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# Potential Health Benefits of Wild Rice and Wild Rice Products: Literature Review

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# TABLE OF CONTENTS

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<b>INTRODUCTION .....</b>	<b>4</b>
<b>PHYTOCHEMICALS IN WILD RICE INCLUDING A LIST OF PHYTOCHEMICALS WITH POTENTIAL HEALTH BENEFITS.....</b>	<b>4</b>
DEFINITION OF PHYTOCHEMICALS.....	4
PRIMARY METABOLITES FROM WILD RICE AFFECTING THE NUTRITIONAL QUALITY OF WILD RICE .....	5
<i>Lipids</i> .....	5
Lipid content .....	5
Fatty acid composition.....	6
<i>Protein</i> .....	6
Protein concentrations .....	6
Amino acid composition and protein quality .....	7
Gluten content .....	7
<i>Starch</i> .....	7
<i>Minerals</i> .....	8
SECONDARY METABOLITES (PHYTOCHEMICALS) FROM WILD RICE .....	9
<i>Vitamins</i> .....	9
Water-soluble vitamins.....	9
Fat-soluble vitamins .....	10
Carotenoids.....	11
<i>Sterols</i> .....	11
Phytosterols .....	11
γ-Oryzanol.....	12
<i>Low molecular weight phenolic compounds</i> .....	13
Phenolic acids .....	13
Hydroxycinnamic acids .....	13
Benzoic acids.....	17
Phenolic aldehydes .....	17
Flavonoids.....	18
Anthocyanins .....	19
Other flavonoids .....	19
Flavonoid oligomers.....	20
<i>Phytic acid</i> .....	20
<i>Constituents of the dietary fiber complex</i> .....	21
<b>FUNCTIONAL PRODUCTS FROM WILD RICE CURRENTLY IN THE MARKET PLACE .....</b>	<b>22</b>
<b>SUMMARY OF ANIMAL AND HUMAN STUDIES CONDUCTED WITH WILD RICE THAT MAY SUGGEST HEALTH BENEFITS ....</b>	<b>23</b>
STUDIES OF HEALTH BENEFITS .....	23

<i>Colon cancer</i> .....	23
<i>Cholesterol lowering</i> .....	24
Experiment 1 – 40% Wild Rice And Cholesterol Lowering In Rats .....	24
Summary of experiment 1 .....	25
Experiment 2 – Different Levels of Wild Rice and Cooked Wild Rice Fed to Rats .....	25
Summary of experiment 2 .....	27
Experiment 3 – Wild Rice Milling Fractions and Cholesterol Lowering in Rats .....	27
Summary of experiment 3 .....	29
Experiment 4 – Chemical Fractions of Wild Rice and Cholesterol Lowering In Hamsters .....	30
Summary of experiment 4 .....	31
Experiment 5 – Cholesterol Lowering in Humans Fed Wild Rice.....	32
Experimental design .....	33
Results .....	34
Summary of experiment 5 .....	36
Other Studies of Cholesterol Lowering .....	36
<i>Glycemic Control</i> .....	37
<i>Summary of Studies of the Health Benefits of Wild Rice</i> .....	39
<b>OPPORTUNITIES FOR PHYTOCHEMICALS WITH POTENTIAL HEALTH BENEFITS FROM WILD RICE</b> .....	<b>40</b>
ANTIOXIDANTS .....	40
APIGENIN .....	41
SUBERIN .....	42
<b>POTENTIAL PROJECTS RELATED TO HEALTH BENEFITS RESEARCH AND DEVELOPMENT</b> .....	<b>42</b>
GLYCEMIC INDEX.....	42
ANTIOXIDANTS .....	42
BINDING OF HETEROCYCLIC AROMATIC AMINES .....	43
<b>REFERENCES</b> .....	<b>44</b>

# TABLE OF TABLES AND FIGURES

---

Figure 1. Aberrant crypt foci (ACF) .....	23
Table 2. Aberrant crypt foci after wild rice feeding <sup>1</sup> .....	23
Table 3. Effect of 40% wild rice diet on food intake, body weight, liver weight, liver cholesterol, bile acid excretion, and oxidative resistance to serum lipids in rats <sup>1</sup> .....	24
Table 4. Daily food intake, final body weight, liver weight in rats fed with Basal diet (B), Basal diet + cholesterol (B+Ch), 10% Wild Rice diet (10%WR), 20% Wild Rice diet (20%WR), 20% Parboiled Wild Rice diet (20%CWR) for 4 weeks <sup>1</sup> .....	25
Table 5. Liver cholesterol, cholesterol absorption, cholesterol synthesis, and oxidative resistance in serum lipids in rats fed wild rice <sup>1</sup> .....	26
Table 6. Final body weight, daily food intake, and liver weight in rats fed different wild rice milling fractions .....	27
Figure 2. Liver cholesterol concentration with wild rice milling fractions .....	28
Table 7. Plasma and liver lipids in hamsters fed wild rice and chemically fractionated wild rice with two different dietary fats .....	29
Table 8. Plasma and liver lipids in hamsters fed wild rice and chemically fractionated wild rice with two different dietary fats <sup>1</sup> .....	31
Figure 5. Liver cholesterol concentration in hamsters fed either soy oil or tallow as dietary fat <sup>1</sup> .....	32
Figure 3. Plasma total cholesterol in hamsters fed wild rice with either soy oil or tallow as the dietary fat <sup>1</sup> .....	32
Figure 4. Plasma HDL cholesterol in hamsters fed either soy oil or tallow as dietary fat <sup>1</sup> .....	32
Table 9. Effect of cultivated wild rice on blood lipid concentrations after two or four weeks of consumption <sup>1</sup> .....	35
Table 10. Effect of cultivated wild rice on serum C-reactive protein concentration after two or four weeks of consumption <sup>1</sup> .....	35
Table 11. Effect of cultivated wild rice on body weight and body mass index after two or four weeks of consumption <sup>1</sup> .....	35
Table 12. Effect of cultivated wild rice on blood pressure after two or four weeks of consumption <sup>1</sup> .....	36
Table 13. Guidelines for interpreting the glycemic index values. ....	38
Table 14. Glycemic index and glycemic load values for wild rice and other forms of rice .....	38

## Introduction

Wild rice (*Zizania* sp.) is an annual cross-pollinated species that grows natively in the northern part of the Mid-West region of the United States (Minnesota, Wisconsin, and Michigan primarily). Next to the annual species (*Z. aquatica*, *Z. palustris*) perennial species (*Z. texana* (Texas wild rice), *Z. latifolia*) exist, the latter one being cultivated in Asia. Wild rice grows as reeds 2-4 meters tall in water about 1-2 meters deep. Wild rice traditionally was the most important food eaten by Native Americans in the Great Lakes region where it grew. The grain of cultivated wild rice is somewhat similar to 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 wild rice was viewed as a sacred food by Native Americans, revered for its life-giving properties, only a modest number of scientific studies of its potential health benefits have been carried out. There has been no comprehensive review of the phytochemicals in wild rice that may have health benefits, or of experimental studies conducted to investigate the potential health benefits of consuming wild rice. In this report we will review the literature of the phytochemical content of wild rice, summarize the published literature and our own unpublished studies of the potential health benefits of wild rice, discuss opportunities for the use of phytochemicals from wild rice for products to provide health benefits, and suggest research studies related to health benefits.

## Phytochemicals in wild rice including a list of phytochemicals with potential health benefits

### Definition of phytochemicals

Phytochemicals are often referred to as plant compounds with potential health benefits for humans. This “definition” is, however, misleading as it is focused on potential advantages for the (human) consumer and not on the benefits of the plant. At first glance, this consideration seems to be of academic interest only. However, it becomes important if we want to increase or decrease the concentrations of certain phytochemicals in plant-based food products. Also, this is a key consideration for the evaluation of the safety of phytochemicals in enriched food products or in dietary supplements.

Although the above-mentioned definition is certainly unsatisfactory and easy to critique, it is much more complicated to develop a precise and comprehensive definition of phytochemicals. In plant biochemistry, phytochemicals are often referred to as secondary metabolites of plants. Whereas primary metabolites such as lipids including phytosterols, amino acids, nucleotides, certain carbohydrates, organic acids etc. perform essential metabolic roles in the plant and are required for the growth and development of all plants, secondary metabolites are not necessarily involved in the development and growth of a plant. Phytochemicals often play a crucial role in a plant's defense system, for example against insects, pathogenic microorganisms and UV-irradiation, but can also help to increase animal-plant interactions, e.g. in pollination attraction. Since phytochemicals are involved in defense mechanisms and sometimes act as antifeedings it is unwise to define phytochemicals as plant compounds that have protective or disease preventive properties for humans. Phytochemicals such as certain benzopyranons (to which coumarins belong) can cause adverse effects, e.g. internal bleeding in mammals or photosensitization causing blistering and other reactions. Also, it should always be kept in mind that the ingestion of some phytochemicals may be beneficial in low doses, but toxic in high doses. The current trend of increasing the concentrations of phytochemicals to abnormally high levels must therefore be scrutinized.

Defining phytochemicals as secondary metabolites brings up the problem that the boundary between primary and secondary metabolites can be blurry. Whereas wild rice constituents which are without doubt primary metabolites, e.g. lipids, amino acids/proteins and starch are, just as minerals, only briefly covered in this review, other primary metabolites such as certain vitamins and phytosterols are described in more detail, together with secondary metabolites such as hydroxycinnamic acids and flavonoids.

### **Primary metabolites from wild rice affecting the nutritional quality of wild rice**

Since the focus of this review is on phytochemicals this section is covered only briefly.

#### ***Lipids***

##### *Lipid content*

The lipid content of wild rice is generally described to be low if compared to other cereal grains. In the early literature, the wild rice lipid content is described to be between 0.5 and 0.8% [1]. Slightly higher lipid content was reported in more recent publications. Przybylski et al. published as a lipid content

between 0.7 and 1.1% for commercial wild rice samples from the US and Canada [2], and a comparison between Chinese (*Z. latifolia*) and North American (*Z. aquatica*) wild rice showed only slightly different lipid content for these wild rice samples of different species and origin (0.94 – 1.23% vs. 0.72 – 0.94%) [3].

### *Fatty acid composition*

The lipid composition of wild rice is very different from the lipids of other cereals since about one third of the fatty acids is comprised of the essential fatty acid linolenic acid [1, 4, 5]. Since the content of linoleic acid is high, more than two-thirds of the fatty acids are polyunsaturated. This was confirmed by more recent studies, finding 20.1 – 31.5% linolenic acid and 35.0 – 37.8% linoleic acid as lipid constituents in commercial wild rice samples from the US and Canada [2]. The authors of this study calculated an  $\omega_6$  to  $\omega_3$  ratio between 1.1 and 1.8 and claim that these low ratios may have beneficial effects on blood lipids. Accordingly, a Japanese study on the triacylglycerol composition of *Z. palustris* indicates that up to 60% of the triacylglycerol species are palmitoyl dilinolein (PLL), palmitoyllinoleoyl linolenin (PLLn), dilinoleoyl linolenin (LLLn), trilinolein (LLL), and oleyllinoleoyl linolenin (OLLn) [6].

### **Protein**

#### *Protein concentrations*

Published protein content for wild rice needs to be evaluated carefully since different nitrogen/protein conversion factors were used and protein concentrations are either based on dry or wet weight. This crucial information is often not given, which makes it difficult to rank the published protein concentrations. The used nitrogen/protein factors widely range from 5.7 to 6.25, with 6.25, being the universal correction factor, 5.7 [7], trying to more specifically consider the amino acid composition of the wild rice proteins, and 5.95, being the accepted factor for rice (*O. sativa*) [8, 9]. Keeping this in mind, protein concentrations of different wild rice species grown in different locations range between ca. 12 and 18% [1, 3, 5, 7-14]. One of the best defined studies performed by Terrell and Wiser in 1978 described protein concentration of 13.14% for *Z. aquatica* var. *aquatica* (average of four samples), 13.41% for *Z. palustris* (48 samples) and 12.88% for *Z. latifolia*, using the correction factor 5.95 and reporting the data on a wet weight basis of 11%. High protein concentrations between 15.2% and 17.0% were reported, for example, by Wang and co-workers in 1978 who, however, used a correction factor of 6.25 and reported the data on a dry basis.

### *Amino acid composition and protein quality*

Generally, the amino acid composition of wild rice proteins is described as being more favorable than the amino acid composition of other cereal grains such as rice, corn, barley, or rye [1, 3, 7-9, 12, 13]. Regarding its nutritional value, the amino acid composition of wild rice protein is often compared with oats. The amino acid score of wild rice proteins was determined to be 81 – 84 [3, 7, 13], depending among others on the wild rice species [3]. Lysine is most often described as the first limiting amino acid and threonine as second limiting [3, 7, 12]. However, in some Chinese species threonine can become first limiting and lysine second limiting [12].

The protein efficiency ratio (PER), which was often used in the past to describe the nutritional value of proteins, was analyzed in several studies for wild rice. It was found that wild rice PER is usually at the higher end of the range of PER's for cereal grains although it is considerably lower than casein, which is used as a standard protein. Wang and co-workers determined PER's averaging 1.77 for four different wild rice samples (casein 2.50) [13]. For comparison they listed PERs of other cereal grains: oats 1.8, barley, 1.6, corn, 1.4, rye 1.3, wheat 0.9. More recently, Zhai et al. compared the nutritional value of Chinese wild rice (*Z. latifolia*) and North American wild rice (in the paper indicated as *Z. aquatica*) [3]. Unfortunately, only the PER for Chinese wild rice was analyzed and was found to be 2.75, which is much higher than the PER reported by Wang and co-workers [13].

### *Gluten content*

At the request of the Minnesota Cultivated Wild Rice Council, one of us (DDG) had cultivated wild rice analyzed for gluten content by a commercial laboratory (Bia Diagnostics, LLC, Burlington, VT). Three samples of wild rice and one sample of brown rice were analyzed – Dawn SR, Itasca C12, and Franklin wild rice and Full Circle brown rice. Gluten concentration for all four samples was below the limit of detection of <5 ppm. This indicates that wild rice is gluten-free. See Appendix 1 for the Certificate of Analysis from Bia Diagnostics.

### **Starch**

From a nutritional point of view, starch digestibility might be of interest since a low digestibility would lower the postprandial blood glucose level and, on the other hand, would increase the amount of resistant starch. Resistant starch is considered a type of dietary fiber. It has been demonstrated that the fermentation of resistant starch by gut microorganisms results in the formation of comparably high



concentrations of butyrate in the large intestine. Butyrate is a short chain fatty acid that is believed to play a key role in the maintenance of gut health. Two papers were published describing wild rice starch in more detail [15, 16]. Although these publications are contradictory in structural characteristics of utter importance (e.g. amylose to amylopectin ratios (Hoover's group determined 21.1% apparent amylose and 29.4% total amylose, whereas Lorenz analyzed only unusually low 2% amylose) neither studies give hints that wild rice starch might be less degradable by starch degrading enzymes in the human gastrointestinal tract than starches from other sources. Lorenz found that enzyme susceptibility of native wild rice starch was even higher than that of wheat starch [15]. Only the higher gelatinization temperature of wild rice (end points at 73 °C vs. 61°C) starch as compared to wheat starch may indicate that less starch is gelatinized during grain cooking and therefore more slowly degraded by starch degrading enzymes in the human gastrointestinal tract. However, this is a) hypothetical and b) it needs to be considered that the wild rice gelatinization temperature is higher than that of wheat but comparable to rice (*O. sativa*). Also, Hoover's data do not support this hypothesis, since they determined a much lower gelatinization range for wild rice starch (51 – 64 °C). In the same study they determined a gelatinization range of 70 – 79 °C for long grain brown rice (*O. sativa*). Just as found by Lorenz, Hoover's group found native wild rice starch to be well degraded by amylases. Finally, Hoover and co-workers found that wild rice starch is resistant to retrogradation [16]. Retrogradation describes the formation of crystalline regions of the starch polysaccharides, which, upon cooling and storing of gelatinized starch, makes starch harder to digest, thus increasing the amount of resistant starch.

### **Minerals**

Swain and co-workers analyzed 34 wild rice samples of different variety and/or grown at different locations for their mineral concentrations and composition [14]. Zinc levels were between 4.0 and 12.1 mg/100 g, a level twice as high as that found in oats and wheat and three times higher than in corn [1]. The range of zinc concentrations analyzed for this set of samples embrace the zinc levels which are summarized by Anderson from early literature [5]. However, zinc concentrations reported for wild rice samples more recently are lower. Zhai et al. reported zinc concentrations of 2.8 and 2.5 mg/100 g for two North American wild rice samples [3] and zinc levels between 1 and 4 mg/100 g were found in commercial wild rice samples bought in the US [17]. Other essential minerals such as iron and copper seem to occur in comparable (sometimes slightly lower) levels in wild rice if compared to other cereal grains, whereas calcium concentrations can be slightly lower [1, 3, 4, 14, 17].

## **Secondary metabolites (phytochemicals) from wild rice**

As mentioned above, phytochemicals are secondary metabolites of the plant; however, the boundary between primary and secondary metabolites can be blurry. Therefore, the classification of some of the compounds discussed here is ambiguous. Other compounds described in this chapter are clearly primary metabolites, e.g. phytosterols, but are often referred to as phytochemicals in literature dealing with functional foods. The same is true for the group of vitamins in which we find compounds that are generally considered as primary metabolites of the plant, e.g. ascorbic acid, but which also includes compounds such as tocopherols/tocotrienols, which are often included into the group of phytochemicals.

### ***Vitamins***

#### ***Water-soluble vitamins***

In a paper published in 1924 it was already stated that “wild rice has a greater food value than the cultivated polished rice, because its proteins are of better quality and because it contains adequate amounts of vitamin B for animal growth, which is not true of the polished cultivated rice” [11]. These observations were later confirmed by analyses of the B vitamins.

Among the B vitamins the published levels for ***thiamin*** show large variations with concentrations ranging between 0.02 – 0.63 mg/100 g [1, 3-5, 14]. The lower numbers were often found in early studies. Methodological difficulties may have led to underestimations. In the most recent study, Zhai et al. analyzed average concentrations of 0.59 mg/100 g (n = 5) for Chinese wild rice (*Z. latifolia*) and 0.43 mg/100 g for North American wild rice [3]. In this study, thiamin concentrations were analyzed fluorimetrically using an AOAC method. In an earlier large study by Swain et al. [14], the vitamin concentrations were analyzed in 34 wild rice samples. By using a microbiological assay, they analyzed thiamin concentrations between 0.02 and 0.25 mg/100 g with an overall mean of 0.10 mg/100 g.

Thiamin concentrations of other (whole) grains are reported to be between 0.33 mg/100 g (rice) and 0.70 mg/100 g (oats) [18]. Thiamin concentrations for polished rice seem to be lower (0.07 mg/100 g [5]).

***Riboflavin*** concentrations ranging between 0.05 and 0.63 were published. Focusing again on two detailed studies, Swain et al. analyzed between 0.2 – 0.4 mg/100 g with an overall mean of 0.27

mg/100 g (n=34) [14], whereas Zhai et al. determined average levels of 0.11 mg/100 g for Chinese wild rice (n=5) and 0.20 mg/100 g for North American wild rice (n=2) [3]. Riboflavin of other (whole) grains range between 0.09 mg/100 g (rice) - 0.22 mg/100 g (barley) [18].

**Niacin** concentrations range between 4.6 and 10.3 mg/100 g [4, 5, 14]. The overall mean of 34 wild rice samples from the US (Minnesota) was 6.98 mg/100 g [14]. Niacin concentrations of other (whole) grains range between 1.5 (rye) and 6.4 (wheat, barley) mg/100 g [18].

Finally, **pantothenic acid** was analyzed in a concentration of 1.01 mg/100 g in wild rice [1] (range of other (whole) grains 0.7 (corn) – 1.36 (wheat)) and the amounts of free **folates** were determined as 0.04 mg/100 g (overall mean, n=34) [14].

**Ascorbic acid** was not detected in wild rice [5].

Overall, wild rice contains water-soluble vitamin in concentrations comparable to other whole grains. Some vitamins in wild rice, such as riboflavin and niacin, are at the upper end of the spectrum of whole grains, but the levels are not dramatically higher than in other grains.

#### *Fat-soluble vitamins*

Only very little information is available about the occurrence and levels of fat-soluble vitamins in wild rice. In a review from 1976 it was stated that **Vitamin A** does not exist in wild rice [5] similar to other cereal grains, which is expected since vitamin A does not occur in plants.

**Vitamin E** is mixture of structurally related **tocopherols** and **tocotrienols**, which also exist as differently active stereoisomers. Two publications describe the analysis of vitamin E active compounds in wild rice. Zhai and co-workers analyzed vitamin E in Chinese and North American wild rice. However, from their description of the analytical procedure it is not possible to deduce which compounds they analyzed as vitamin E. Whereas it is obvious that they did not analyze tocotrienols it is not clear whether or not they analyzed different tocopherol forms ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ) or whether they analyzed just  $\alpha$ -tocopherol. They indicate “vitamin E” levels of 0.29 mg/100 g for Chinese wild rice (overall mean, n=5) and 0.20 mg/100 g for the North American wild rice (overall mean, n=2). Przybylski’s group analyzed seven North American wild rice samples for tocopherols and tocotrienols [2] and compared them with medium and long grain brown rice (*O. sativa*). Different from Zhai’s study, the different forms of vitamin E active compounds were analyzed. Total tocopherol and tocotrienol concentrations varied

significantly among the wild rice samples. Total tocopherols ranged between 251 and 3,682 mg/kg lipids and total tocotrienols ranged between 540 and 9,378 mg/kg lipids (lipid concentrations of the samples ranged between 0.7 and 1.1%). Total tocopherol and tocotrienol concentrations in medium grain rice were 2,565 and 4,478 mg/kg lipids (lipid content of 2.8%). The concentrations in long grain rice were considerably lower (seven times less tocopherols and twenty times less tocotrienols). If tocopherol and tocotrienol concentrations are calculated on grain base and not on lipid base it becomes obvious that medium grain brown rice contains higher levels of tocopherols (7.18 mg/100 g grains) and tocotrienols (12.54 mg/100 g grains) than the wild rice sample richest in tocopherols (4.05 mg/100 g grains) and tocotrienols (10.32 mg/100 g). The tocopherol levels of wild rice are also comparable with the amounts found in oats (1.53 – 4.75 mg total tocopherols/100 g) and rye (0.5 -1.8 mg/100 g  $\alpha$ -, 0.3 – 0.7 mg/100 g  $\beta$ - and 0.6 mg/100 g  $\gamma$ -tocopherol) but considerably lower than in corn [19].  $\alpha$ -Tocopherol is the dominating tocopherol (52.8 – 81.1% of total tocopherols), just as  $\alpha$ -tocotrienol is the dominating tocotrienol (68.4 – 97.8% of total tocotrienols). These trends are also found in other cereal grains such as oats [19].

### *Carotenoids*

**Carotenoids** can play an important role as biological antioxidants and, depending on their chemical structure, they can be provitamins A. Diets rich in carotenoids are being investigated to determine whether they may reduce the risk of certain diseases such as coronary heart disease and certain forms of cancer.

Information about carotenoids in wild rice is scarce. The only carotenoid mentioned in literature is **violaxanthin** that was tentatively identified from 12 wild rice carotenoid fractions [1].

### *Sterols*

#### *Phytosterols*

It is well established that diets rich in **phytosterols** can lower serum cholesterol levels. A linear relationship between phytosterol uptake and lowering of serum cholesterol was demonstrated for phytosterol intakes between 500 mg and 2.5 g per day [20]. Since the daily intake of natural phytosterols is estimated to be about 200 to 300 mg only [21], phytosterol-enriched food products are offered to serve this purpose.

Generally, cereals belong to the most important sources of phytosterols in our diet but little information about the occurrence of phytosterols in wild rice is available. Osamu and co-workers reported that free sterols, sterol esters, steryl glycosides, and acylsteryl glycosides exist in wild rice [6]. The different phytosterols were found in the order **sitosterol** > **campesterol** > **stigmasterol**. More details on phytosterol concentrations and composition in wild rice was recently published by Przybylski's group [2]. The total sterol concentrations in seven North American wild rice samples ranged between 70 and 145 g/kg lipid (lipid concentrations of the samples ranged between 0.7 and 1.1%). This translates into phytosterol concentrations of up to 129 mg/100 g wild rice. This seems to be a high concentration if compared with other cereals and pseudo cereals. Normen and co-workers analyzed the concentrations of phytosterols and phytostanols in different food products from Sweden and the Netherlands. The phytosterol data are not fully comparable with the data from Przybylski's group since they only quantified the phytosterols campesterol, sitosterol and stigmasterol. For these phytosterols, which are most often the dominant phytosterols, they found concentrations of 99 mg/100 g buckwheat flour, 37 mg/100 g corn flour, 23 mg/100 g rice flour, 68 mg/ 100 g rye flour, and 60 mg/100 g whole wheat flour [22]. If the stanols campestanol and sitostanol are added to the phytosterol concentrations the total of sterols and stanols in these samples are calculated as 99 mg/100 g buckwheat, 52 mg/100 g cornflour, 23 mg/100 g rice flour, 86 mg/100 mg rye flour and 70 mg/100 g whole wheat flour [22].

Campesterol,  $\beta$ -sitosterol and **cycloartenol** (which is actually a stanol) were named the dominant phytosterols in wild rice lipids [2]. These sterols/stanols made up between 54 and 75% of the phytosterols in the different wild rice samples. Next to these three compounds stigmasterol, **clerosterol**, **23-dehydrositosterol**,  $\Delta^5$ -avenasterol, **gramisterol**,  $\Delta^7$ -avenasterol, **24-methylenecycloartanol**, and **citrostadienol** were detected in the wild rice samples [2].

#### *$\gamma$ -Oryzanol*

**$\gamma$ -Oryzanol** is a term used for **steryl ferulates** in rice. However, steryl ferulates do not only occur in rice but also in other cereals and were identified, for example, in rye and wheat [23].  $\gamma$ -Oryzanol was described to have a cholesterol-lowering effect in animals [24, 25] and in men [26]. It was assumed that mainly the free 4-desmethylsterols are responsible for the cholesterol-lowering effect [26]. This requires deferuloylation by gastrointestinal esterases, liberating phytosterols and ferulic acid. Next to

the phytosterols, ferulic acid itself may contribute to the health beneficial effects of the steryl ferulates ( $\gamma$ -oryzanol).

Przybilski and co-workers analyzed  $\gamma$ -oryzanol concentrations in commercial North American wild rice samples. Although the quantification of steryl ferulates described in this paper is somewhat ambiguous, the authors reported  $\gamma$ -oryzanol concentrations of 459 to 730 mg/kg lipid in the analyzed wild rice samples. In brown rice (*O. sativa*) samples, which were analyzed concurrently, they found  $\gamma$ -oryzanol concentrations of 459 and 613 mg/kg lipid.

### ***Low molecular weight phenolic compounds***

Phenolic compounds in cereal grains have gained considerable attention in recent years. Several favorable physiological effects have been suggested for the different classes of phenolic compounds including antioxidant, anti-inflammatory, anti-microbial, blood cholesterol lowering, blood glucose lowering and enzyme modulating effects [27]. Although not all suggested effects are supported by unambiguous data, phenolic compounds seem to be among the most potent phytochemicals in cereal grains, including wild rice. Besides the occurrence of phenolic phytochemicals their bioavailability (i.e. absorption by the small intestine) is a very important factor to consider, as phenolic acids are often attached to polymers making them less bioavailable.

#### *Phenolic acids*

Among the phenolic acids, ***hydroxycinnamic acids***, which are formed in the phenylpropanoid pathway from the aromatic amino acids phenylalanine and, in grasses, also from tyrosine, are the most abundant in cereal grains including wild rice. Next to the hydroxycinnamic acids other aromatic acids such as ***benzoic acids*** (generally classified as phenolic acids as well) are found in wild rice and other cereal grains.

#### *Hydroxycinnamic acids*

The most common hydroxycinnamic acids in plant-based food products are ***ferulic acid, caffeic acid, p-coumaric acid*** and ***sinapic acid***. In cereal grains ferulic acid is generally most abundant with lower amounts of *p*-coumaric and sinapic acid and negligible amounts of caffeic acid. The vast majority of the hydroxycinnamic acids do not occur in their free forms but are ester-linked to cell wall polymers, usually arabinoxylans. In lignified tissues or in tissues that contain lignin-like compounds *p*-coumaric

acid is attached to the lignin monomers via an ester-linkage and ferulic acid can be additionally ether-linked to lignin units. Of special interest for wild rice could be that hydroxycinnamic acids also form the polyaromatic domain of suberin. **Suberin** (see also below) is a waterproofing polymer, which is found in specialized tissues of a plant (for example in the endodermis) and which is also formed as part of the wound response.

Hydroxycinnamic acids can form dimers and higher oligomers. **Hydroxycinnamate dimers** in the plant are formed by photochemical and by a oxidative, radical mechanisms [28, 29]. The formation of **higher oligomers** is possible by the radical mechanism only [30]. Cell wall components, especially arabinoxylans, are cross-linked by the formation of hydroxycinnamate oligomers contributing to its rigidity.

Hydroxycinnamic acids and their dimers are well described antioxidants [31-40]. Next to its direct antioxidant effect, ferulic acid (and *p*-coumaric acid) were described to induce antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and catalase in rats [41]. Also, the induction of detoxifying phase II enzymes such as glutathione-S-transferase in rats by supplementing diets with either 1% ferulic acid or ferulic acid ethyl ester was described. Induction of phase II enzymes was also suggested as a potential mechanism for the protective effects of ferulic acid in the azoxymethane-induced colon carcinogenesis in rats [42]. Ferulic acid and its dimers as well as some microbial metabolites of these compounds have been described to have anti-inflammatory effects [43-45]. Antioxidant and anti-inflammatory effects may also contribute to the glucose-lowering effect of ferulic acid [46, 47]. These effects depend, however, on the bioavailability of ferulic acid. Whereas free ferulic acid is highly bioavailable the bioavailability of ferulic acid attached to cell wall polymers is usually low [48-54].

Just as in other cereal grains, ferulic acid is the dominant hydroxycinnamic acid in wild rice. The ferulic acid content of wild rice insoluble dietary fiber was determined after alkaline hydrolysis to about 3.9 mg/g (3744 µg/g *trans*-ferulic acid/g; 198 µg/g *cis*-ferulic acid/g) by using an HPLC-UV method [55] and 4.5 mg/g (4358 µg/g *trans*-ferulic acid; 167 µg/g *cis*-ferulic acid) by using a GC-FID method [56]. The ferulic acid concentration in soluble wild rice dietary fiber was 129 µg/g (101 µg *trans*-ferulic acid; 28 µg/g *cis*-ferulic acid) (GC-FID method) [56]. Using the GC-FID data for ferulic acid and wild rice fiber concentrations (3.34 g insoluble dietary fiber/100 g flour and 0.79 g soluble dietary fiber/100 g flour)

the ferulic acid concentrations of wild rice flour can be calculated as 14.6 mg *trans*-ferulic acid/100 g flour and 0.6 mg *cis*-ferulic acid/100 g flour. By analyzing wild rice flours directly for their “insoluble” phenolic acid concentrations Qiu and co-workers analyzed considerably higher alkaline extractable ferulic acid concentrations (24 - 36 mg/100 g) for different raw North American wild rice samples [57]. The only processed sample analyzed showed a ferulic acid content of 23 mg/100 g in the (methanol) “insoluble fraction”. The ferulic acid concentrations in the (methanol) “soluble fractions” after alkaline hydrolysis were between 0.3 and 0.6 mg/100 g in the raw samples and 0.2 mg/100 g in the quick cooking wild rice. Although Qiu and co-workers used white rice (*O. sativa*) as a “control” and analyzed lower ferulic acid concentrations in white rice (10 mg/100 g flour in the “insoluble fraction” and 0.9 mg/100 g in the “soluble fraction”) the ferulic acid content of wild rice is at the lower end of the spectrum of cereal grains. Because the bran is removed in the production of white rice (most of the ferulic acid is located in the bran), white rice is not a particularly good grain to compare with. In our own studies using brown rice (*O. sativa*) we determined about 26 mg total ferulic acid/100 g brown rice (via the analysis of ferulic acid concentrations in soluble and insoluble dietary fiber). In the same study the total ferulic acid concentrations for whole grain wheat were 60 mg/100 g and 292 mg/100 g for whole grain popcorn [56].

Just as in other cereal grains ferulic acid in wild rice is mainly attached via ester-linkages to arabinoxylans. By using enzymatic and acidic hydrolyses, characteristic feruloylated oligosaccharides, demonstrating the attachment of ferulic acid to the arabinose-side chains of arabinoxylans, were liberated and, after chromatographic isolation, structurally characterized [55].

Concentrations of *p*-coumaric acid and sinapic acid are much lower than ferulic acid concentrations in wild rice. Qiu and co-workers reported *p*-coumaric acid concentrations of 3 - 4 mg/100 g wild rice in the “insoluble fraction” and 2 – 5 mg/100 g in the “soluble fraction”. Also, a *p*-coumaric acid content of 142 µg/g wild rice insoluble fiber demonstrates much lower *p*-coumaric acid concentrations than ferulic acid concentrations in wild rice, which is very common for cereal grains [56].

Wild rice is, however, somewhat different from other cereal grains by containing higher amounts of sinapic acid. The sinapic acid content in wild rice insoluble dietary fiber was determined to about 518 µg/g by using an HPLC-UV method [55] and 454 µg/g by using a GC-FID method [56]. The insoluble dietary fibers of other cereal grains (wheat, rye, corn and barley) contained less than 100 µg sinapic



acid/g insoluble fiber (wheat, rye, corn, barley) or less than 200 µg/g (oats, rice). Considering wild rice insoluble fiber concentrations of 3.34 g /100 g wild rice, flour contains at least 1.5 mg sinapic acid/100 g flour. Again, Qui et al. analyzed higher amounts of sinapic acid in their commercial wild rice samples (ca. 6 – 10 mg/100 g (“insoluble fraction”)) by analyzing the hydroxycinnamic acids directly [57].

Although the isolation and identification of defined sinapic acid-oligosaccharides could not be achieved, the solubilization of sinapic acid from insoluble wild rice fiber by using a mixture of carbohydrases indicated that sinapic acid is at least partially bound to cell wall polymers [55].

The extraction of a rather unusual cinnamic acid, **3,4,5-trimethoxycinnamic acid** was described in a Japanese patent [58]. This compound was extracted from wild rice by using ethanol [59]. Additionally, two compounds containing esters of 3,4,5-trimethoxycinnamic acid with sugars were identified by the same group. These compounds were described as a **phenolic glycoside** with 3,4,5-trimethoxycinnamate and *p*-hydroxy acetophenone as the aglycone moieties, and a flavonoid glycoside with 3,4,5-trimethoxycinnamate and luteolin as the aglycone [58, 59].

**Ferulic acid dehydrodimers** were identified in wild rice soluble and insoluble dietary fiber after alkaline hydrolysis. The total dehydrodiferulic acid concentrations in the wild rice insoluble dietary fiber was 2,840 µg/g; only trace amounts were measured in soluble dietary fiber [60]. For comparison, total dehydrodiferulic acid concentrations in the insoluble dietary fibers of rice, wheat and popcorn were 4,042 µg/g, 2,372 µg/g, and 12,596 µg/g. If the dietary fiber concentrations of these samples (3.34 g/100 g for wild rice, 3.21 g/100 g for rice, 8.76 g/100 g for wheat and 11.73 g/100 g for corn) are considered it becomes obvious that wild rice contains comparably low amounts of ester-bound dehydrodiferulic acids. Several regioisomers of dehydrodiferulic acids have been identified in the past, e.g. 8-8-linked, 8-5-linked, 8-O-4-linked, and 4-O-5-linked dimers. Wild rice is somewhat different from the other cereal grains since about 26% of the total dimers are 8-8-linked whereas only about 16% of the total dimers are 8-8-linked in the other cereal grains [60]. **Ferulic acid trimers** (5-5/8-O-4-triferulic acid and 8-O-4/8-O-4-triferulic acid) have been identified in wild rice insoluble fiber, but quantification was not possible [61].

Whereas the amounts of ferulic acid oligomers are rather low in wild rice, wild rice contains the highest amounts of oxidatively formed dehydrodisinapic acids among common cereal grains [62]. Due to the additional aromatic methoxyl group less regioisomers can be formed when sinapic acid esters are

dimerized as compared to the dimerization of ferulic acid esters. Two 8-8-coupled **dehydrodisinapic acids** were identified and quantified. 8-O-4-Coupled dehydrodisinapic acid was not found. Total dehydrodisinapic acid concentrations of insoluble dietary fibers were 481 µg/g for wild rice but only 44 µg/g for rice and 33 µg/g for wheat. Only trace amounts were found in rye and barley insoluble dietary fiber. Disinapic acids were also identified and quantified by Qiu and co-workers in North American wild rice samples. However, as they used an ambiguous quantification strategy, these data do not seem to be relevant [57]. Although higher amounts of dehydrodisinapic acids are somewhat unique for wild rice among the commonly used cereal grains, we do not know yet whether these compounds have physiological effects of interest.

Finally, **cross-coupling products comprised of radically coupled ferulic acid and coniferyl alcohol** were identified in the alkaline hydrolyzates of wild rice dietary fiber just like in all, with the exception of corn, analyzed cereal grain insoluble dietary fibers [63]. From a plant physiological point of view these compounds can be cross-links between arabinoxylans and lignin or lignin-like components. Whether these compounds have any physiological benefits for humans (beyond the logical antioxidant effect of phenolic compounds) is unknown.

#### *Benzoic acids*

Low amounts of benzoic acids were identified in the alkaline hydrolysates of wild rice insoluble dietary fiber [55], the methanol “soluble fraction” and the methanol “insoluble fraction” of wild rice [57]. Qiu and co-workers identified **p-hydroxy benzoic acid, vanillic acid** and **syringic acid** in individual quantities up to 3 mg /100 g (vanillic acid) in the “insoluble fractions” and up to about 5 mg/100 g (*p*-hydroxy benzoic acid) in the “soluble fractions” [57]. These benzoic acids were also identified in the alkaline hydrolyzates of wild rice insoluble dietary fiber (individual amounts up to 34 µg/g (syringic acid)), but additionally **protocatechuic acid** was identified and determined to 128 µg/g insoluble fiber.

#### *Phenolic aldehydes*

Phenolic aldehydes, which partially show the same aromatic substitution pattern as the corresponding benzoic and/or hydroxycinnamic acids, occur either naturally and/or are generated from hydroxycinnamic acids during alkaline hydrolysis. Asamarai and co-workers fractionated the methanol extract of wild rice hulls which showed in-vitro antioxidant activity in the ammonium thiocyanate assay [64]. The most active fraction contained the phenolic aldehydes **m-hydroxybenzaldehyde**, 4-hydroxy-3-

methoxybenzaldehyde (**vanillin**), and 4-hydroxy-3,5-dimethoxybenzaldehyde (**syringaldehyde**). Next to these three phenolic aldehydes the antioxidant active fraction contained **2,3,6-trimethylanisole** and **2,3-dihydrobenzofuran**, the latter being a pro-oxidant. Phenolic aldehydes were also determined in the methanol “soluble fraction” and the methanol “insoluble fraction” of wild rice of commercially available North American wild rice kernels [57]. Although the authors report the identification of **p-hydroxybenzaldehyde** and vanillin quantitative data were not given. In addition to vanillin and p-hydroxybenzaldehyde, **protocatechuic aldehyde** was identified in the alkaline hydrolyzates of wild rice insoluble dietary fiber. The amounts of these aldehydes in the insoluble fibers were determined to 56 µg/g (p-hydroxybenzaldehyde), 89 µg/g (protocatechuic aldehyde), and 27 µg/g (vanillin) [55]. Although phenolic aldehydes can be formed from hydroxycinnamic acids under alkaline conditions by oxidative degradation [65] oxidation can be largely suppressed by taking precautions such as nitrogen purging of the sodium hydroxide solution and the headspace during the hydrolysis. Whereas some vanillin and 4-hydroxybenzaldehyde may be formed from p-coumaric acid and ferulic acid during alkaline hydrolysis, the pre-cursor of protocatechuic aldehyde, caffeic acid, was not identified in the wild rice insoluble fiber. Thus, it is also possible that the identified aldehydes are indeed natural products that are linked to cell wall polymers, e.g. structural proteins or uronic acid containing polysaccharides in the wild rice kernel.

### *Flavonoids*

Flavonoids describe various groups of phytochemicals that can be classified according to their structure into chalcones, aurones, isoflavones, flavonones, flavones, flavonols, leucoanthocyanidins, catechins, and anthocyanins. Multiple potential health benefits have been discussed for flavonoids. Some of these benefits were demonstrated for most or all of the flavonoids, e.g. antioxidant effects, whereas some of physiological effects are more specific for one group of the flavonoids only, for example binding of isoflavones to estrogen receptors, an effect that may reduce breast cancer risk.

The antioxidant and anti-inflammatory effects (interactions with enzymes and transcription factors) seem to be most important in the prevention of certain types of cancer and cardiovascular disease. However, other factors such as anti-platelet activity also seem to be involved in the prevention of cardiovascular diseases [66-69].

### *Anthocyanins*

The occurrence of two anthocyanins, ***cyanidin 3-glucoside*** and ***cyanidin 3-rhamnoglucoside***, was described for the staminate florets and the leaf sheath of wild rice [70]. Information about the occurrence of anthocyanins in wild rice kernels is, however, inconsistent. In an early review [1] a contribution of flavonoid pigments (anthocyanidins) to seed pigmentation in early developmental stages was mentioned. ***Cyanidin*** and the colorless ***leucocyanidin*** were identified and ***delphinidin*** was indicated as tentatively identified. Abdel-Aal and co-workers analyzed colored cereal grains including wild rice kernels for their anthocyanin compositions. In their wild rice sample, which was not further specified, a low amount of total anthocyanins was determined (27 µg/g) in the unspecific total anthocyanin assay. This assay is a spectrophotometric test measuring all compounds that are extractable and absorb light at 535 nm. If analyzed more specifically for anthocyanins by HPLC-UV/vis no distinct anthocyanin signals were detected [71]. Different from Abdel-Aal's study, Kim and co-workers detected three different pigments, two of them being anthocyanins, in a wild rice sample, which was not further specified [72]. By using LC-MS they identified cyanidin 3-glucoside and tentatively identified cyanidin-fructoside. However, since the other samples analyzed in this study were black and red rice, and due to the confusion of the term "wild rice", it is not clear whether their sample was a *Zizania* or an *Oryza* sample.

### *Other flavonoids*

Qiu and co-workers partially fractionated acidic acetone-water extracts from commercially available North American wild rice samples on Sephadex LH-20. By using HPLC-MS/MS they identified three apigenin (a flavone) glycosides (diglucosyl apigenin, glucosyl-arabinosyl apigenin, diarabinosyl apigenin) [73]. This might be of interest since apigenin is discussed as a phytochemical with potential anticancer activities [74, 75]. However, currently quantitative data about apigenin and its glycosides in wild rice is not available.

In addition, catechin and epicatechin, which are flavanols, were identified in the acidic acetone-water extract [73].

### *Flavonoid oligomers*

Procyanidins, oligomeric compounds comprised of catechin and/or epicatechin, were detected in acidic acetone-water extracts from North American wild rice samples (see 3.2.3.2) [73]. Qiu and co-workers found oligomerization products up to pentamers. Their occurrence was, however, dependent on the analyzed wild rice samples; some samples contained only dimers and trimers, other samples contained all oligomers.

### ***Phytic acid***

Phytic acid serves as a storage form of phosphorus in the plant. It is a chelator of metal ions including essential minerals such as iron, calcium, magnesium, and zinc, reducing their bioavailability in the human body [76]. On the other hand, due to the complexation of metal ions, which can promote lipid oxidation, phytic acid is also an antioxidant [77]. The antioxidant activity of wild rice and extracts thereof has often been studied in food products or in artificial test systems, using several different methods [57, 64, 73, 78-81]. Besides phenolic compounds, phytic acid was described as one of the key antioxidants in food systems [78, 81]. In addition to its importance for food quality, phytic acid has also been suggested as a health beneficial food constituent [76], potentially protecting against colon cancer and inflammatory bowel diseases due to its ability to suppress oxidative reactions [77, 82, 83]. However, in other studies the contribution of phytic acid to cancer protective effects of, for example, wheat bran was described to be marginally only [84]. Phytic acid was also suggested to flatten the blood glucose response due to the complexation of calcium [85], which is a cofactor of  $\alpha$ -amylase, the primary enzyme involved in starch digestion. Other proposed health beneficial effects include a preventive role of phytic acid in coronary heart disease and renal lithiasis. Although phytic acid was often discussed as a major contributor to the antioxidant activity of wild rice, quantitative data are scarce. Phytic acid concentrations of mature, partially mature and immature kernels (*Z. aquatica* L.) were published as 2.17, 2.18 and 2.17 g/100 g (dry weight basis) with small kernels showing higher concentrations (2.41 g/100 g) than larger kernels (2.08 g/100 g) [86]. These concentrations are higher than the concentrations described for wheat, corn, barley and oats (0.8 – 1.1 g/100 g), comparable to peanuts and sunflower seeds (1.9 g/100 g) but lower than in Lima beans (2.5 g/100 g), corn germ (6.4 g/100 g), and wheat bran (4.8 g/100 g) [87].

### ***Constituents of the dietary fiber complex***

Dietary fiber is defined as “the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation” [88]. Major components of cereal grain dietary fibers are cell wall polysaccharides such as arabinoxylans and cellulose. Besides cell-wall polysaccharides, non-carbohydrate cell-wall components such as lignin, suberin, and cutin as well as resistant starch as a storage polysaccharide contribute to cereal dietary fiber.

In an early publication, Capen and LeClerc reported crude fiber concentrations for 36 wild rice samples (33 of them having their origin from Minnesota) between 0.9 and 1.35 g/100 g in unparched samples and 0.55 to 1.18 g/100 g in parched samples. Crude fiber concentrations of 1.2 g/100 g were reported for 12 samples of Canadian lake and paddy wild rice (*Z. aquatica* L.) [7]. Zhai et al. reported comparable concentrations (1.15 and 1.24 g/100 g) for two North American commercial wild rice samples whereas the concentrations for some of the analyzed Chinese wild rice samples (*Z. latifolia*) were higher (1.15 – 1.93 g/100 g, average 1.70 g/100 g). Crude fiber concentrations do not reflect, however, dietary fiber concentrations since the determination of this parameter includes treatments which are not relevant from a physiological point of view, thus largely underestimating dietary fiber concentrations. Without mentioning any detail, a fiber content of 4.5 g/100 g is indicated in a review written by Oelke and co-workers [4]. By using a preparative enzymatic gravimetric procedure insoluble and soluble dietary fiber concentrations of a commercial wild rice sample (*Z. aquatica*) were determined to 3.34 g/100 g and 0.79 g/100 g, respectively. These fiber fractions were analyzed for their neutral monosaccharide composition. The neutral sugar composition of the wild rice insoluble dietary fiber was 52.7% glucose, 17.7% arabinose, 17.7% xylose, 6.5% galactose, and 5.4% mannose; the composition of the soluble fraction was 8.6% arabinose, 6.3% xylose, 42.9% manose, 23.5% glucose, 18.7% galactose and trace amounts of fucose [55, 56]. Tahara and Misaki describe the cell wall polysaccharide composition of wild rice (*Z. palustris*) as 7% **pectin**, 71% **hemicelluloses**, and 22% **cellulose**. In addition, they identified neutral **arabinoxylans** but also **glucuronoarabinoxylans** as constituents of the soluble hemicellulose fraction [89]. Non-carbohydrate constituents of the dietary fiber complex are lignin, a phenolic polymer, suberin, a polymer comprised of a polyaliphatic domain and a polyaromatic domain, cutin, a

polyaliphatic polymer, and waxes. Lignified and suberized dietary fibers were suggested to adsorb heterocyclic aromatic amines [90-93], which are putative procarcinogens, thus limiting their absorption and activation to the ultimate carcinogenic species in the human body [94]. Although cereal brans were often described as highly lignified in the past, this assumption is probably not true since it is based on an unspecific analytical methodology only [95, 96]. On the other hand it is likely that hulls contain higher amounts of *lignin*, although this has not been analyzed for wild rice hulls yet. Hulls could therefore potentially be used to prepare a dietary fiber rich preparation suitable to absorb heterocyclic aromatic amines. Min and Ding describe a technology based on “biological and chemical methods” and on an “extrusion technique” to produce a dietary fiber product with “increased total dietary fiber and more soluble fiber” [97]. However, since this publication is available in Chinese only, no details about this process could be gathered.

In addition to lignified cell walls, suberized cell walls were proposed to be good adsorbers for heterocyclic aromatic amines. Although no details about *suberin* (or *cutin*) in wild rice kernels were found, Anderson describes the seed coat of wild rice as “suberized impregnated with polyphenols” [10]. Also, the pericarp was described to be cutinized and the outer epidermal wall of the pericarp to be covered by a thick layer of *wax* [10].

## **Functional products from wild rice currently in the market place**

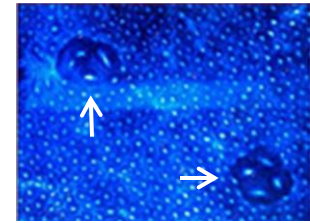
Functional products can display functionality in two different ways. One is to add a desirable characteristic or quality to a food product. An example of a functional product by this meaning is adding gelatin to yogurt to give it firmness. Another, quite different way is to impart a health benefit. An example of this would be to consume plant sterols to reduce plasma cholesterol.

We have conducted an extensive on-line search for examples of either type of functional products that incorporate wild rice or wild rice components. We were unable to identify any such products that are currently available. The only type of product in which wild rice potentially displays functionality could be in meat products. Several papers described an antioxidant effect of wild rice in meat products [78-81], thus contributing to increased shelf-life of these products. Wild rice containing meat products in the market are, for example, wild rice bratwurst, wild rice sausage, and wild rice meatballs.

## Summary of animal and human studies conducted with wild rice that may suggest health benefits

### Studies of Health Benefits

**Colon cancer.** To date, only one study of the effect of wild rice on colon cancer risk appears available, the unpublished work of Gallaher and Gallaher. In this study, rats (12 per group) were fed a standard purified diet (control) or the purified diet containing 40% wild rice, ground to a flour. After 10 days of feeding, rats were administered two doses of a colon-specific carcinogen (dimethylhydrazine; 50 mg/kg body weight), one week apart. Eleven weeks after the last carcinogen dose, the rats were killed, the colons collected and fixed to preserve them, and processed for counting of pre-cancerous lesions (aberrant crypt foci, ACF). An image of a stained rat colon, in which the ACF are indicated by the arrows, is seen in Figure 1, which shows two ACF, each composed of 4 aberrant crypts.



**Figure 1. Aberrant crypt foci (ACF)**

As can be seen in **Table 2**, there was no statistically significant difference in the number of ACF between the control group and the wild rice-fed group. However, there was a strong trend towards fewer large ACF (ACF with 4 or more aberrant crypts per focus) with wild rice feeding, compared to the control group. This may be of importance, as several studies strongly suggest that large ACF are more likely to progress to tumors than small ACF [98, 99]. To our knowledge, this unpublished work represents the only study of the effect of wild rice on colon cancer risk. Given this trend toward a lowered risk, this is an area that may warrant further investigation.

**Table 2. Aberrant crypt foci after wild rice feeding<sup>1</sup>**

Group	ACF (number/cm <sup>2</sup> )	Large ACF (number/cm <sup>2</sup> )
Control	10.41 ± 0.93	1.15 ± 0.17
Wild Rice	11.46 ± 1.19	0.74 ± 0.12*

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SEM, n=12 per group.

\*Trend towards a difference from the control group, p = 0.062.

**Cholesterol lowering.** A number of studies have examined the effect of wild rice on cholesterol lowering in animal models, and one (unpublished) study has examined the effect in humans.

*Experiment 1 – 40% Wild Rice And Cholesterol Lowering In Rats.* Our research group has examined the effect of diets containing wild rice flour in several studies using cholesterol-fed rats. In our first study, rats were fed for 5 weeks either a control diet, a control diet containing 0.12% cholesterol, or a diet containing 40% wild rice and 0.12% cholesterol.

**Table 3. Effect of 40% wild rice diet on food intake, body weight, liver weight, liver cholesterol, bile acid excretion, and oxidative resistance to serum lipids in rats<sup>1</sup>.**

Parameter	Control No Chol	Control + 0.12% Chol	40%Wild Rice + 0.12% Chol
Daily food intake, g/day	18.4 ± 0.4	20.4 ± 0.7	19.1 ± 0.6
Final body weight, g	281.5 ± 10.0	280.7 ± 11.4	280.7 ± 10.0
Liver weight, g	8.39 ± 0.44 <sup>a</sup>	9.72 ± 0.45 <sup>b</sup>	8.80 ± 0.30 <sup>ab</sup>
Liver cholesterol, mg/g	3.01 ± 0.09 <sup>b</sup>	11.33 ± 0.95 <sup>a</sup>	3.95 ± 0.26 <sup>b</sup>
Bile acid excretion, μmole/day	9.51 ± 0.69 <sup>b</sup>	25.69 ± 1.78 <sup>a</sup>	22.59 ± 1.56 <sup>a</sup>
Oxidative resistance of serum lipids, min	47.44 ± 6.61 <sup>b</sup>	139.56 ± 20.15 <sup>a</sup>	188.78 ± 38.44 <sup>a</sup>

<sup>1</sup>Values are mean ± SEM, n= 6 for the control group, and 9 for the control + cholesterol and wild rice groups. Values that do not share a superscript are significantly different, p<0.05. Abbreviation: Chol, cholesterol.

Food intake and final body weight did not differ between the wild rice-fed rats and those fed the control diets (**Table 3**). Rats fed the control diet containing cholesterol had a greater liver weight than those fed the control diet without cholesterol, a common finding in these types of studies. Liver weight in the wild rice group showed a trend towards lowering. Liver cholesterol concentration was greatly elevated in rats fed the control diet + cholesterol compared to rats fed the cholesterol-free control diet, as expected. The rats fed wild rice diet, containing cholesterol, showed a *dramatic* reduction in liver cholesterol concentration, to a value that did not differ from that of the animals fed the cholesterol-free control diet. Bile acids, which are synthesized from cholesterol, are typically excreted

in greater amounts when the dietary cholesterol intake is increased. This was found in the present experiment, as can be seen by comparing the cholesterol-free and cholesterol-containing control diets. Increasing bile acid excretion is a mechanism for lowering cholesterol, one that has been employed pharmacologically. However, rats fed wild rice showed no further increase in bile acid excretion, eliminating this as a mechanism by which wild rice may lower liver cholesterol. Finally, resistance of serum lipids to oxidation was examined, as wild rice has considerable antioxidant activity in vitro. Since increases in serum lipid oxidation, leading to an increase in oxidative stress, are believed to play an important role in the development of atherosclerosis [100], a finding that consuming wild rice increased resistance to lipid oxidation would suggest a greater ability to keep oxidative stress at a low level, and thereby reduce the risk for heart disease. Adding cholesterol to the control diet increased resistance to lipid oxidation. However, consuming wild rice did not lead to a significant increase in resistance to serum lipid oxidation relative to the control + cholesterol group. This would suggest that antioxidants within the wild rice are not well absorbed.

*Summary of experiment 1.* This study demonstrates that wild rice has a dramatic effect on reducing liver cholesterol in rats. The lack of an increase in bile acid excretion with wild rice indicates that bile acid excretion is not the mechanism by which wild rice lowers cholesterol. In addition, the lack of increase in the antioxidant capacity of plasma from rats fed wild rice suggests that antioxidants in wild rice have limited bioavailability.

*Experiment 2 – Different Levels of Wild Rice and Cooked Wild Rice Fed to Rats.* In the next experiment we examined the effect of feeding two different levels of wild rice on cholesterol lowering in rats. In addition, we included one group fed cooked wild rice in order to determine whether the effect of wild rice on cholesterol lowering is altered by cooking. In addition, in this study, we examined the effect of wild rice on intestinal cholesterol absorption and on liver cholesterol synthesis.

Rats were fed diets containing either 10% wild rice, 20% wild rice, or 20% cooked wild rice. All wild rice diets contained 0.075% cholesterol. In addition, one group was fed a cholesterol-free control diet and another group fed the control diet containing 0.075% cholesterol. Diets were fed for 4 weeks.

**Table 4. Daily food intake, final body weight, liver weight in rats fed with Basal diet (B), Basal diet + cholesterol (B+Ch), 10% Wild Rice diet (10%WR), 20% Wild Rice diet (20%WR), 20% Parboiled Wild Rice diet (20%CWR) for 4 weeks<sup>1</sup>**

Parameter	Control	Control +	10%WR +	20%WR +	20% CWR +
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	No Chol	0.075% Chol	0.075% Chol	0.075% Chol	0.075% Chol
Daily food intake, g	20.4 ± 0.6	19.6 ± 0.6	19.6 ± 2.1	20.8 ± 1.0	19.5 ± 0.7
Final body weight, g	294 ± 9	291 ± 5	315 ± 8	304 ± 10	314 ± 6
Liver weight, g	11.99 ± 0.73 <sup>a</sup>	13.55 ± 0.46 <sup>ab</sup>	14.31 ± 0.62 <sup>b</sup>	14.05 ± 0.57 <sup>b</sup>	14.13 ± 0.54 <sup>b</sup>

<sup>1</sup>Values are means ± SEM, n=9-12. Values within a row that do not share a letter differ significantly,  $P < 0.05$ .

As in our first study on cholesterol lowering, wild rice had no effect on daily food intake or final body weight (**Table 4**). Inclusion of cholesterol in the diet led to greater liver weights, but wild rice had no effect on liver weight.

As shown in **Table 5**, all wild rice-containing diets reduced liver cholesterol concentration compared to the cholesterol-containing control diet. The 20% wild rice diet showed a trend towards a greater reduction than 10% wild rice, with the 20% cooked wild rice group achieving a lower liver cholesterol concentration than the 10% wild rice group. Cholesterol absorption did not differ among the groups, indicating that wild rice is not lowering liver cholesterol by reducing cholesterol absorption. The cholesterol-fed control group had a lower rate of cholesterol synthesis compared to the cholesterol-free control group. This result was expected, as dietary cholesterol is well known to suppress liver cholesterol synthesis in rats. Cholesterol synthesis rates did not differ between the cholesterol-containing control group and any of the wild rice groups, although the values were numerically greater. Thus, a reduced cholesterol synthesis also does not appear to explain the reduction in liver cholesterol found in the wild rice-fed groups. As in the previous study, wild rice did not increase the oxidative resistance of serum lipids. This again indicates that consumption of wild rice does not lead to an increase in lipid-soluble antioxidants in the serum, and that the antioxidants in wild rice may have limited bioavailability.

**Table 5.** Liver cholesterol, cholesterol absorption, cholesterol synthesis, and oxidative resistance in serum lipids in rats fed wild rice<sup>1</sup>.

Parameter	Control	Control + 0.075% Chol	10%WR + 0.075% Chol	20%WR + 0.075% Chol	20% CWR + 0.075% Chol
Liver cholesterol, µmol/g	11.3 ± 0.6 <sup>d</sup>	25.9 ± 1.8 <sup>a</sup>	19.6 ± 2.1 <sup>b</sup>	17.6 ± 1.0 <sup>bc</sup>	15.2 ± 0.9 <sup>c</sup>
Cholesterol absorption, %	-	41.4 ± 1.4	40.1 ± 1.6	43.7 ± 1.8	41.9 ± 1.9

Cholesterol synthesis, pmol mevalolactone /min/mg liver	6.20 ± 0.87 <sup>a</sup>	2.51 ± 0.28 <sup>b</sup>	3.69 ± 0.52 <sup>b</sup>	3.37 ± 0.3 <sup>b</sup>	2.81 ± 0.39 <sup>b</sup>
Oxidative resistance of serum lipids, min	136 ± 14	166 ± 11	166 ± 10	172 ± 12	159 ± 9

<sup>1</sup>Values are means ± SEM, n=9-12. Means that do not share a letter differ significantly, P<0.05.

*Summary of experiment 2.* This experiment confirmed the cholesterol lowering effect by wild rice observed in the first study, and showed that the lowering occurred at much lower levels than in that first experiment. In addition, it demonstrated that the cholesterol lowering effect was not lost with cooking. Finally, we confirmed the lack of increase in serum oxidative resistance seen in experiment 1 with wild rice feeding, again suggesting limited absorption of antioxidants from wild rice.

*Experiment 3 – Wild Rice Milling Fractions and Cholesterol Lowering in Rats.* In this experiment, we examined different milling fractions of wild rice to determine whether its cholesterol lowering property was located in the endosperm or bran. In addition, we included brown rice as a point of comparison.

Wild rice and brown rice were incorporated into the diet at 20% by weight. Wild rice endosperm was incorporated at 19% of the diet, and wild rice bran included at 1% of the diet. The dietary amounts for wild rice endosperm and bran were chosen to represent that amount of each that would be present in a diet containing 20% wild rice. All diets except for the cholesterol-free control diet contained 0.125% cholesterol. Rats were fed the experimental diets for 4 weeks. **Table 6** shows the final body weight, average food intake, and liver weight. There were no differences among the groups in any of these parameters.

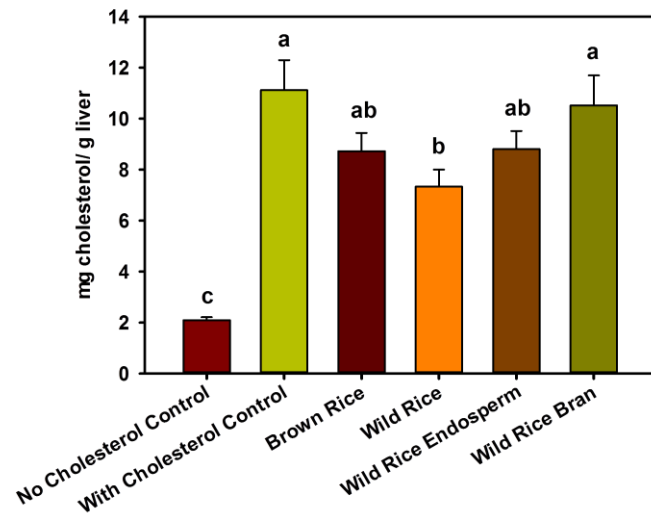
**Table 6. Final body weight, daily food intake, and liver weight in rats fed different wild rice milling fractions**

Parameter	Control	Control + 0.125% Chol	20% Brown Rice	20% Wild Rice	19% Wild Rice Endosperm	1% Wild Rice Bran
<b>Final body</b>	340.8 ±	339.2 ±	350.0 ± 7.0	346.4 ±	357.8 ± 9.5	359.2 ± 9.0

<b>weight, g</b>	10.6	12.9		14.1		
<b>Average</b>						
				21.2 ± 0.7	20.6 ± 0.6	20.7 ± 0.6
				12.5 ± 0.8	13.8 ± 0.5	13.3 ± 0.6

Liver cholesterol

**Figure 2. Liver cholesterol concentration with wild rice milling fractions**



concentration is shown in **Figure 2**. The groups fed 1% wild rice bran, 19% wild rice endosperm, or 20% brown rice did not differ significantly from the cholesterol-fed control group, although both the wild rice endosperm and brown rice groups showed a trend towards a reduction in liver

cholesterol. The group fed 20% wild rice showed a significant reduction in liver cholesterol compared to the cholesterol-fed control group, thus confirming our previous studies.

Finally, in this study, we examined whether feeding wild rice would decrease body fat in these rats. This was examined two ways. The first was to measure the weight of the fat pads, which are discrete bodies of fat (adipose) tissue. It is well established that the weight of the fat pads is proportional to total body fat [101]. The second approach was to measure plasma leptin concentrations. Leptin is a hormone that is secreted from the adipose tissue in proportion to the amount of body fat [102]. Thus decreases in plasma leptin indicated decreased body fat. Finally, we determined the plasma concentration of adiponectin. This hormone is also secreted from fat tissue, and correlates inversely with insulin resistance. That is, as insulin resistance goes down (a desirable situation), plasma adiponectin increases. Thus, greater values for adiponectin are viewed positively.

As can be seen in **Table 7**, there were no differences among the diet groups in either fat pad weight or plasma leptin. This would indicate that neither wild rice nor its milling fractions altered the amount of body fat in the animals. However, wild rice significantly increased plasma adiponectin, and there was a trend for an increase with the 1% wild rice bran. This is a very positive finding for wild rice and, as explained above, suggests that wild rice may act to improve insulin resistance.

Parameter	Control	Control + 0.125% Chol	20% Brown Rice	20% Wild Rice	19% Wild Rice Endosperm	1% Wild Rice Bran
Perirenal fat pad, g/100 g body wt.	0.15 ± 0.02	0.15 ± 0.02	0.19 ± 0.01	0.18 ± 0.03	0.17 ± 0.02	0.21 ± 0.02
Epididymal fat pad, g/100 g body wt.	0.65 ± 0.05	0.59 ± 0.03	0.60 ± 0.04	0.69 ± 0.09	0.56 ± 0.05	0.78 ± 0.05
Plasma leptin, ng/mL	2.67 ± 0.46	3.02 ± 0.80	3.82 ± 0.39	4.20 ± 0.14	3.65 ± 0.65	6.19 ± 0.81
Plasma adiponectin, ng/mL	--	5.85 ± 0.41 <sup>b</sup>	7.62 ± 0.59 <sup>ab</sup>	9.77 ± 0.74 <sup>a</sup>	7.68 ± 0.74 <sup>ab</sup>	8.03 ± 0.42 <sup>ab</sup>

Table 7. Plasma and liver lipids in hamsters fed wild rice and chemically fractionated wild

rice with two different dietary fats

<sup>1</sup>Values are means ± SEM, n=10-11. Means that do not share a letter differ significantly,  $P < 0.05$ .

*Summary of experiment 3.* In this experiment, 20% wild rice was found to reduce liver cholesterol, whereas wild rice endosperm only showed a trend towards a reduction and 1% wild rice bran had no effect on liver cholesterol. Thus, the wild rice needs to be intact in order for its cholesterol lowering property to be expressed. Neither wild rice nor its milling fractions had an effect on body fat. However, wild rice increased adiponectin, which suggests that it may reduce insulin resistance.

*Experiment 4 – Chemical Fractions of Wild Rice and Cholesterol Lowering In Hamsters.* In the previous experiment, we fractionated wild rice based on milling. This next study was conducted to determine whether the agent(s) responsible for cholesterol lowering were present in a fraction that could be extracted with organic solvents or were in a water-soluble fraction. In addition, wild rice was fed in the presence of two different fat sources – soybean oil or beef tallow. In this study, we chose to use hamsters as the animal model, as some investigators have suggested that hamsters are a superior animal model to rats for examining cholesterol lowering. Further, in hamsters, plasma cholesterol responds to dietary changes that influence cholesterol metabolism. This is in contrast to rats, where plasma cholesterol is very resistant to change [103], and one must examine liver cholesterol instead.

The chemical fractionation was carried out by grinding wild rice to flour and extracting this flour with methanol. The methanol extract was then evaporated and the residue remaining incorporated into a diet (wild rice extract diet). The wild rice residue after extraction was also put into a diet (wild rice residue diet). There were two diets containing 20% wild rice – one in which soybean oil was used as the fat source and the other in which beef tallow was used as the diet. As positive controls, there was a soybean diet with no wild rice and a beef tallow diet with no wild rice. All of the diets just described contained 0.125% cholesterol. Finally, there was one cholesterol-free diet (with soybean oil as the fat source) to serve as a negative control. The animals were fed their respective diets for 4 weeks, and then killed and blood and tissues collected.

Final body weight and daily food intake did not differ among any of the groups (data not shown). Plasma and liver lipid concentrations are shown in **Table 8**.

The plasma lipid values for the wild rice extract were quite similar to that of the native wild rice, whereas the wild rice residue (remainder after methanol extraction) was somewhat lower than the native wild rice. However, neither the wild rice extract diet nor the wild rice residue diet lowered plasma cholesterol, plasma triacylglycerol, or liver cholesterol concentration below that of the soy oil + cholesterol control group.

Table 8. Plasma and liver lipids in hamsters fed wild rice and chemically fractionated wild rice with two different dietary fats<sup>1</sup>.

Parameter	Negative Control	Soy oil Control + 0.125% Chol	Tallow Control + 0.125% Chol	20% Wild Rice – Soy Oil	20% Wild Rice - Tallow	Wild Rice Extract	Wild Rice Residue
Total plasma cholesterol, mg/dL	120 ± 9	181 ± 9	242 ± 12	200 ± 9	225 ± 9	207 ± 15	184 ± 9
HDL cholesterol, mg/dL	86 ± 5	128 ± 7	165 ± 10	131 ± 7	158 ± 8	133 ± 12	128 ± 8
Plasma Triacylglycerol, mg/dL	58 ± 6	80 ± 7	133 ± 15	112 ± 14	144 ± 19	114 ± 11	88 ± 8
Liver cholesterol, mg/g	3.08	18.56	16.03	15.78	13.92	16.83	21.62

<sup>1</sup>Values are means ± SEM, n=10-11. Means that do not share a letter differ significantly, P<0.05.

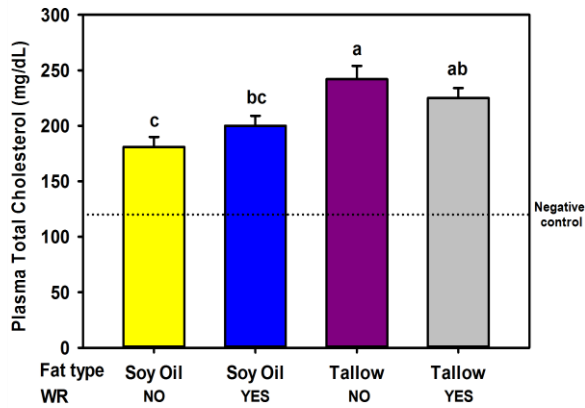
The effect of wild rice on plasma and liver lipids as influenced by dietary fat type is shown in **Figures 3, 4, and 5**. Plasma cholesterol was increased by tallow compared to soy oil, but was not influenced by wild rice (**Figure 3**). Similarly, plasma HDL cholesterol was increased by tallow in the diet, compared to soy oil, but wild rice was without effect (**Figure 4**). Finally, liver cholesterol concentration was not significantly affected by either fat source or wild rice (**Figure 5**), although there was a tendency for lower liver cholesterol with tallow feeding and with wild rice feeding.

*Summary of experiment 4.* Using hamsters as the experimental animal model, wild rice did not reduce plasma or liver cholesterol, as we had seen in previous experiments using rats. The reason for this difference is not clear, but may be due to the well-known difference in the capacity for cholesterol synthesis between the two species [103]. Whereas rats can alter their rate of cholesterol synthesis over a wide range, hamsters can vary it over only a narrow range. Thus, if wild rice is reducing cholesterol by reducing cholesterol synthesis, then the hamster, with its limited capacity to alter

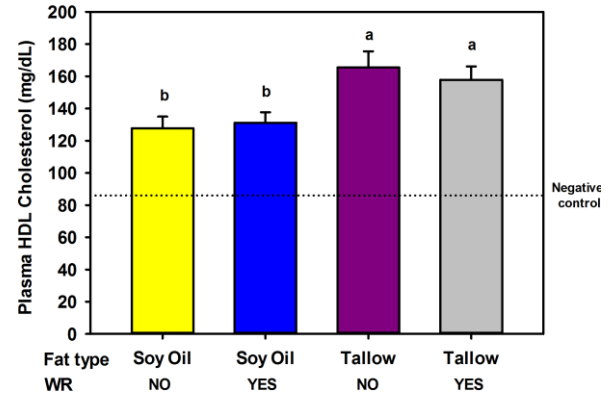


cholesterol synthesis, would not be able to respond to the wild rice. Further work will be needed to confirm this possibility.

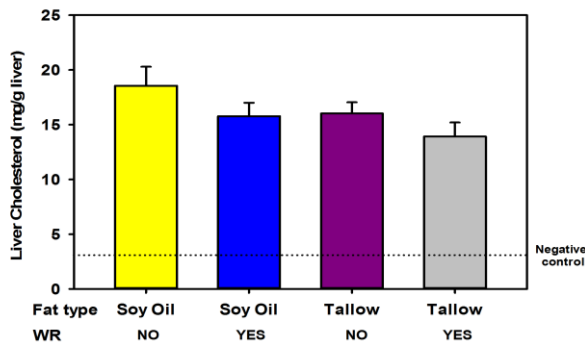
**Figure 3. Plasma total cholesterol in hamsters fed wild rice with either soy oil or tallow as the dietary fat<sup>1</sup>.**



**Figure 4. Plasma HDL cholesterol in hamsters fed either soy oil or tallow as dietary fat<sup>1</sup>.**



**Figure 5. Liver cholesterol concentration in hamsters fed either soy oil or tallow as dietary fat<sup>1</sup>.**



Abbreviation: WR, wild rice.

*Experiment 5 – Cholesterol Lowering in Humans Fed*

*Wild Rice.* Based on the findings that wild rice lowered cholesterol dramatically in rats, an experiment was conducted to determine whether cholesterol lowering would occur in humans fed wild rice. The

goal for the study was to determine the effect of cultivated wild rice consumption in humans on the following parameters related to coronary heart disease. The specific objectives were as follows:

- Serum cholesterol concentrations, including LDL and HDL cholesterol
- Serum marker of inflammation (C-Reactive protein)

#### *Experimental design*

A four-week feeding study of parallel design was utilized to determine whether consumption of cultivated wild rice affects the parameters of interest. Subjects (25 per group) meeting the minimum criteria for entry (moderately elevated serum cholesterol, normal body weight, no cholesterol lowering drugs, etc.) had the parameters described above measured at entry (baseline). Subjects came to the General Clinical Research Center (GCRC) to be given their wild rice and white rice-containing foods and instructed how to incorporate rice (either white rice or cultivated wild rice) into their diet (two servings a day). One serving was 45 g dry weight, or ¼ cup. The control subjects consumed white rice while those in the treatment group consumed cultivated wild rice. Subjects were asked to consume the rice six days out of every week.

Subjects were asked not to make other changes in their diet, and a food frequency questionnaire was administered at baseline and between weeks two and three to confirm this. At two weeks and four weeks after the subjects began consuming the rice, blood was drawn. Serum lipid concentrations (total and HDL cholesterol, triacylglycerol) were measured at baseline, two and four weeks. The other parameters were measured at baseline and four weeks only. Differences in values for the measurements made at entry and at completion were statistically analyzed, as well as differences between the cultivated wild rice and white rice groups at the end of the experiment.

Serum total and HDL cholesterol, serum triacylglycerol (for calculation of LDL cholesterol) and C-reactive protein were measured by the GCRC laboratory using standard methods. The GCRC laboratory is certified for measurement of serum lipids.

Subjects were randomly assigned to consumption of either white rice (control) or cultivated wild rice (treatment). Subjects were asked to consume one-half cup (90 g dry weight) a day of dried rice for 6 days out of each week, which was consumed cooked into foods in a variety of ways. The rice was

consumed at several meals over the day. Subjects were asked to maintain their normal dietary patterns.

Twenty-five subjects per groups, for a total of 50 subjects, were used in this study. Subjects were both men and women between the ages of 18 and 80 who were generally healthy, but had serum cholesterol concentration in the range of 195-250 mg/dL. In order to participate, each subject had to be willing to be randomized into one of the two treatment groups.

There were a total of 50 subjects that completed the study, with 25 participants in each of the rice groups. A total of 27 women and 23 men finished the study.

### *Results*

Compliance was excellent. Of the 50 subjects that started the study, none of the subjects dropped out for their own reasons. Nine individuals were taken out of the study shortly after the baseline total cholesterol measurement, as their total cholesterol values were outside of the acceptable range (195-250 mg/dL) to qualify. Compliance with consuming the rice dishes was quite good. There were 8 subjects that missed 1-2 days of the 24 days of rice consumption that were required.

The results of the blood lipid determinations are shown in **Table 9**. There was a modest reduction in total cholesterol in the wild rice group compared to white rice at 4 weeks. However, this difference did not achieve statistical significance. Within the wild rice group, there was also a modest, but nonsignificant reduction in total cholesterol at 4 weeks compared to baseline. The results for LDL-cholesterol were similar to those for total cholesterol. There was a modest, but nonsignificant decrease in LDL-cholesterol in the wild rice group compared to white rice at 4 weeks. There was a strong trend for a difference in HDL-cholesterol between the two groups at 4 weeks ( $p=0.051$ ), which is an unfavorable difference.

**Table 10** shows the effect of the rice-containing diets on serum C-reactive protein, a protein associated with inflammation. Elevated concentrations of serum C-reactive protein are associated with a greater risk of heart disease. The concentration of C-reactive protein declined in both groups from baseline to four weeks. However, neither the declines nor the differences between the groups were statistically significant.

**Table 9. Effect of cultivated wild rice on blood lipid concentrations after two or four weeks of consumption<sup>1</sup>.**

Group	Total Cholesterol			LDL Cholesterol			HDL Cholesterol			Triglycerides		
	Baseline	2 Weeks	4 Weeks	Baseline	2 Weeks	4 Weeks	Baseline	2 Weeks	4 Weeks	Baseline	2 Weeks	4 Weeks
White Rice	220	222	225	146	147	150	50	51	53	115	118	107
Wild Rice	223	221	219	150	148	147	49	49	48	118	125	124

<sup>1</sup>Values are means, n=25, expressed as mg/dL.

**Table 10. Effect of cultivated wild rice on serum C-reactive protein concentration after two or four weeks of consumption<sup>1</sup>.**

Group	Serum C-Reactive Protein	
	Baseline	4 Weeks
White Rice	0.32	0.16
Wild Rice	0.25	0.20

<sup>1</sup>Values are means, n=25, expressed as mg/dL

The effect of the rice-containing diets on body weight and body

mass index is shown in **Table 11**. Although both groups lost weight, and therefore had a reduced body mass index, the loss was greater in subjects consuming wild rice. However, this difference did not achieve statistical significance.

Systolic and diastolic blood pressure at each time point is shown in **Table 12**. Blood pressure was essentially unchanged over the course of the trial.

**Table 11. Effect of cultivated wild rice on body weight and body mass index after two or four weeks of consumption<sup>1</sup>.**

Group	Body Weight			Body Mass Index		
	Baseline	2 Weeks	4 Weeks	Baseline	2 Weeks	4 Weeks
White Rice	147.8	147.4	147.6	23.9	23.8	23.9

Wild Rice	159.4	158.8	158.4	24.2	24.1	24.0
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<sup>1</sup>Values are means, n=25, expressed as mg/dL.

**Table 1. Effect of cultivated wild rice on blood pressure after two or four weeks of consumption<sup>1</sup>.**

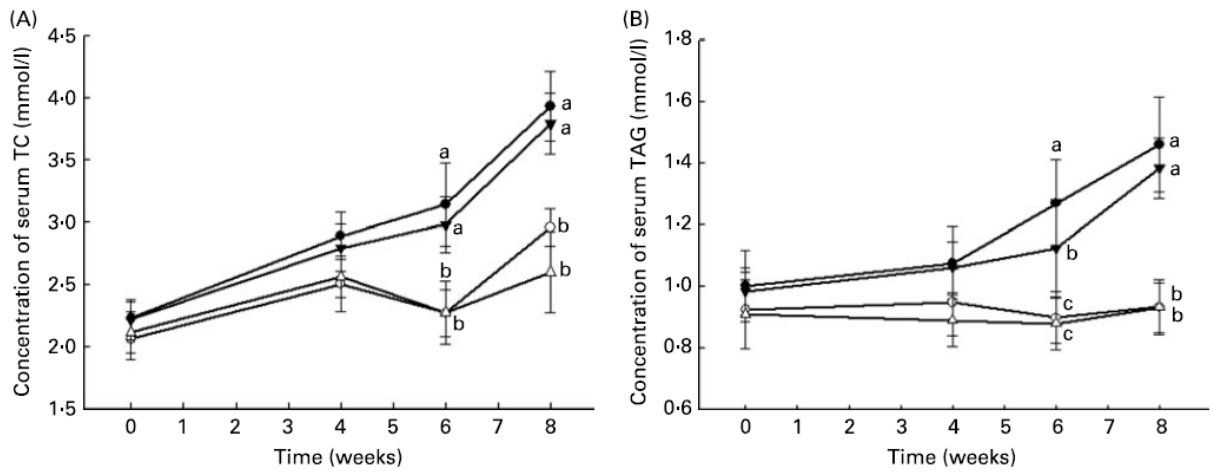
Group	Systolic Blood Pressure			Diastolic Blood Pressure		
	Baseline	2 Weeks	4 Weeks	Baseline	2 Weeks	4 Weeks
White Rice	119.4	118.8	117.5	71.5	70.0	69.2
Wild Rice	122.2	119.9	122.3	68.3	67.8	69.6

<sup>1</sup>Values are means, n=25, expressed as mm Hg.

*Summary of experiment 5.* Wild rice, when fed to humans with elevated serum cholesterol, failed to demonstrate a cholesterol lowering effect. This was a surprising and disappointing finding, given the highly positive effects of wild rice on cholesterol lowering in rats. In terms of the response to wild rice, humans appear to be similar to hamsters. It is believed that the capacity of humans to alter their cholesterol synthesis rate is somewhere between that of a rat and a hamster. If it were more similar to hamsters, this would suggest that wild rice is unlikely to be an effective cholesterol lowering agent in humans. However, without an understanding of how wild rice functions to lower cholesterol in rats, it will be uncertain whether wild rice has any potential to lower cholesterol in humans.

*Other Studies of Cholesterol Lowering.* Only one study, a short communication, has been published examining the cholesterol lowering effect of wild rice. This study, conducted in rats fed a high cholesterol (1.75%) /high fat (13%) diet reported that Chinese wild rice (*Z. latifolia* (Griseb) Turcz) fed at 52.3% of the diet, lowered plasma cholesterol and triacylglycerols [104]. Their findings are shown in **Figure 6**.

**Figure 6.** The lipid profiles of rats (n=11) fed the experimental diets for 8 weeks. (A) Concentration of serum total cholesterol (TC) and (B) concentration of serum TAG. Values are means and standard deviations. a,b,c Values with unlike letters are significantly different (P,0.05; ANOVA followed by the Tukey post hoc test). ●, High fat/cholesterol; ▼, city diet; ○, wild rice; ▲, low fat.

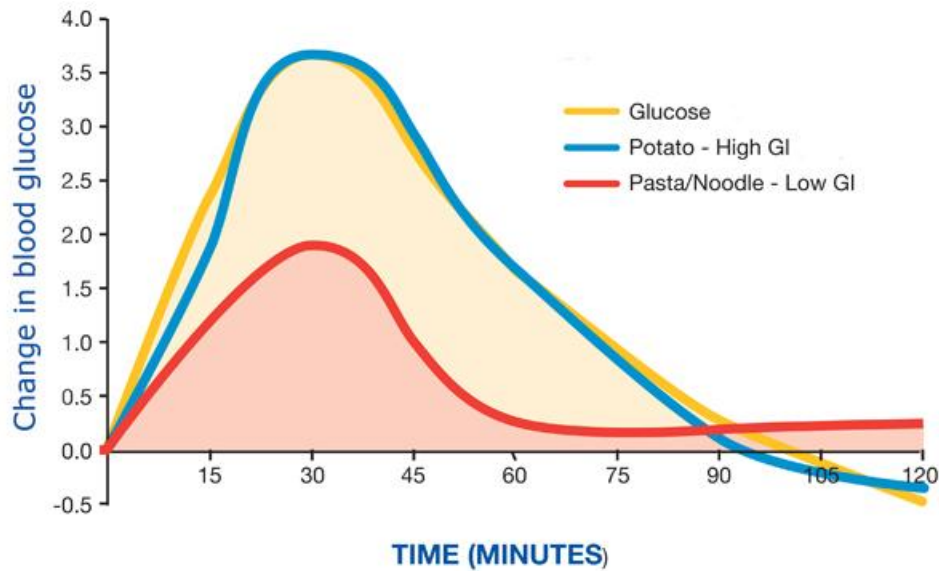


Thus, the results of this study confirm the cholesterol lowering effect noted in our studies in rats fed wild rice from Minnesota. However, in contrast to our findings in rats, the authors reported that the antioxidant capacity of the serum was significantly increased by the wild rice diet. They also reported that two markers of oxidative stress in plasma, thiobarbituric acid reactive substances (TBARS) and superoxide dismutase activity, were altered in a way that indicated lower oxidative stress in rats fed Chinese wild rice. That is, serum TBARS decreased and serum superoxide dismutase activity increased.

### ***Glycemic Control***

Appropriate control of blood sugar (glycemic control) is considered important for maintaining proper levels of insulin sensitivity, and failure to do so has been linked to an increased risk of diabetes, heart disease, and obesity, and is one of the defining characteristics of metabolic disease. Although it remains controversial, considerable evidence indicates that consumption of foods that result in a slower absorption of glucose are important in maintaining glycemic control. This has been quantitated as the **glycemic index**, which is defined as a ranking of foods on a scale of 0 to 100, based on the degree to which they raise blood glucose, compared to a standard food (either pure glucose or white bread). Therefore, foods with a lower glycemic index are considered to be more beneficial in terms of blood glucose control. A related measure, glycemic load, is the glycemic index multiplied by the quantity of carbohydrate in a serving. As with the glycemic index, a lower value for the glycemic load is viewed as better. This measure thus incorporates the amount of glucose in the meal with the estimate of the blood glucose response.

The blood glucose response to both a high glycemic index and low glycemic index food are illustrated below:



From <http://www.gisymbol.com.au/using.php>

Guidelines for the glycemic index are shown in Table 13 below:

Table 2. Guidelines for interpreting the glycemic index values.

Low glycemic index food	<b>&lt;55</b>
Intermediate glycemic index food	<b>55 - 70</b>
High glycemic index food	<b>&gt;70</b>

Tables of the glycemic index of foods have been published. There is one entry for wild rice. Table 14 shows the glycemic index for this one entry of wild rice as well as several other foods of interest for comparison. All values are taken from reference [105].

Table 3. Glycemic index and glycemic load values for wild rice and other forms of rice

Item	Glycemic Index (Glucose=100)	Glycemic Index (Bread=100)	Glycemic Load (per serving)
<b>Wild rice, Saskatchewan</b>	<b>59</b>	<b>81 ± 8</b>	<b>18</b>
Brown rice, steamed	50	72	16

(USA)			
Brown rice (Canada)	66 ± 5	94	21
Calrose brown rice (Australia)	87 ± 8	124	33
Calrose, white, medium grain, boiled (low-amylose) (Australia)	83 ± 13	119	36
Doongara, white (high-amylose) (Australia)	56 ± 4	78 ± 7	22
Instant rice	69 ± 12	98 ± 17	29
Parboiled rice (Canada)	48	68 ± 6	18
Parboiled rice (USA)	72	103	26

As one can see from Table 14, wild rice has a glycemic index and glycemic load lower than most other rice products. This would suggest that wild rice would be a useful food to control blood glucose. However, as can be seen from the table, there is considerable variation within the same type of food. For example, parboiled rice has a glycemic index of 48 (low glycemic index) and 72 (high glycemic index). Thus, a single value, such as there is for wild rice, must be viewed cautiously, and ideally additional determinations of the glycemic index should be made.

### ***Summary of Studies of the Health Benefits of Wild Rice***

From our **unpublished** studies and the single published study of the health benefits of wild rice consumption, the following conclusions may be drawn:

- Wild rice may reduce the risk of colon cancer in rats. However, further work is necessary to confirm and extend our preliminary findings.
- Wild rice feeding lowers cholesterol in rats to an impressive degree. The mechanism by which it does so is unknown.



- Wild rice does not lower plasma cholesterol in hamsters, another often-used animal model to study cholesterol metabolism.
- The results regarding reduction in oxidative stress with wild rice feeding in rats is conflicting; our studies did not find an increase in antioxidant capacity in the serum, whereas others reported a decrease in markers of oxidative stress with wild rice feeding.
- Wild rice does not lower plasma total or LDL cholesterol or raise HDL cholesterol in humans with elevated plasma cholesterol. It does not alter C-reactive protein, a measure of inflammation, compared to subjects fed white rice. Finally, neither blood pressure nor body mass index, a measure of body fat, were altered by consuming wild rice.
- Based on a single determination, wild rice has a low glycemic index, which suggests that it would be a food to be recommended for maintaining proper glucose control. However, because there is only this one determination, the value must be viewed with caution.

## **Opportunities for phytochemicals with potential health benefits from wild rice**

Overall, based on our review of the literature and our own studies, it is our view that the phytochemical content of wild rice does not differ dramatically from other cereals. For only a few phytochemicals, for example sinapic acid and its dimers, does wild rice appear to be a more concentrated source. For other phytochemicals wild rice is relatively lower. However, compared to white rice, which has the phytochemical-rich bran layer removed, wild rice has greater amounts of phytochemicals. Since “regular” rice is most often consumed milled, as white rice, whereas wild rice is always consumed intact, there is some justification to comparing wild rice to white rice.

The following represents our views on potential opportunities for health benefits for wild rice, focusing primarily, but not exclusively, on the phytochemicals in wild rice.

### **Antioxidants**

Wild rice contains a number of phytochemicals that have a demonstrated antioxidant effect in vitro. These antioxidant compounds are of two types. First are those that directly act as antioxidants by eliminating oxidants. Ferulic acid is an example of this type. Second are those that act indirectly by

binding metals such as iron that may lead to formation of oxidants. Phytic acid, which binds iron, is an example of this type of antioxidant.

There is currently considerable interest in foods with high antioxidant activity. Wild rice is clearly such a food. One approach is to promote wild rice solely on this basis. Various commodity groups have done so aggressively with their products (e.g. blueberries). However, to do this one would need to have wild rice analyzed by a certified laboratory for antioxidant activity using one of the commonly used assays for antioxidant capacity, such as the oxygen radical absorbance content (ORAC) assay. Also, it has to be considered that a large portion of antioxidants in wild rice cannot easily be extracted by just using water or organic solvents. While most antioxidants in, for example, blueberries are extractable, a large portion of the wild rice antioxidants is bound to polymers and cannot be extracted by using the general procedures to prepare samples for the ORAC assay. While the polymer-bound antioxidants can be liberated by, for example, alkaline extraction procedures, this is not routinely done and may also bias the data, since polymer-bound antioxidants are not well (if at all) absorbed.

A second approach would be to develop a wild rice concentrate that would have an increased concentration of these antioxidants. This would likely involve grinding the wild rice to flour and using amylases to remove the starch. Additional processing could be employed to increase the bioavailability of the antioxidants, as many of them are, as mentioned earlier, bound to cell wall material, primarily arabinoxylans, and therefore very likely not well absorbed. Such additional processing steps could include either addition of dilute alkali, which is well established to release phenolics such as ferulic acid from arabinoxylans, or enzymes that will hydrolyze the linkages.

### **Apigenin**

Apigenin is a potentially interesting phytochemical due to its suggested anti-cancer activity. Before any potential apigenin related effects are tested for wild rice, the occurrence and especially the concentrations of apigenin in wild rice should be (re)evaluated. If apigenin is indeed a wild rice phytochemical its concentrations in wild rice and its milling fractions should be compared with other apigenin sources such as oats and sorghum.

## **Suberin**

Although it is mentioned that wild rice seeds are suberized, original papers describing suberin composition and concentrations of wild rice seeds could not be found. Suberized dietary fiber is of potential interest since suberized cell walls were described to adsorb carcinogens such as heterocyclic aromatic amines (see above). Adsorption of heterocyclic aromatic amines to suberized cell walls/dietary fiber in the small intestine would interfere with their absorption and thus activation to carcinogens in the liver. Due to the growing conditions of wild rice suberization of wild rice kernels is likely but needs to be confirmed by detailed studies.

## **Potential projects related to health benefits research and development**

### **Glycemic index**

The glycemic index, while still somewhat controversial, is accepted by many as an important aspect of a food. For examples, the Glycemic Index Foundation has established a certification system that allows foods with a low glycemic index to use a specific seal, allowing consumers to readily identify them. Allow this seal is not in use in the United States yet, it is being used in Australia and South Africa.

Currently there is only one determination of the glycemic index for wild rice, for Saskatchewan wild rice. It would seem quite appropriate for additional determinations of the glycemic index to be conducted using Minnesota cultivated wild rice, so that there is a value tied directly to it. There are commercial laboratories that will carry out this determination for a fee. Doing so insures that the glycemic index value will be accepted and entered into tables of the glycemic index of foods.

### **Antioxidants**

Given the antioxidant content of wild rice, several different types of projects are recommended. First would be to have the antioxidant capacity of several samples of wild rice determined, using the ORAC assay. This work should be done by a commercial laboratory that specializes in this type of measurement in order to insure that the values will be well accepted. However, most nutritionists understand that the antioxidant capacity of a food may not translate to its antioxidant effect in animals or humans. This is because the bioavailabilities of the antioxidant compounds in a food differ considerably. Given the conflicting results regarding the effect of wild rice on markers of oxidative

stress in animals, it would be prudent to further examine the antioxidant effect in an animal model or humans before wild rice is promoted for its antioxidant properties.

### **Binding of heterocyclic aromatic amines**

If suberization of wild rice cell walls can be confirmed, projects studying the adsorption properties of wild rice dietary fiber for heterocyclic aromatic amines are recommended. These studies would start with in-vitro tests, in which the adsorption properties of wild rice fiber are analyzed simulating the conditions of the small intestine. If wild rice fibers adsorb more heterocyclic aromatic amines than other cereal fibers, these studies can also potentially be performed in animals to confirm the significance of the adsorption of heterocyclic aromatic amines to suberized fiber in-vivo.

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