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Minnesota Cultivated Wild Rice

Protein Quality, Effect on the Gut Microbiome, and Hardening When Cooked with a Sweetener

Final Report
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Introduction

Wild rice (Zizania sp.) is an annual cross-pollinated species that grows natively in the northern part of the Midwest region of the United States (Minnesota, Wisconsin, and Michigan primarily). The grain of cultivated wild rice is somewhat like the grain of white rice (Oryza sativa) though it is longer and its color after processing is between black and brown. After harvesting, wild rice is dried, parched, winnowed, milled, and treaded.

Although the literature about the phytochemical content of wild rice has been reviewed, there are major gaps in the research team's understanding of some of its important nutrition characteristics [1]. One of these gaps is an understanding of the protein quality of wild rice.

Wild rice contains approximately 13-15% protein, high among cereals, and similar to hard red spring wheat. However, little is known about the quality of protein in wild rice. Using an older method to analyze protein quality, the protein efficiency ratio (PER), wild rice was determined to have a high protein quality, higher than wheat and similar to oats [2]. However, PER has limitations which make it no longer an acceptable method for determining protein quality. Currently, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) recommended method for determining food protein quality is the protein digestibility-corrected amino acid scored (PDCAAS) assay [3]. The protein quality of wild rice has not been determined using the PDCAAS assay.

Another important nutritional characteristic of foods is how they influence the large intestinal bacterial population, often referred to as the gut microbiome. A large body of literature now demonstrates that specific patterns of bacterial abundance are associated with several disease conditions, including obesity, fatty liver, control of blood glucose, and possibly other adverse health conditions. Thus, analyzing the microbiome of animals fed cultivated wild rice would be of great interest in terms of understanding potential health benefits.

Additionally, cultivated wild rice may have great utility as a food ingredient. However, an impediment to the use of wild rice in food products is that wild rice hardens within a short time after cooking with a sweetener, such as sugar. Scientists do not understand this hardening phenomenon. Gaining an understanding of why it occurs is important, as it limits the ability to use wild rice as an ingredient in sweetened foods such as energy bars.

Objectives

There were three objectives to this study, as follows:

1. To determine the protein quality of cultivated wild rice, compared to brown rice, using the protein digestibility-corrected amino acid score (PDCAAS) method.
2. To determine the large intestinal microbial profile (i.e., the gut microbiome) in rats fed cultivated wild rice or brown rice.
3. To investigate the hardening of wild rice when cooked with a sweetener (sugar).

Methods

Measurement of protein quality

The PDCAAS evaluation was carried out as described by others [3-5]. Briefly, there were four groups of male Sprague-Dawley rats, with an initial body weight of 55-66 g. Researchers fed the rats diets containing one of two rice flours (cultivated wild rice or brown rice) and a casein control, or a protein-free control. There were eight rats in each of the rice flour groups and casein control group, and four rats in the protein-free control group. Each rat was offered 15 g/d of their respective diets for nine days. The research team then collected feces quantitatively from each rat for the last five days. At the end of the study, the five-day fecal collection for each rat was composited, dried, and analyzed for nitrogen. Then, the team calculated the true protein digestibility (TD) for casein and the rice flours (test groups) by using the equation below:

$$TD = \frac{I - (F - F_k)}{I} X\ 100$$

Where I= intake of dietary nitrogen (g) for the test and casein control groups, F= fecal nitrogen (g) from the test and casein control groups, and F_k= endogenous fecal nitrogen from the protein-free group.

F_k is calculated by the equation below:

$$F_k = total\ diet\ consumed\ by\ test\ protein\ group\ (g) \times \frac{mg\ of\ fecal\ nitrogen\ for\ the\ protein-free\ group}{g\ of\ diet\ consumed\ by\ the\ protein-free\ group}$$

PDCAAS is then calculated as follows:

$$PDCAAS = amino\ acid\ score\ \times true\ digestibility$$

The amino acid score is calculated as the first limiting essential amino acid of the protein, relative to the essential amino acid requirements of a reference pattern. The reference pattern is based on the essential amino acid requirements for preschool children aged one to three years, as published in Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (2005).

Researchers analyzed differences in PDCAAS by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test using the Statistical Analysis System (SAS), version 9.14.

Measurement of the microbiome

Upon completion of the evaluation of protein quality, the rats in the casein control, cultivated wild rice flour, and brown rice flour groups continued consuming their diets for eight more weeks. After this time, the rats were euthanized, and researchers collected cecal contents (first part of the large intestine) and flash froze on dry ice and stored at -80° C until ready for use. Samples were thawed on ice and mixed prior to use. DNA was extracted from approximately 180-220 mg of the samples using the Qiagen Stool Extraction kit and DNA eluates quantified on a DU 730 Life Science UV/Vis Spec (Beckman Coulter). DNA eluates were then submitted to the University of Minnesota Genomics Center for sequencing of the 16S ribosomal subunit with the V5/V6 region amplicon on an Illumina MiSeq using 2x300 bp paired end reads. Microbiome

data were analyzed in DADA2 with R plugins for alpha diversity, beta diversity, taxonomical abundance, and statistical analyses. Microbiome analysis provides details on changes in alpha- and beta-diversity of the gut microbial populations as well as changes in taxonomy.

Hardening of wild rice by sugar

One cultivated wild rice variety was used for this study. The Minnesota Cultivated Wild Rice Council provided the wild rice used for this experiment. Long grain white rice was purchased from the supermarket and compared with the wild rice. This project analyzed the characteristics of extracted starches from wild rice compared to long grain white rice. At the recommendation of the Council, three to four cups of boiling water were added to one cup of cultivated wild rice and returned to boil with stirring. The wild rice simmered for 60 minutes and then excess water was drained. After draining, the cooked rice cooled to room temperature. White rice was cooked in a rice cooker. One (1) portion of the white rice was added to one-and-a-half portions of water and cooked for 25 min and allowed to cool to room temperature. For both rice types, 20% sucrose was added and mixed after they were cooled to room temperature. Textural profile analysis (TPA) of the cooked rice was conducted using a texture analyzer (TA Exponent 32 Stable Micro Systems version 5.0.3.0. Texture Technologies Corp., Scarsdale, New York, USA) with a 5 kg load cell and a two-cycle compression according to the procedure by Mohapatra and Bal [6]. The parameter recorded was hardness. Volume expansion and water uptake ratios were also determined.

The team then extracted starches from raw rice kernels and characterized them for their thermal properties, unit chain profiles and pasting characteristics.

Results

Section 1. Protein quality, health benefits, and effects on the microbiome

Protein quality

Protein quality, shown as protein digestibility-corrected amino acid scored (PDCAAS) values is shown in Table 1 below.

Table 1. *Protein quality of wild rice and brown rice, determined in this study, and of other plant protein sources.*

Protein Source	PDCAAS [ref]	Protein Content (%, as is basis)
Wild rice, cooked and dried (present study)	**0.60 ± 0.01**	**13.0***
Brown rice, cooked and dried (present study)	**0.61 ± 0.02**	**7.96***
Wheat, hard red spring	0.41 [3]	15.4
Wheat, soft white	0.50 [7]	10.7 [7]
Oats, Regular and Quick	0.49 [8], 0.67 [9], 0.57 [3]	13.2
Yellow or White Corn	0.60 [10]	9.42
Brown rice	0.53 [11]	7.54
Barley, pearled	0.44 [12]	9.91
Red kidney beans	0.65 [9], 0.68 [3]	25.9
Lentils, Pink or Red	0.52 [3]	25.93
Peas, green	0.68 [3], 0.61 [3]	23.1
Yellow peas, cooked	0.69 [13]	22.9 [13]
Green peas, cooked	0.72 [13]	23.9 [13]
Black beans	0.53 [3]	21.6
Chickpea Flour	0.66 [3]	22.4
Pinto beans	0.57 [3]	21.4

*Values determined by Medallion laboratories. All other values for protein content are from the USDA Food Composition database, except where otherwise indicated.

The PDCAAS value for brown rice was equivalent to the PDCAAS for wild rice. Both wild rice and brown rice had good PDCAAS scores for plant protein sources. Wild and brown rice had better protein qualities than several other cereals, such as wheat and barley, and possibly oats. Brown and wild rice also had better PDCAAS scores compared to several legumes.

PDCAAS is the product of the amino acid score and the protein digestibility values. These values are shown in Table 2 below.

Table 2. *Limiting amino acid, amino acid score, and protein digestibility of wild rice and brown rice.*

Sample	Limiting amino acid	Amino acid score	Protein digestibility
Wild rice	Lysine	0.716	83.70 ± 1.79*
Brown rice	Lysine	0.780	77.67 ± 1.97

* Significantly different from brown rice, $p<0.001$. N=8 for each group.

Lysine is the limiting amino acid in both wild rice and brown rice, as expected from reports in the literature, and as found, generally, with cereals. The amino acid score of wild rice is slightly less than that of brown rice, but its protein digestibility is somewhat greater, a difference that was statistically significant.

Potential health benefits of wild rice

After the five-day feeding trial to determine protein digestibility, the rats were placed on high fat diets containing either casein, wild rice, or brown rice. Wild rice and brown rice diets contained 40% rice by weight. Researchers matched the diets for protein, digestible carbohydrate, fat, and dietary fiber. The diet composition and nutrient composition is in Table 3. Rats were fed these diets for eight weeks, then euthanized and tissues and other samples collected.

Rats fed wild rice or brown rice gained the same amount of weight as the casein control fed rats (Fig. 1). There were no differences among the groups in average daily food intake (Fig. 2).

Liver weight decreased significantly in groups fed wild rice and brown rice (Fig. 3). Reductions in liver weight often occur when liver lipids decline (see figures 5 & 6). There was no observable change in the weights of epididymal fat pads among the groups (Fig. 4). Since epididymal fat pad weight correlates highly with total body fat (Abernathy & Gallaher, unpublished), this indicates neither wild rice nor brown rice caused reductions in body fat.

Both wild rice and brown rice reduced total liver cholesterol (Fig. 5) and total liver lipids (Fig. 6). Although researchers noted reductions in total liver cholesterol by wild rice previously, this is the first report the team is aware of where wild rice reduces liver lipids. LECT2 is a serum protein which reportedly correlates with liver fat. Although LECT2 serum concentrations did not differ statistically in this study (Fig. 7), it showed the same pattern as liver fat.

Table 3. *Diet composition for 8-week study of health benefits of wild and brown rice*

Nutrient (g/kg)	AIN-93G (20% protein)	Wild Rice	Brown Rice
Sucrose	100	100	100
Corn Starch	176.486	18.5	2.7
Maltodextrin	172	0	0
Lard	187.5	184.5	179.1
Soybean Oil	62.5	61.5	59.7
Casein	200	148	168.2
Cellulose	50	36	38.8
Mineral Mix	35	35	35
Vitamin Mix	10	10	10
L-Cystine	3	3	3
Choline bitartrate	2.5	2.5	2.5
BHT	0.014	0.014	0.014
Cholesterol	1	1	1
Wild Rice	0	400	0
Brown Rice	0	0	400
Total Weight	1000	1000.014	1000.014
Nutrient Composition			
% Carbohydrates	45%	41.97%	40.47%
% Lipids	25%	25.00%	25.00%
% Protein	20%	20.00%	20.00%
% Fiber	5.00%	5.00%	5.00%
% Sum	95%	91.97%	90.47%
kcal/g	4.919	4.804	4.744

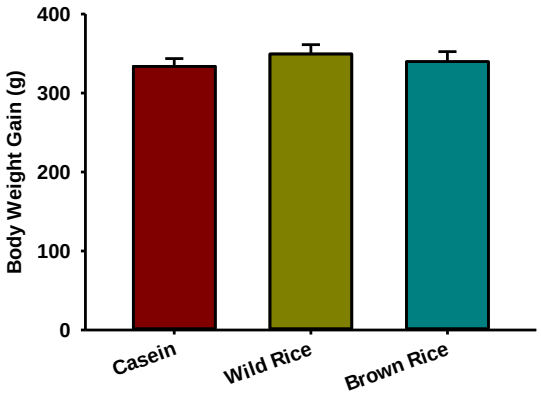

Figure 1. Body weight gain.

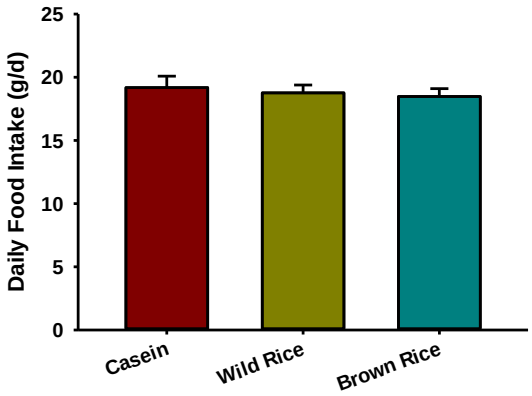

Figure 2. Daily average food intake

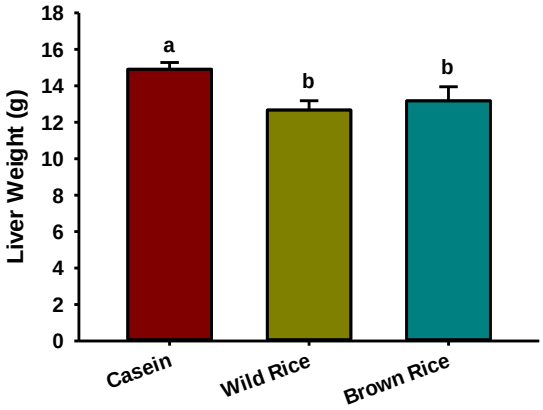

Figure 3. Liver weight

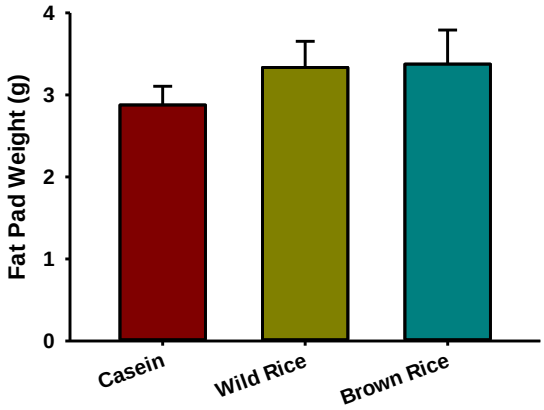

Figure 4. Epididymal fat pad weight

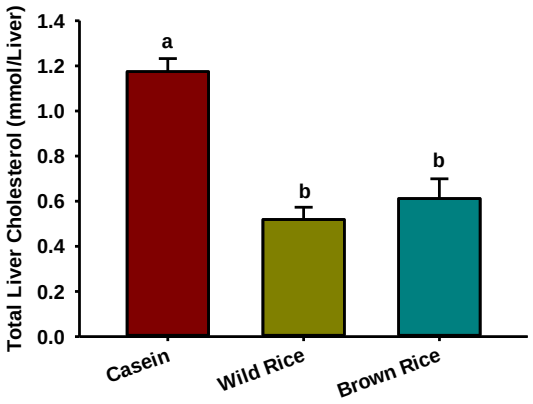

Figure 5. Total liver cholesterol

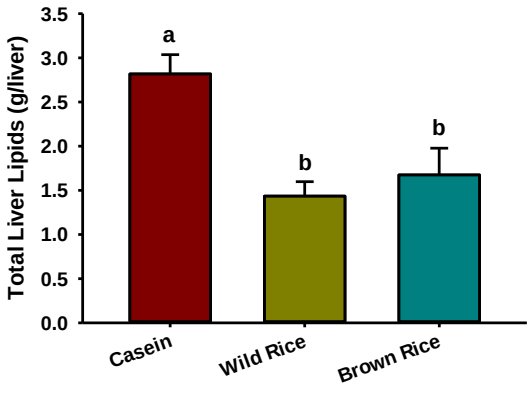

Figure 6. Total liver lipids

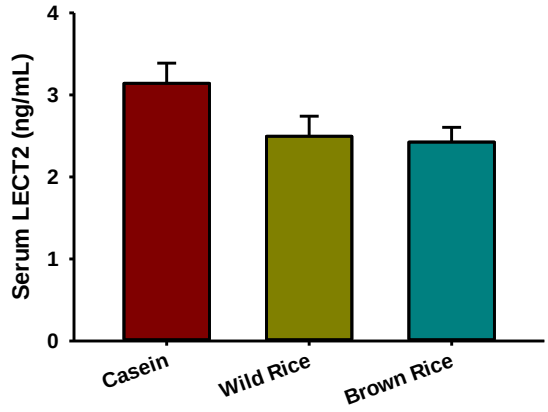

Figure 7. Serum LECT2 concentration

Microbiome analysis

Bacterial DNA was extracted from the cecal contents. The cecum is the first section of the large intestine of the rat and is the site of the most active fermentation of undigested diet that enters the large intestine. After determining the DNA extracts were of sufficient quality for sequencing, researchers sent them to the University of Minnesota Genomics Center (UMGC) for sequencing. Researchers completed an initial analysis of this sequence data.

The team examined beta diversity of the groups. Beta diversity describes the difference in bacterial composition between samples. The project team employed a commonly used method called Bray-Curtis dissimilarity, shown in figure 8. The wild rice samples (captured by the pink circle) were tightly associated with each other and were different than the casein control samples (orange circle). This means the microbiome of the wild rice group is different than the microbiome of the casein group. In contrast, the brown rice samples (green circle) were highly scattered and did not differ from the casein group. Thus, the microbiome of the brown rice group did not differ from the casein group.

The relative abundance of the different bacterial taxonomic groups is available in figure 9 as a heatmap. Each rectangle represents a single animal's microbiome. The color of the rectangle indicates the abundance of the bacteria shown on the vertical (Y) axis. Heatmaps are complex. However, the one bacteria worth focusing on is lactobacillus (see arrow), which is considered a probiotic bacteria. Thus, a greater abundance of lactobacillus is a very positive finding.

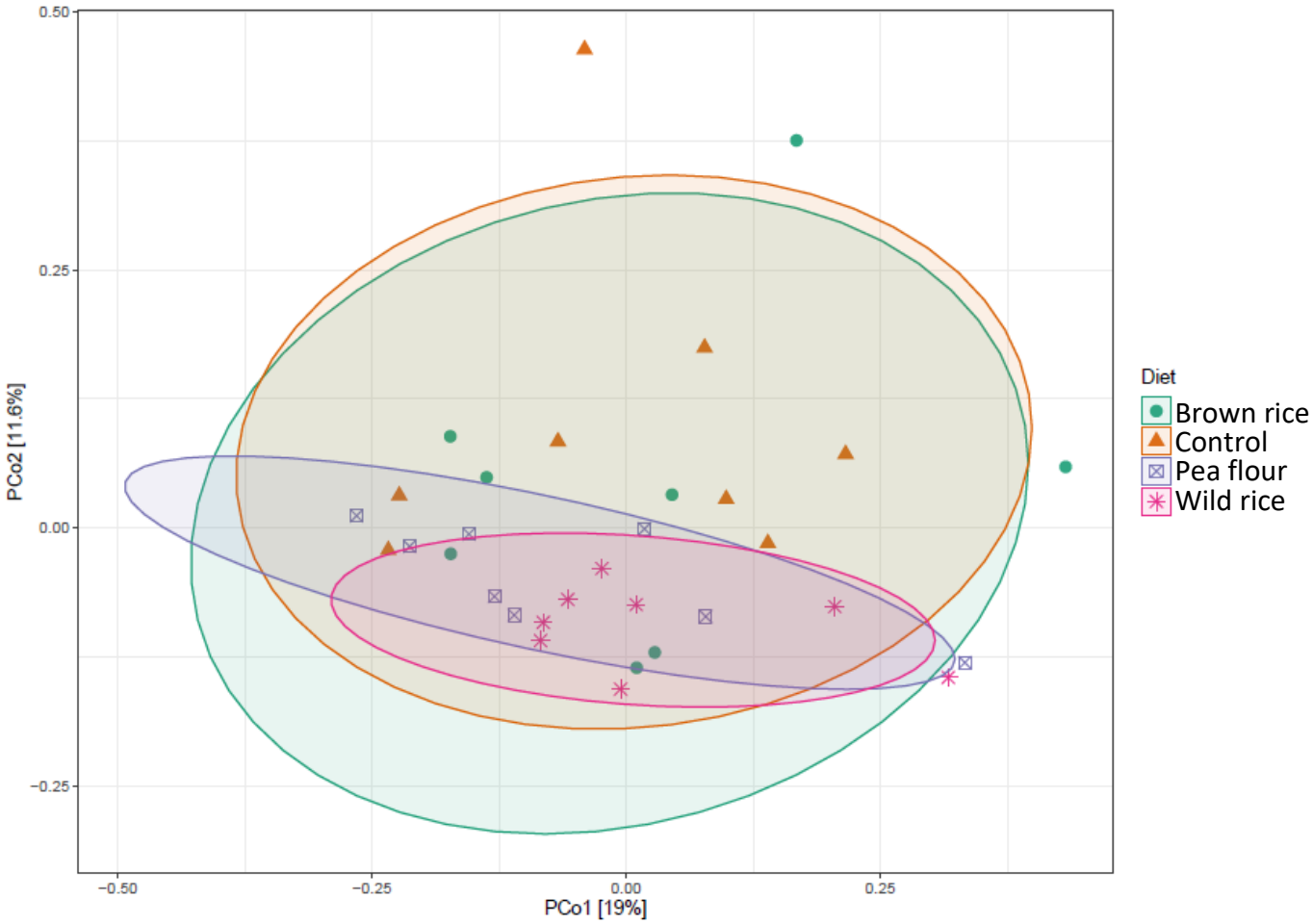

Figure 8. Bray-Curtis dissimilarity of microbial composition in rats fed a control diet, brown rice, or wild rice.

We will be continuing our analysis of the microbiome results and will keep the Minnesota Cultivated Wild Rice Council updated as to our findings.

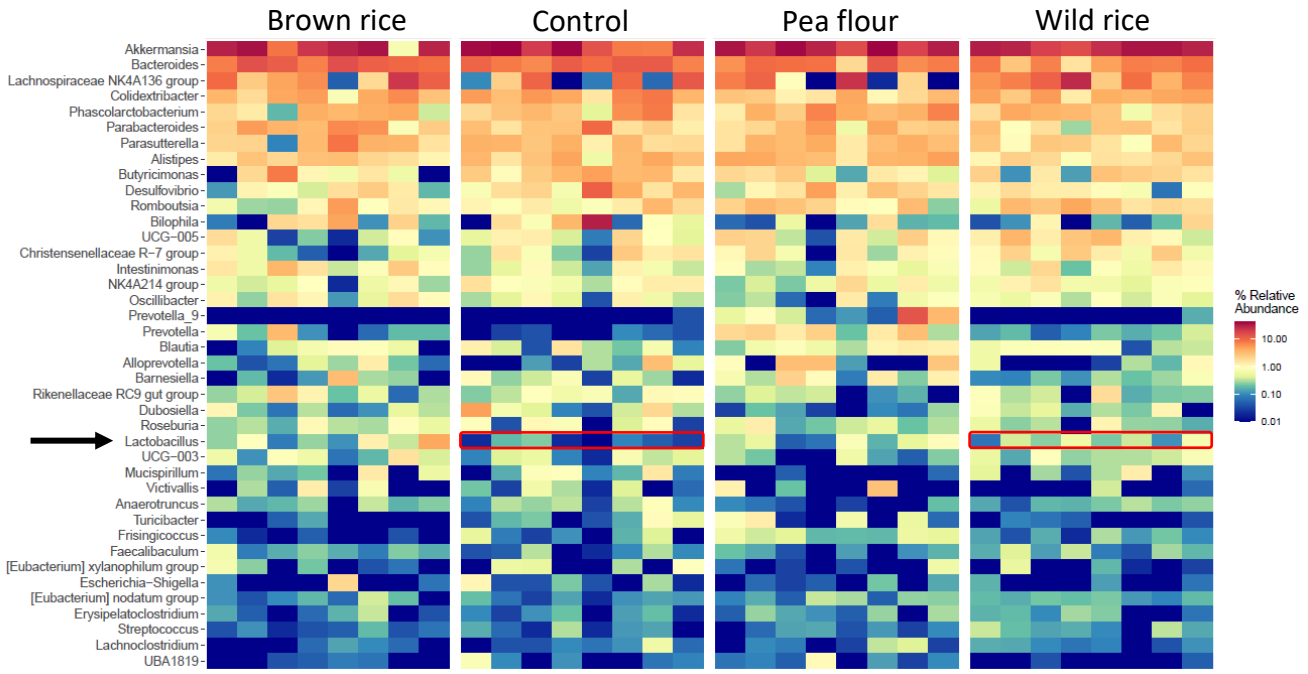

Figure 9. Heatmap indicating relative abundances of different bacteria in the cecal contents of rats fed the control diet, brown rice diet, or wild rice diet.

Section 2. Hardening of Wild Rice When Cooked with a Sweetener

This part of the project investigated the hardening of wild rice observed when cooked with a sweetener such as sucrose. Figure 1 and 2 shows the cooked wild rice without and with 20% sugar added.

Figure 1. Cooked wild rice

Figure 2. Cooked wild rice with 20% sucrose

As seen from the pictures, the wild rice with the 20% sugar mixed into it had a shiny surface compared to the wild rice with no sucrose added. The cooked wild and white rice were divided into 2 parts with one part kept on a tray (Figure 3) and the other part put in sealed Ziplock bags (Figure 4) stored overnight at room temperature.

Figure 3. Cooked rice samples on a tray

Figure 4. Cooked rice samples in sealed Ziplock bags

The Ziplock bags reduced the loss of moisture during overnight storage of the samples. The hardness of rice samples with and without sucrose were determined immediately after the addition of the sucrose and after being kept at room temperature overnight on a tray (Figure 3) and in Ziplock bags (Figure 4). Researchers measured the hardness as the force needed to cut

through the cooked rice samples. Researchers took measurements on five rice grains lined together and done in triplicates. As shown in Figure 5, the cooked wild rice samples were generally harder than the white rice. Before the addition of sucrose, the cooked wild rice was seven times harder than the white rice. This difference could come from the differences in the cooking methods used. The different cooking methods may also be the reason for the differences in the weight and volume change of the rice samples after cooking. As seen in table 1, the weight and the volume changes observed for the white rice sample was about twice that observed for the wild rice. This means the white rice absorbed about twice as much water than the wild rice. The hardness of the rice samples measured immediately after the addition of 20% sucrose was similar to that of the rice samples without the addition of sucrose. However, after storing the samples overnight on the trays, significant increases in the hardness were observed for both the wild rice and the white rice.

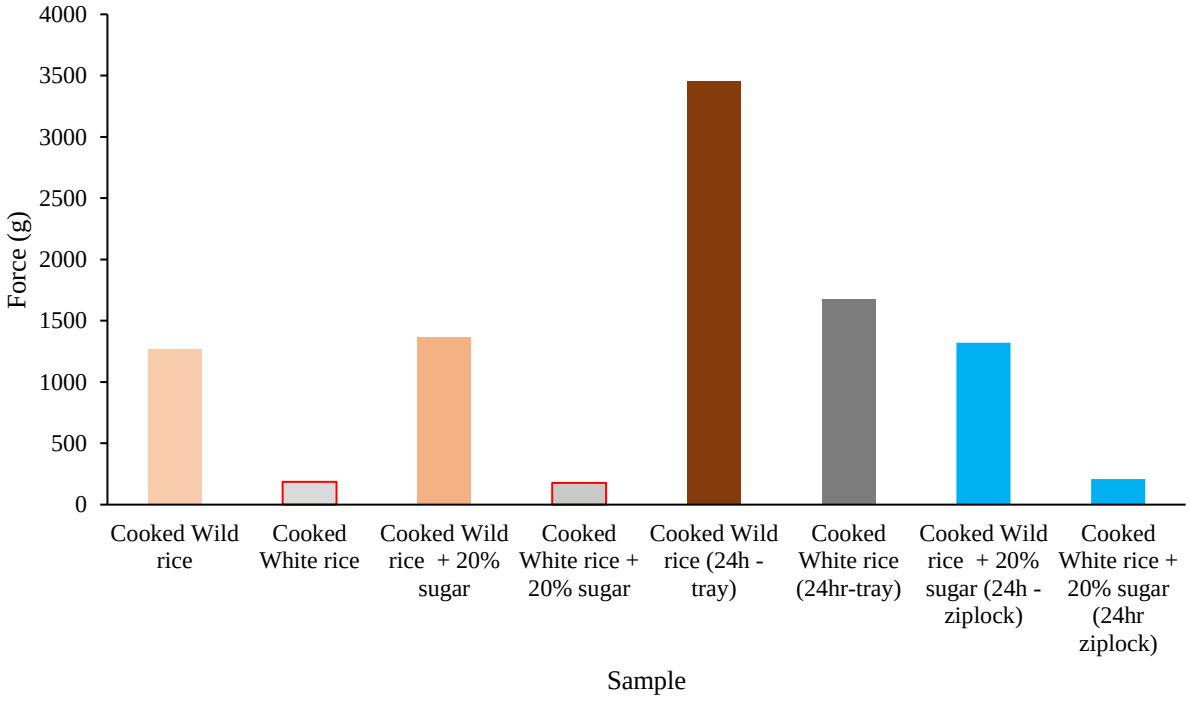

Figure 5. Changes in the hardness of wild and white rice with and without 20% sucrose

Table 1. Changes in the weight and volume of cooked rice samples

Sample	Weight change (g)	Volume change (mL)
White rice	169	480
Wild rice	84.3	2.5

When held overnight, the wild rice sample became significantly harder than the white rice after the addition of the 20% sucrose. While the white rice with 20% sucrose added needed a force of 1680 g to cut through the grains, the wild rice with 20% sucrose needed as much as 3450 g. It is interesting to note that the hardness of the white rice samples with 20% sucrose stored overnight was comparable to the hardness of the freshly cooked wild rice. When storing the rice samples in Ziplock bags to reduce the loss of moisture, both samples maintained their hardness overnight even when 20% sucrose was added to both. This observation shows the importance of controlling moisture loss to prevent the rice samples from becoming hard overnight. This means that for food products having wild rice and sucrose as ingredients, moisture control is critical. Packages that will prevent or significantly reduce the loss of moisture should be recommended for such products.

This study also investigated the cooked pasting properties of the wild rice flour and their extracted starch using the Brabender Micro Visco Amylograph. An alkaline buffer was used to extract the starches. In these tests, 15% starch or flour slurries were made and heated to 95^{o}C, kept at 95^{o}C for 15 min and then cooled down to 50^{o}C at a rate of 7.5^{o}C per minute. Figure 6 and table 2 shows the pasting profiles of the wild rice flour and starch samples in comparison to white rice.

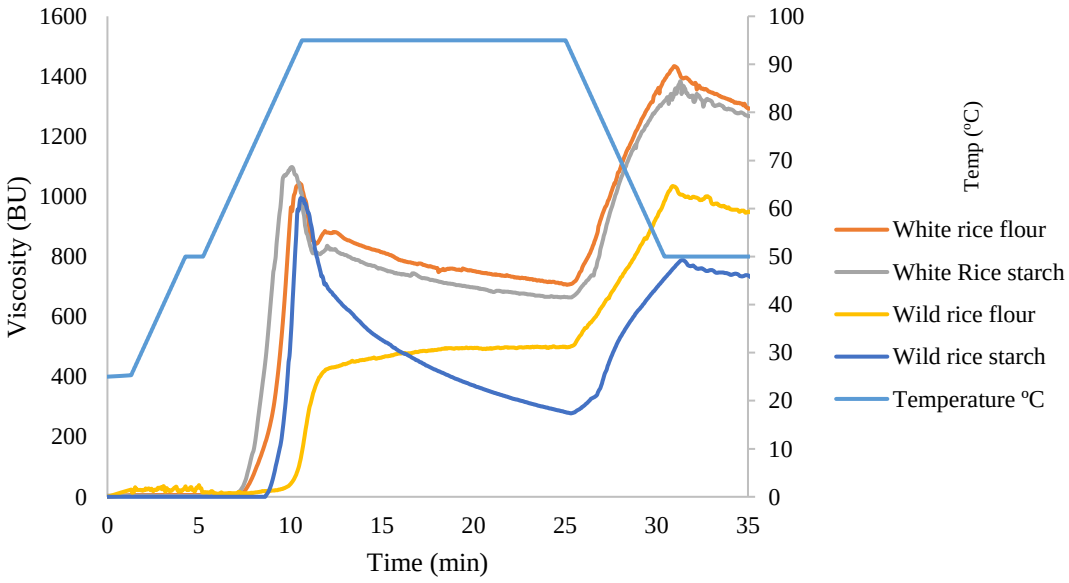

Figure 6. Starch pasting profiles of wild rice and white rice flour and starches.

Wild rice flour and starch has a much higher pasting temperature when compared to white rice (table 2). This means the starches in wild rice cook at a much higher temperature compared to white rice. The slurry from wild rice flour was also not as viscous (peak viscosity as seen in table 2) as that of the starch in white rice. This means wild rice flour is usable for food applications where a lower thickness is needed compared to white rice. The thickness of the wild rice flour was also very stable (breakdown on table 2) when heated. This makes it ideal for foods where the same viscosity is necessary during processing. The wild rice starch on the other had similar peak viscosity but its viscosity decreased significantly during heating. When cooled, wild rice flour and starch had lower viscosities than white rice. This means they underwent less retrogradation compared to white rice. Table 3 shows the thermal properties of the wild rice and white rice flours after the addition of 20% sucrose as well as their extracted starches. The thermal property was determined using the Differential Scanning Calorimeter (DSC). This measures the temperature at which the molecules of uncooked starches melt, and the energy needed to melt these molecules [indicated as Normalized (Jg^{-1}) in table 3]. In theory, if starch is gelatinized or cooked, there should not be any gelatinization temperatures and no energy should be expended in the DSC. Table 3 shows all starches in the white rice samples were gelatinized after they were cooked in the rice cooker. That cannot be said for the wild rice samples. These samples still had ungelatinized starches even after simmering in boiling water for 60 min. This observation explains the increased hardness of the cooked wild rice when left overnight. Therefore, the research team recommends a cooking protocol ensuring the complete gelatinization of the starches in the wild rice be investigated. Table 3 also indicated the addition of the 20% sucrose increased the onset and end set temperatures of gelatinization.

Table 2. Pasting parameter of wild rice and white rice flour and extracted starch

Sample	Pasting temperature (°C)	Peak Viscosity (BU)	Breakdown (BU)	Setback (BU)	Final Viscosity (BU)
White rice flour	67.1	1347	466	709	1590
White rice starch	67.1	1347	466	709	1590
Wild rice Flour	80.2	526	1	425	945.5

Table 3. Thermal properties of wild rice flour and starches

Sample	Onset (°C)	Peak (°C)	End set (°C)	Normalized (Jg^{-1})
Wild rice flour	69.3	74.2	78.7	2.0
Wild rice starch	66.0	70.7	74.9	1.8
White rice flour	61.8	66.8	72.3	2.2
White rice starch	62.8	67.8	72.6	3.2
Wild rice without sugar 1st day	69.7	74.3	78.4	1.6
Wild rice without sugar 2nd day	70.0	74.4	78.4	1.3
Wild rice 20% sugar 1st day	70.1	75.0	79.5	2.0
Wild rice 20% sugar 2nd day	70.2	74.8	79.3	1.6
White rice without sugar 1st day	-	-	-	-
White rice without sugar 2nd day	-	-	-	-
White rice 20% sugar 1st day	-	-	-	-
White rice 20% sugar 2nd day	-	-	-	-

The last part of the study involved the characterization of amylopectin of white and wild rice. For this part, amylopectin was fractionated from the extracted starches of both white and wild rice, hydrolyzed with starch debranching enzymes and their unit chain profiles determined using a High-Performance anionic exchange chromatographic system. The unit chain profiles of the amylopectin of the wild rice and white rice samples are shown in Figure 7.

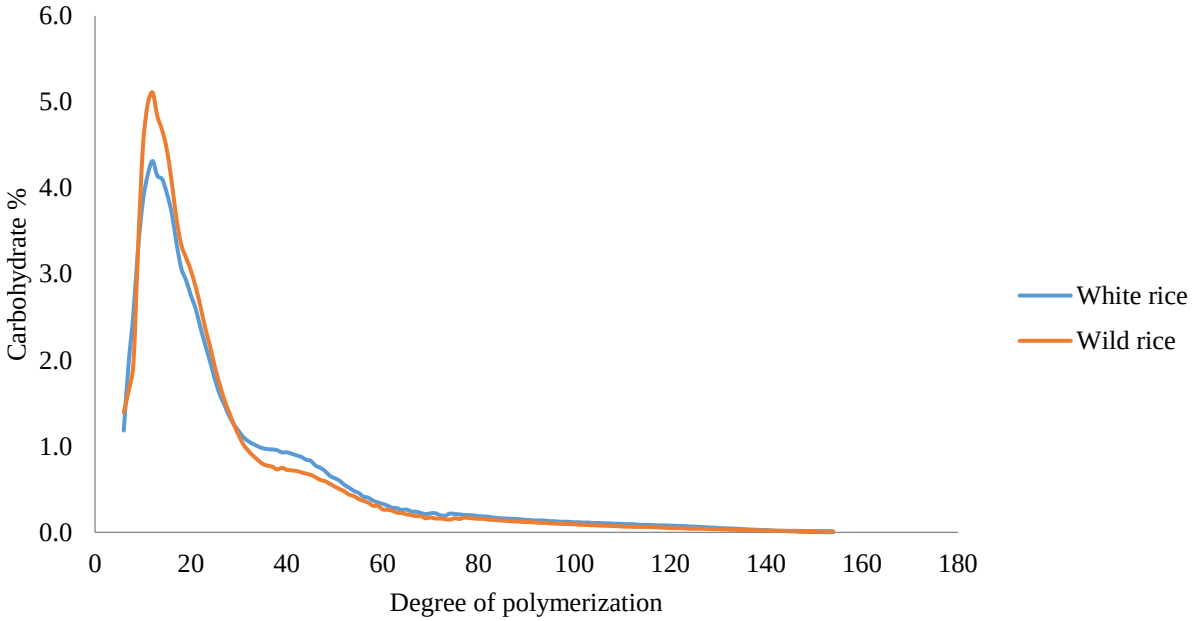

Figure 7. Unit chain profiles of amylopectin from wild rice and white rice starches

The unit chain results showed the amylopectin from white rice had longer chains (DP of 18.4) than that of wild rice (DP of 17.5). Though this difference seems small, it can significantly impact the properties of the starches from the various sources. Figure 8 also shows wild rice starches have more short chains than long chains. These short chains with DP up to 36 are known to contribute to the crystalline structure of starches. This means wild rice has more crystals in its amylopectin structure when compared to white rice. This is also evident from the Normalized (Jg^{-1}) values obtained from the DSC where more energy was necessary to melt the crystals in wild rice starches.

In conclusion, observations include moisture control playing a significant role in maintaining the hardness of cooked wild rice with a sweetener (sucrose) when stored overnight. This means the use of packing systems which serve as a barrier to moisture loss in products containing cooked wild rice and a sweetener is necessary. Another observation was the cooking protocol recommended by the wild rice council did not result in the complete gelatinization of the starches in the wild rice. In other words, the cooking protocol did not lead to the complete cooking of the wild rice. This may also cause further hardening of the cooked wild rice when a

sweetener is added. The amylopectin of wild rice starch seems to be structurally different from the amylopectin of white rice, which may explain the differences in the response to added sugar, as observed in this research.

Summary and Conclusions

- The protein quality of wild rice is particularly good for a plant protein. Wild rice has a high protein digestibility, higher than brown rice. However, its somewhat lower value for the limiting amino acid lysine, resulting in a lower amino acid score, leads to it having an equivalent PDCAAS to brown rice.
- In the context of a high fat diet, feeding wild rice greatly reduces cholesterol and total fat in the liver of rats. Although the team previously found that feeding wild rice lowers liver cholesterol, the finding that it lowers liver fat is new and highly significant. Excess liver fat is the early stage of non-alcoholic fatty liver disease (NAFLD). NAFLD is a serious and growing health issue for humans, for which there is no drug treatment. Thus, wild rice may represent a treatment for NAFLD.
- Analysis of the effect of wild rice on the microbiome indicates the microbiome of rats fed wild rice differs from that of rats fed the casein control diet. Further, rats fed wild rice have a greater abundance of lactobacillus, a probiotic bacteria, than rats fed the casein diet. Thus, our preliminary analysis indicates wild rice has a positive influence on the microbiome.
- Moisture loss appears to be a major reason for the hardening of wild rice in the presence of sucrose over time. Packaging systems that reduce moisture loss will likely be necessary in food products containing both wild rice and sucrose.
- Incomplete cooking of wild rice, leading to incomplete starch gelatinization, may contribute to the hardening of wild rice in the presence of sucrose. Thus, cooking protocols that completely gelatinize the starch in wild rice may also help reduce hardening in the presence of sucrose.

Acknowledgement

The authors wish to thank Lolly Occhino of the Agricultural Utilization Research Institute for her careful reading of this report and many helpful comments.

Appendix

Wild Rice as a Good Source of Protein Labeling Claim

The reference amounts customarily consumed (RACC) per eating occasion for grains, plain, including rice, is set to 140 g prepared or 45 g dry (i.e., uncooked) [14].

To make a “good source” claim for protein, a food must contain 10 to 19 percent of the RDI or the Daily Reference Value (DRV) per RACC [15].

For protein, a value of 50 grams of protein is the DRV for adults and children 4 or more years [16].

The “corrected amount of protein (gram) per serving” is the actual amount of protein per serving multiplied by the amino acid score corrected for protein digestibility (i.e., the PDCAAS value) [16].

Our wild rice sample (cooked and dried) was found to contain 13.0% protein on an as is basis. Thus, a RACC of 45 g would provide 5.85 g of protein in a RACC serving. Multiplying by the wild rice PDCAAS value of 0.60 gives a corrected amount of protein per RACC serving of **3.51 g**.

As the DRV for protein is 50 g and a claim as a ‘good source of protein’ requires that a food must provide at least 10% protein in the RACC, this would mean a serving of wild rice would need to provide **5 g** of protein per RACC.

Thus, based on our analysis of the protein content of wild rice (determined by a commercial laboratory) and our measurement of the PDCAAS, wild rice does not provide a sufficient corrected amount of protein in a reference amount customarily consumed to meet the requirement for a claim of a good source of protein.

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