing at -10  $^{\circ}$ C for 18 h were selected to save time and energy; this treatment was identified as V1018 in the subsequent analyses.

#### **Cooking quality**

The cooking quality of mung bean vermicelli was indicated by the cooking time, water absorption, cooking loss, and volume increase (Table 2). The cooking time was recorded as the time taken for the white core inside the vermicelli strand to disappear. The sample V1018 was totally cooked after 12.69 min, while commercial vermicelli needed cooking for 8.56 min. This may have been affected by the diameter of the vermicelli strands being 0.1 cm for V1018 and 0.06 cm for the commercial vermicelli.

Water absorption is an indicator of the water absorption ability of noodles during cooking, which is one of the important factors determining the cooking quality of noodles (Chung et al., 2012). There was a significant difference in water absorption between the V1018 and commercial vermicelli (502.97 and 347.34%, respectively). The higher water absorption in V1018 might have been due to the freezing and thawing process, resulting in improved porosity inside the vermicelli strands, as well as a bigger pore size. Consistent with water absorption, the volume increase was 19.13% and 15.19% for the V1018 and commercial samples, respectively. These results were similar to the research results of Kang et al. (2017) who found that the volume increase in wheat flour noodles was highest with the highest water absorption.

Cooking loss is an important property for the evaluation of good quality noodles, as it shows the percentage of the solid content that leaches into the cooking water, including starch and other soluble components (Pongpichaiudom and Songsermpong, 2018). Therefore, cooking loss has a negative effect on the sensory property of the noodle product (Kang et al., 2017). A good quality noodle has less cooking loss, which

Sample	Freezing conditions	Resistant starch (%, dry weight)		
	Temperature (°C)	Time (h)		
Commercial	- 3	8	$9.73 \pm 0.92^{c}$	
VO	4	1.5	$10.04 \pm 0.92^{bc}$	
V56	-5	6	10.27 ± 1.57 <sup>bc</sup>	
V512	-5	12	$11.91 \pm 1.50^{abc}$	
V518	-5	18	$12.07 \pm 0.81^{ab}$	
V524	-5	24	$12.11 \pm 0.81^{ab}$	
V106	-10	6	$11.18 \pm 1.44^{abc}$	
V1012	-10	12	$11.34 \pm 0.46^{abc}$	
V1018	-10	18	$12.83 \pm 0.46^{\circ}$	
V1024	-10	24	12.86 ± 0.65 <sup>°</sup>	
V156	-15	6	$11.59 \pm 1.05^{abc}$	
V1512	-15	12	$11.80 \pm 1.35^{abc}$	
V1518	-15	18	11.82 ± 1.28 <sup>abc</sup>	
V1524	-15	24	$11.54 \pm 0.62^{abc}$	
V206	-20	6	10.19 ± 2.29 <sup>bc</sup>	
V2012	-20	12	10.76 ± 1.38 <sup>abc</sup>	
V2018	-20	18	$11.30 \pm 0.72^{abc}$	
V2024	-20	24	11.34 ± 0.63 <sup>abc</sup>	

Table 1. Resistant starch content of dried mung bean vermicelli treated using different freezing conditions.

Mean ± SD values with different, lowercase superscripts in same column are significantly different at p ≤ 0.05, according to Duncan's multiple range test.

Table 2. Cooking qualities and resistant starch content of cooked mung bean vermicelli.

Sample	Cooking time (min)	Water absorption (%)	Cooking loss (%)	Volume increase (%)	Resistant starch (%)
V1018	12.69 <sup>ª</sup> ± 0.14	502.97 ± 4.26 <sup>a</sup>	$1.08 \pm 0.24^{b}$	19.13 ± 0.31 <sup>ª</sup>	7.32 ± 0.35 <sup>ª</sup>
Commercial	8.56 <sup>b</sup> ± 0.17	347.34 ± 5.00 <sup>b</sup>	$2.12 \pm 0.05^{a}$	15.19 ± 0.00 <sup>b</sup>	5.58 ± 0.66 <sup>b</sup>

V1018 = freezing at -10°C for 18 h. Mean ± SD values with different, lowercase superscripts in same column are significantly different at p ≤ 0.05, according to Duncan's multiple range test.

#### Table 3. Texture properties of mung bean vermicelli.

Sample	Tensile strength (N)	Elasticity (mm)
V1018	$0.0101^{b} \pm 0.010$	11.96 <sup>b</sup> ± 2.81
Commercial	$0.0117^{a} \pm 0.001$	25.82 <sup>a</sup> ± 5.98

V1018 = freezing at  $-10^{\circ}$ C for 18 h. Mean ± SD values with different, lowercase superscripts in same column are significantly different at  $p \le 0.05$ , according to Duncan's multiple range test.

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Sample	mple Color parameter									
	L <sup>*</sup>		a*		<i>b</i> *		<i>∆</i> Ε <sup>*</sup>			
	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked		
V1018	89.49 <sup>Aa</sup> ± 1.32	$77.62^{Ba} \pm 0.16$	$0.18^{Aa} \pm 0.03$	$-1.66^{Ba} \pm 0.04$	$7.12^{Aa} \pm 0.74$	$-2.09^{Ba} \pm 0.13$	$5.10^{A} \pm 0.77$	$2.50^{B} \pm 0.17$		
Commercial	83.91 <sup>Ab</sup> + 2.16	$70.20^{Bb} + 0.17$	$-0.39^{Ab} + 0.06$	$-2.28^{Bb} + 0.05$	$5.63^{Ab} + 0.30$	$-2.26^{Ab} + 0.16$	-	-		

V1018 = freezing at -10°C for 18 h. Mean ± SD values with different, lowercase superscripts in same column and uppercase superscripts in rows are significantly different at  $\rho \le 0.05$ , according to Duncan's multiple range test.

should not be more than 10% (Tan et al., 2009). Table 2 indicates the V1018 had a lower cooking loss (1.08%) compared to the commercial product (2.12%), which in V1018 was related to the RS content and indicated a strong structure due to the high degree of starch retrogradation (Satmalee et al., 2009) resulting in fewer soluble components leaching from the vermicelli. As can be seen from Table 2, the higher the water absorption, the lower the cooking loss. This result was similar to that from the study by Sirichokworrakit et al. (2015), where the water absorption of noodles supplemented with Riceberry flour decreased with increased amounts of Riceberry flour, while the cooking loss showed an increasing trend. The RS of the mung bean vermicelli after cooking decreased in all treatments. Notably, the RS of the cooked V1018 vermicelli was higher than for the commercial product (7.32 and 5.58%, respectively). The decrease in the RS content could have been due to the re-gelatinization of the starch during cooking and the instability of retrograded amylopectin under heating (Niba, 2003). A similar result was reported by Photinam et al. (2016), where the RS in mixed mung bean vermicelli was reduced after cooking (from 23.83 to 13.23%).

#### Texture

The texture of mung bean vermicelli is described in Table 3, based on tensile strength and elasticity. The tensile strength of

V1018 was 0.0101 N, while that of the commercial product was 0.0117 N. Showing a similar trend, the elasticity of V1018 (11.96 mm) was lower than for the commercial product (25.82 mm). Lower values for these properties of V1018 could be explained by its weak structure (Nura et al., 2011) that was caused by the more porous structure inside the vermicelli strand leading to less elasticity. In addition, the presence of protein in the extracted starch (0.19%) might have affected the V1018 texture, because the lower the protein content, the softer the texture, due to less interaction between the starch and protein molecules. Puhin et al. (2021) found that the percentage elongation of rice flour noodles was higher than for starch noodles because of the protein network. This was supported by the research of Sun et al. (2013), who reported that the elongation of pea starch film increased when peanut protein isolate was added, due to the starch-protein interaction. Furthermore, the different techniques and machinery used in production between factory and handmade vermicelli products could affect the texture of the vermicelli.

#### Color

Color plays an important role in the quality evaluation of materials due to its visual impact on customers (Ritthiruangdej et al., 2011), with a dark or gray color often being rejected by consumers. Mung bean vermicelli with a transparent and white color is preferred in Asian countries. In the current study, the color of the dried mung bean vermicelli was indicated by the values for  $L^*$ ,  $a^*$ ,  $b^*$ , and  $\Delta E^*$  (compared with the  $L^*$ ,  $a^*$ , and  $b^*$  values of the commercial vermicelli) in Table 4. The lightness ( $L^*$ ) value of V1018 was 89.49, while that of the commercial product was 83.91. The  $a^*$  value (0.18)

indicated the redness in V1018 and the greenness in the commercial product (-0.39). The  $b^*$  values indicated yellowness in both vermicelli samples (7.12 and 5.63 for V1018 and the commercial product, respectively). The differences in these color parameters between V1018 and the commercial product might have been due to the protein content in the starch causing a Maillard reaction between the amino acid and reducing sugar components, resulting in a dark color (Mohamed et al., 2010). The higher the protein content, the lower the lightness value ( $L^*$ ).

The parameter values for each treatment decreased in terms of the color of the mung bean vermicelli after cooking, especially the  $a^{\prime}$  and  $b^{\prime}$  values of V1018 that changed to greenness and blueness, respectively, while only the  $b^{T}$  value of the commercial product changed its blueness value. Generally, the color of vermicelli was greener and bluer and not light after cooking, which could be explained by the diffusivity of color compounds in the water; hence, the lightness value decreased. In addition, the starch was gelatinized during cooking in hot water so that the vermicelli was more transparent, resulting in increased greenness and blueness (Mohamed et al., 2010). The total color difference for the commercial product was higher than for V1018 before cooking than after cooking (5.10 and 2.50, respectively). It was noticed that after cooking, the V1018 vermicelli was not much different from the commercial product.

#### **Materials and Methods**

#### Plant and food materials

The hulled-split mung bean (KhaoThong Brand; Thai Food Industry Co. Ltd.; Thailand) was purchased from a supermarket. A supermass colloider (Masuko Sangyo Co. Ltd.; Japan) and basket centrifuge (Centurion Scientific Ltd.; UK) were provided for starch extraction, the handle extruder (Thailand made) was used to make the mung bean vermicelli and an ice cream freezer (EFI2153; Liebherr; Germany) was used for freezing. The experiment was conducted at the Food Processing Pilot Plant, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand.

#### Mung bean starch preparation

Mung bean starch was prepared following the method of Park et al. (2012), with a slight modification. The dehulled mung bean seeds were soaked in tap water (seeds-to-tap water ratio, 1:3 by weight) at room temperature for 12 h. Then, the soaked mung bean seeds were washed and ground using the supermass colloider at low speed with a ratio of beans-towater of 1:3 by weight. The starch slurry was passed through a basket centrifuge filter and settled for 3 h. The supernatant was removed using suction and the settled starch layer was washed using the tap water until the starch became white. Finally, the starch was collected and dried in an oven at 50 °C for about 10 h to obtain a moisture content of 11–12% (wet basis). The mung bean starch was finely ground, sieved and packaged in plastic bags and kept at room temperature for the further experiments.

#### Pure mung bean starch vermicelli preparation

The mung bean vermicelli was prepared according to the procedure of Nguyen et al. (2022). The mixture (95.5 g raw starch and 4.5 g of pre-gelatinized starch, with a raw starch-towater ratio of 1:10 weight per weight) was boiled for 5 min. Hot water (120 g) was mixed into the starch mixture to adjust the moisture content of the dough to 55%. The starch mixture was extruded into boiling water using a handle extruder with a diameter of 1.5 mm. The mung bean vermicelli strands were cooked for 3 min and then cooled under tap water. The cooled noodles were placed on a stick to facilitate water drainage for 5 min. Then, the vermicelli samples were incubated by placing the noodles in a refrigerator at 4 °C for 1 h 30 min for the retrogradation process (Photinam et al., 2016) before being frozen in the ice cream freezer at -5, -10, -15, or -20 °C. At each freezing temperature, a sample of the vermicelli was taken after 6, 12, 18, and 24 h and thawed in tap water to separate the noodle strands before drying at 45 °C in a hot-air oven for 8-12 h to reduce the moisture content to below 12% (wet basis). The dried samples were kept in polyethylene bags until being analyzed for their resistant starch content and physical properties. Pure mung bean starch vermicelli with the highest RS content was compared with the commercial vermicelli (Lion brand; Tharuea Phrathaen Co. Ltd.; Kanchanaburi, Thailand) used as the control sample.

#### Resistant starch content

RS content in the instant mung bean noodles was analyzed using a Megazyme Resistant Starch Assay Kit (Megazyme International Ireland Ltd, Bray, Ireland) according to the manufacturer's instructions.

#### **Cooking qualities**

The cooking qualities of the mung bean vermicelli were determined following the method 60–55 (AACC, 2000). Water absorption was determined by immersing 25 g (dry basis) of dried vermicelli in 300 mL hot water at 90 °C until the white core inside the vermicelli strands had disappeared. After that, the vermicelli strands were cooled in 50 mL distilled water for 1 min and then drained for 5 min. Immediately, the cooked vermicelli strands were weighed to calculate the percentage of water absorption based on equation (1):

$$= \frac{\text{Water absorption (\%)}}{\text{Weight of cooked sample} - \text{Weight of uncooked sample}} \times 100$$
(1)

where all weights were measured in grams.

The remaining cooking water from above was transferred to a moisture can and dried at 105  $^{\circ}$ C for 24 h. Cooking loss was calculated based on equation (2):

$$= \frac{\text{Weight of dry matter in cooking water}}{\text{Weight of dried noodles}} \times 100$$
(2)

where all weights were measured in grams.

The volume increase was calculated by adding 20 g of dried vermicelli before and after cooking into 300 mL distilled water in a 500 mL cylinder. The volume increase was calculated based on equation (3):

$$Volume increase(\%) = \frac{Volume of cooked vermicelli - Volume of dried vermicelli}{Volume of dried vermicelli} \times 100$$
 (3)

where all volumes were measured in milliliters.

#### Texture

The tensile strength (maximum force, measured in newtons) and elasticity (distance at maximum force, measured in millimeters) of the mung bean vermicelli were measured using a texture analyzer (TA.XT. Plus; Stable Micro Systems Ltd.; Godalming, UK) following the method of Nguyen et al. (2022). The dried vermicelli (25 g dry basis) was immersed in 300 mL hot water at 90 °C until the white core inside the vermicelli strands had disappeared. The vermicelli strands were cooled in 50 mL distilled water for 1 min and drained for 5 min. The sample was cut into 30 cm lengths and placed around a parallel spaghetti rig probe (A/SPR). The instrument settings for each treatment of 30 specimens were: extension mode; trigger type, auto 5.0 g; pre-test speed, 3.0 mm/s; post-test speed, 5.0 mm/s; test speed, 3.0 mm/s: and trigger distance, 70 mm.

#### **Color parameters**

The Hunter Lab colorimeter (Ultra Scan Pro; HunterLab; USA) was used to measure the color of the mung bean vermicelli samples at ambient temperature, based on the CIELAB

measuring system. About 20 g of ground sample were placed in separate plastic bags before measurement. The colorimeter was calibrated using white and black standards. The color values of  $L^*$  (100 for white and 0 for black),  $a^*$  (rednessgreenness), and  $b^*$  (yellowness-blueness) were measured using a D65 illuminant. The total color differences ( $\Delta E^*$ ) between the commercial vermicelli (control sample) and the instant mung bean vermicelli samples in the study were determined using equation (4):

 $\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$ (4) where  $\Delta L^*$  is  $L^*_{sample} - L^*_{control}$ ,  $\Delta a^*$  is  $a^*_{sample} - a^*_{control}$ , and  $\Delta b^*$  is  $b^*_{sample} - b^*_{control}$ .

#### Statistical analysis

Analysis of variance was performed using the SPSS software package Version 17 (IBM Corp.; Chicago, IL, USA). Comparisons between means were examined using Duncan's multiple range test at the  $p \le 0.05$  significance level. The results from three replications were presented as mean ± SD.

#### Conclusion

RS formation in the mung bean vermicelli was influenced by the chilling and freezing processes. The recommended suitable conditions for increasing the RS content for the fresh mung bean vermicelli were incubation at 4 °C for 1 h 30 min and then freezing at -10 °C for 18 h (V1018) to produce an RS content of 12.83%. Compared to commercial vermicelli, the quality of V1018 with its suitable freezing conditions was significantly different; in particular, the RS content of the cooked V1018 vermicelli was higher than for the cooked commercial vermicelli. This knowledge could be transferred to manufacturers to help them increase the RS content of vermicelli for health benefits.

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