様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　６ 年　 ６ 月 ２０ 日現在

機関番号：１０１０５
研究種目：基盤研究(C)（一般）
研究期間：2021～2023
課題番号：２１Ｋ０５４８２

研究課題名（和文）ウコンでんぷんに新たな機能性はあるのか

研究課題名（英文）New functionality of turmeric starch

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交付決定額（研究期間全体）：（直接経費）　　2,300,000円

研究成果の概要（和文）：ウコンデンプンはリンをエステル結合の形で含み、ジャガイモデンプンよりもはるかに高いリン含量を持ち、大腸のアルカリホスファターゼ（ALP）活性を増加させることで腸の防御機能を強化することが予想される。本研究では、ウコンデンプンに含まれる高リン型デンプンが腸内環境に及ぼす影響を調査した。動物実験により、ウコンデンプンが盲腸内の短鎖脂肪酸の生成を促進し、有益な腸内細菌の増加も観察された。しかし、ALP活性には有意な影響は見られなかった。以上の結果から、ウコンデンプンは腸内健康の改善に寄与する可能性が示唆され、健康食品への応用が期待される。今後、抗炎症効果の詳細な評価が必要である。

研究成果の学術的意義や社会的意義
本研究は、ウコンデンプンが腸内環境に及ぼす影響を解明した。ウコンデンプンはレジスタントスターチとして機能し、腸内の短鎖脂肪酸の生成を促進し、脂肪蓄積を抑制する効果が確認された。また、特定の有益な腸内細菌の増加も観察された。これにより、ウコンデンプンは腸の健康を改善し、肥満予防に寄与する可能性がある。今後、腸内発酵に伴う抗炎症効果を明らかにすることで健康食品や医療への応用が期待される。本研究は、社会全体の健康増進に重要な意義を持つ成果を提供している。

研究成果の概要（英文）：Turmeric starch contains phosphorus in ester bond form, significantly higher than potato starch (PS), and is expected to enhance gut defense by increasing colonic Alkaline Phosphatase (ALP) activity. Therefore,this study investigated the effects of high-phosphorus turmeric starch on the intestinal environment. It was shown that turmeric starch promotes the production of short-chain fatty acids (SCFAs) in the cecal contents and suppresses fat accumulation. Beneficial gut bacteria also increased, although there was no significant impact on the intestinal ALP activity in rats. These findings suggest that turmeric starch may improve gut health with potential applications in functional foods. However, further evaluation of its anti-inflammatory effects is necessary.

研究分野：機能性食品科学

キーワード：ウコンでんぷん　腸内発酵

１．研究開始当初の背景

Resistant Starch (RS) is a well-known ingredient in functional foods, known for its high fermentability in the colon. It offers numerous health benefits by altering the colonic environment. In recent years, the identification of new starches with different properties has been increasingly studied due to the interest in health promotion and RS has attracted a great deal of attention. In addition to dietary fiber, turmeric (*Curcuma aromatica S.)* rhizomes contain a large amount of starch composed of about 30% amylose. The turmeric starch contains phosphorus in the form of ester bonds, which is much higher than that of potato starch (PS), which is called a high-phosphorus type starch. When potato starch is decomposed by amylase as high phosphorus-type starch, the decomposition rate is low because amylase cannot act near the phosphate groups, and oligosaccharides with phosphate groups are produced at the end of the reaction. Therefore, it is strongly expected that turmeric starch may also have the properties of high-phosphorus type RS. Furthermore, it is anticipated that turmeric starch will significantly enhance the function of intestinal defense by increasing colonic Alkaline Phosphatase (ALP) activity. This enhancement is achieved by its dual role as a phosphorus source and as a source of indigestible oligosaccharides.

２．研究の目的

In this study, three sub-themes are established to elucidate the functional properties of turmeric starch. Three animal experiments are then conducted 1) to understand turmeric starch's fermentability and ALP activity in different doses, and 2) to understand a comparative analysis on colonic fermentation characteristics of TS, PS was chosen as a high-phosphorus starch, high-amylose corn starch (HAS) as the positive control, and yam starch (YS) was likely chosen due to its high RS with comparatively low phosphorus content. And experiment 3 was designed to evaluate the intestinal anti-inflammatory effect of the turmeric starch in DSS-induces colitis in rats.

３．研究の方法

Experiment 1) Dose-dependent effect of turmeric starch: four experimental diets containing different levels of TS (5%, 10%, and 20% w/w) were formulated and fed to male Fischer 344 rats for two weeks and compared with rats fed 0% TS diet (TS0).

Experiment 2) A comparative analysis of the effects of turmeric starch: Five experimental diets, corn starch (CS), HAS, PS, TS, and YS were formulated based on the AIN-93G diet with a 10% RS content except for the CS diet. Animal experiment was conducted using 7-week-old male F344 rats (7 rats/group) for two weeks.

Experiment 3) Anti-inflammatory effect of Turmeric starch: Three experimental diets, CS, TS, and YS were formulated based on the AIN-93G diet with a 10% of RS content except in the CS diet. CS as the negative control, and YS was chosen due to its high ALP and butyrate production in experiment 2. Animal experiment was conducted using 8-week-old male F344 rats (6 rats/group) for three weeks. Rats were assigned into 6 different groups based on DSS treatment and control. After acclimatization, DSS groups were daily treated 4% (w/v) DSS for first 4 days and 3% (w/v) DSS for following 3 days in drinking water. After DSS treatment period test diet was fed for two weeks and daily body weight, water intake and disease activity index (DAI) was measured. At the time of dissection colon length was recorded. Additionally, cecum and colon tissue were collected for the analysis of myeloperoxidase (MPO) and histological score.

４．研究成果

The results of experiment 1 showed that increasing doses of TS resulted in reduced body weight gain and lower visceral tissue weights compared to the TS0 group. These effects may be attributed to the high content of resistant starch in TS, which contributed to lower caloric intake. Colonic fermentation analysis revealed that higher doses of TS resulted in increased short chain fatty acids (SCFA) production, specifically increased cecal acetate content dose-dependently. ALP improves the intestinal barrier function by regulating tight junction protein expression and dephosphorylating lipopolysaccharide of gram-negative bacteria. Furthermore, fermentable non-digestible carbohydrate affects the increase of colonic ALP activity. However, this study did not observe a positive effect of TS on ALP activity. Notably, beneficial bacteria from family *Oscillospiraceae*, *Lachnospiraceae NK4A136* group, and *Ruminococcus spp.* were enriched in the TS fed groups, further supporting its beneficial effects on gut microbiota and SCFA production. Furthermore, these results suggest that TS may have beneficial effects on colonic fermentation in rats.

Table 1 Intestinal ALP Activity (units/mg protein)

Sample	TS0	TS5	TS10	TS20
Cecum	37.7 ± 4.4	39.2 ± 4.6	37.1 ± 4.8	48.0 ± 5.6
Colon	51.3 ± 8.4	55.9 ± 21.9	51.1 ± 9.1	11.9 ± 3.0

mean ± SE, n = 7. Experiment 1).

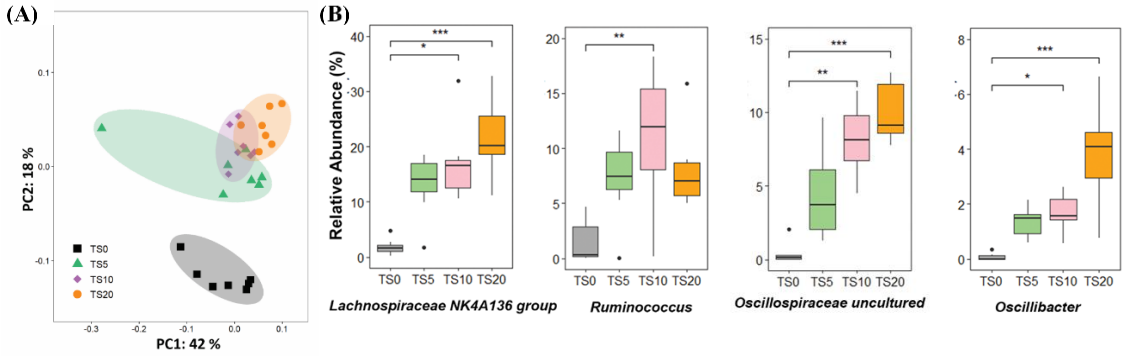

Figure 1. (A) Weighted UniFrac Principle Coordinate Analysis (PCoA) plot for the β-diversity and (B) Box and whisker plot for the relative abundance of selected microbial genera. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ TS0, 0% turmeric starch; TS5, 5% turmeric starch; TS10, 10% turmeric starch; TS20, 20% turmeric starch; n = 7). Experiment 1).

The results of experiment 2 showed that all RS-fed groups (HAS, PS, TS, YS) had significantly lower feed intake than the CS group, but similar feed intake among themselves. Final body weight was consistent across all groups. RS-fed groups had significantly higher cecal content weight and lower visceral fat mass compared to the CS group. The SCFA content in the cecal digesta was significantly higher in the RS-fed groups compared to the CS group. Specially in TS group shows significant higher acetate and propionate content. The cecal pH was significantly lower in HAS and YS groups compared to other groups. Cecal mucin content was significantly highest in the RS-fed groups and TS group shows highest mucin content. Cecal IgA content was significantly highest in the YS group. Starch excretion rate was significantly higher in the PS group compared to all other groups, with minimal starch excretion observed in the HAS and YS groups. ALP activity of the cecum mucosa was not significant among the diet groups, However, in the colon mucosa YS and HAS groups showed significantly higher ALP activity compared to the other diet groups. Interestingly, TS group was not showed significant difference of ALP activity in both cecum and colon, furthermore it was complied with experiment 1. Microbial composition was clearly differentiated between the RS-fed groups and the CS group, indicating that the TS diet had a significant impact on the gut microbial composition in the rats, similar to the other RS diets. At the genus level, relative abundances of genera *Lachnospiraceae NK4A136* group and *Ruminococcus* were significantly higher ($p<0.05$) in the TS group compared to the HAS group. These results suggest that TS may be metabolized at a slower rate by the microbiota, similar to the PS, attributed to the high phosphorus content. Findings of this study suggest that TS might behave similar to other well-characterized prebiotic RS sources such as HAS and PS during colonic fermentation.

Table 2 Resistant starch and phosphorus content of crude starch

Ingredient (g/100g)	CS	HAS	YS	PS	TS
Total starch	83.9	84.8	76.3	81.0	69.7
Resistant starch (RS)	0.79	40.8	57.9	65.4	61.2
RS/Total starch ratio (%)	0.94	48.1	75.9	80.7	87.8
Phosphorous (ppm)	219	168	269	640	1998

Experiment 2).

Table 3 Cecal Parameter (μmol/content)

Parameter	CS	HAS	YS	PS	TS
Acetate	241.8 ± 23.9^{c}	557.4 ± 74.2^{bc}	831.3 ± 105.9^{ab}	684.9 ± 118.7^{ab}	947.7 ± 59.5^{a}
Propionate	31.8 ± 2.32^{c}	93.6 ± 22.4^{ab}	65.9 ± 9.71^{bc}	66.1 ± 11.5^{bc}	128.6 ± 18.4^{a}
n-Butyrate	17.6 ± 1.1^{c}	112.5 ± 29.8^{a}	90.9 ± 19.6^{a}	48.2 ± 7.6^{ab}	81.0 ± 14.8^{ab}
Total-SCFA	291.2 ± 26.3^{b}	763.5 ± 115.6^{a}	988.1 ± 128.1^{a}	799.2 ± 135.1^{a}	1157.2 ± 75.2^{a}
Mucin	21.3 ± 1.9^{b}	75.6 ± 12.1^{a}	87.0 ± 11.1^{a}	105.6 ± 18.4^{a}	118.8 ± 6.9^{a}
IgA	1.00 ± 0.13^{c}	7.38 ± 1.02^{ab}	9.09 ± 2.09^{a}	4.17 ± 1.08^{abc}	3.83 ± 1.13^{bc}
Ammonia	1.22 ± 0.07	2.27 ± 0.36	2.46 ± 0.50	2.04 ± 0.27	2.12 ± 0.44
Cecal pH	7.43 ± 0.04^{a}	5.77 ± 0.09^{b}	5.88 ± 0.22^{b}	6.77 ± 0.28^{a}	6.85 ± 0.17^{a}
Cecal digesta (g)	2.21 ± 0.12^{b}	8.94 ± 1.23^{a}	9.59 ± 1.31^{a}	8.21 ± 1.38^{a}	12.1 ± 0.6^{a}

mean ± SE, $n = 7$. $^{a\text{-}c}p < 0.05$ by Tukey's test. Experiment 2).

Table 4 Intestinal ALP Activity (units/mg protein)

	CS	HAS	YS	PS	TS
Cecum	9.5±1.9	20.7±3.7	21.0±1.6	15.6±3.3	9.1±1.1
Colon	8.5 ± 1.1^{b}	28.8 ± 4.9^{a}	29.6 ± 7.1^{a}	11.0 ± 1.8^{ab}	13.7 ± 2.5^{ab}

mean ± SE, $n = 7$. $^{a\text{-}c}p < 0.05$ by Tukey's test. Experiment 2).

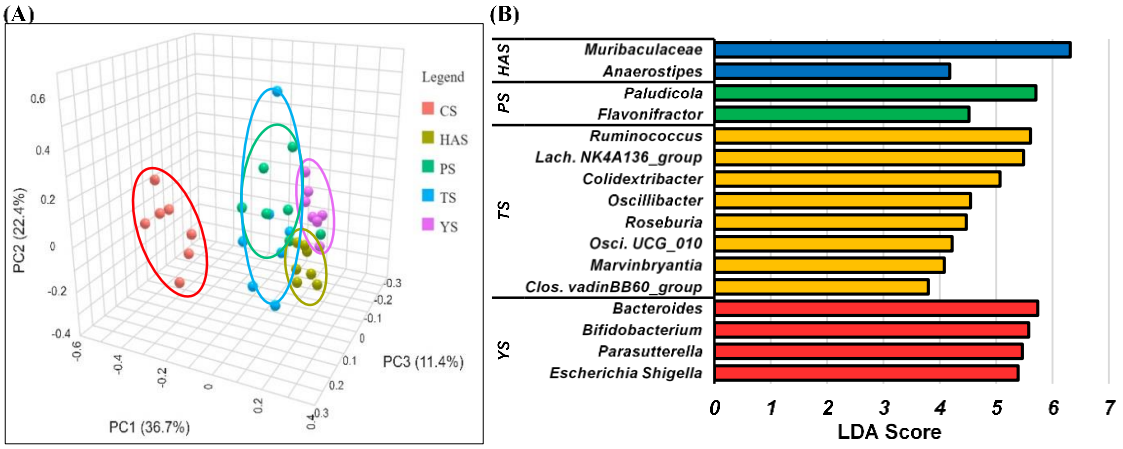

Figure 2. (A) Bray-Curtis Principle Coordinate Analysis (PCoA) plot for the β-diversity and (B) Linear discriminant analysis Effect Size (LEfSe) plot for the microbial genera. (CS – Corn starch, HAS – High amylase starch, PS – Potato starch, YS- Yam starch TS - Turmeric starch; $n = 7$). Experiment 2).

The results of experiment 3 showed that the DAI saw a significant increase during the DSS administration period ($p<0.05$), confirming the induction of colitis. However, during the test diet period, the DAI was drastically reduced and remained unchanged after the 8th day, suggesting the onset of natural recovery. Furthermore, the colon length, MPO activity, and histological scores did not show significant differences among all experimental groups, further confirming this observation.

Turmeric starch has been observed to influence the intestinal environment of rats, functioning as a resistant starch. Furthermore, turmeric starch was found to suppress fat accumulation, reflecting the effects observed in the PS, HAS, and YS diet groups. It triggers increased SCFA production, particularly acetate and propionate production. While there was no significant difference in the ALP activity in the cecum and colon of the turmeric starch group as compared to the control group. This suggests that turmeric starch may have potential benefits for gut health. However, further experiments are needed with adjusted DSS concentration or test diet period to evaluate the intestinal anti-inflammatory effect of the turmeric starch. This will provide a more comprehensive understanding

of the potential health benefits of turmeric starch.

Table 5 Criteria for disease activity index

Score	Disease Activity Index (DAI)		
	Weight Loss (%)	Stool Consistency	Bleeding
0	No loss	Well-formed pellets	No
1	1-5		
2	5-10	Pasty and semi-formed stools	Blood in feces
3	10-15		
4	>15	Liquid stools	Gross bleeding
DAI	Sum of all three components		

Experiment 3).

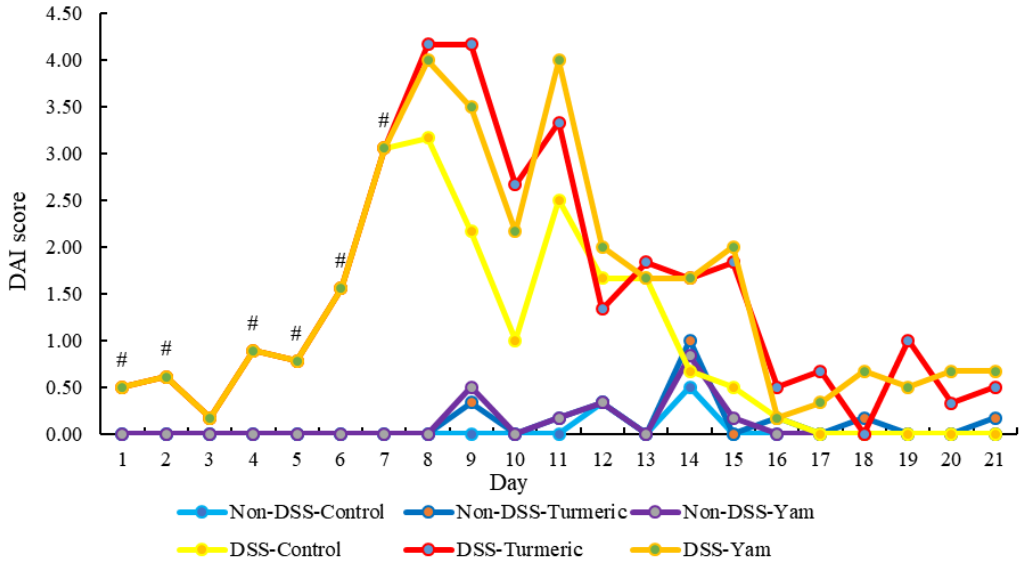

Figure 3. Disease activity index throughout the experimental period. Experiment 3).

５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計2件（うち招待講演　0件／うち国際学会　0件）

１．発表者名 Asanka Ekanayake，石井良汰，上田莉帆，Samanthi Pelpolage，永田龍次，福間直希，島田謙一郎，韓圭鎬，福島道広
２．発表標題 ウコンでんぷんの摂取がラットの脂質代謝および腸内環境に与える影響
３．学会等名 日本食物繊維学会
４．発表年 2022年

１．発表者名 E. M. A. C. Ekanayake, Seiki Deguchi, Ryuji Nagata, Kenichiro Shimada, Kyu-Ho Han, Michihiro Fukushima
２．発表標題 A Comparative Analysis of the Effects of Turmeric Starch on Intestinal Environment in Rats
３．学会等名 日本食物繊維学会
４．発表年 2023年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関