Chapter 161

Effects of parboiled rice and grape marc-based mixtures on rats fed hypercholesterolemic diets

10.56238/tfisdwv1-161

Mônica Lourdes Palomino de los Santos
Coordinator of the Nutrition Course at the University Center of the Campaign Region (URCAMP)
PhD in Agroindustrial Science and Technology (UFPEL)
Rua Coronel Azambuja, 35; CEP: 96400-710; Bagé/RS.
E-mail: monicasantos@urcamp.edu.br

Guilherme Cassão Marques Bragança
Coordinator of Pharmacy and Biomedicine Courses at the Campania University Center (URCAMP)
PhD in Agroindustrial Science and Technology (UFPEL)
Rua Coronel Azambuja, 35; CEP: 96400-710; Bagé/RS.
E-mail: guilhermebraganca@urcamp.edu.br

Vera Maria de Souza Bortolini
Nutritionist, Professor at the Campania Region University Center (URCAMP)
Ph.D. in Food Science and Technology (UFPEL)
Rua Coronel Azambuja, 35; CEP: 96400-710; Bagé/RS.
E-mail: verabortolini@urcamp.edu.br

Reni Rockenbach
Nutritionist, Professor at the Campania Region University Center (URCAMP)
Ph.D. in Agroindustrial Science and Technology (UFPEL)
Rua Coronel Azambuja, 35; CEP: 96400-710; Bagé/RS.
E-mail: renirockenbach@urcamp.edu.br

Roberta Nunes Horta
Pharmacist, PhD in Genetics and Applied Toxicology (ULBRA)
Av. Dom Joaquim 1515, sala 910; CEP: 96020-260; Pelotas/RS.
E-mail: robertaulbra@gmail.com

Jander Luis Fernandes Monks
Chemical Engineer, Coordinator of the Higher Course in Chemical Engineering at Instituto Federal Sul-Rio-Grandense (IFSUL)
PhD in Agroindustrial Science and Technology (UFPEL)
Praça Vinte de Setembro, 455; CEP 96015-360 - Pelotas/RS
E-mail: jandermonks@hotmail.com

William Peres
Pharmacist, Professor at the Federal University of Pelotas (UFPEL)
Ph.D. in Biological Sciences (University of León/Spain)
Av. Eliseu Maciel, s/n; CEP 96160-000 - Capão do Leão-RS
E-mail: noty62@hotmail.com

Moacir Cardoso Elias
Agronomist, Professor at the Federal University of Pelotas (UFPEL)
PhD in Agronomy (UFPEL)
Av. Eliseu Maciel, s/n; CEP 96160-000 - Capão do Leão-RS
E-mail: eliasmc@uol.com.br

ABSTRACT
In today's society, there is evidence of a nutritional transition in food, where poor consumption habits are risk factors for chronic diseases. Due to the functional capabilities of rice and grape marcs in the prevention and treatment of cardiovascular diseases, this sought to evaluate the chemical composition of grape marc and parboiled rice grain and their effects on the biological response of *Wistar* rats fed a hypercholesterolemic diet. This work encompassed two experiments. In the experiment, the antioxidant activity was determined in parboiled rice grains, grape marc, and a mix of both (Mix 1 - 40% of parboiled rice and 60% grape marc and Mix 2 - 60% of parboiled rice and 40% grape marc). In experiment II, we assessed the biological response of adult male *Wistar* rats, Universidade Federal de Pelotas strain, fed hypercholesterolemic diets with 40 and 60% of grape marc and parboiled rice, respectively. The addition of parboiled rice and grape marc improves the nutritional parameters of hypercholesterolemic diets with increased concentrations of protective factors. The increase in the grape marc ratio in the diet increases the availability of antioxidants, reducing blood levels of cholesterol and Low-Density Lipoprotein (LDL) without compromising parameters related to renal function. The presence of parboiled rice in a hypercholesterolemic diet increases the plasma levels of HDL.

Keywords: Cholesterol, diets, grape marc, mixtures, rice, Wistar rats.

1 INTRODUCTION

There is an evidenced nutritional transition in modern society, whose consumption pattern is influenced by socioeconomic, demographic, and behavioral factors, with a predominance of simple carbohydrates rather than fruits and vegetables. Qualitative characteristics of a diet are important in defining the health status of a population, especially concerning chronic degenerative diseases of adulthood (PINHO et al., 2012).

The beneficial effects of certain types of food on health are known for a long time. Functional foods, for example, are those that contain substances that may be considered biologically active and, in addition, contribute to nutrition and are beneficial to health (KOMATSU et al., 2008). Changes in processing and the growing exigence of the consumer for food with high sensory quality, nutritional and what bring benefits to health, encourage the study of new ingredients for the food industry (SILVA et al., 2009). The combination of the parboiled Rice and the grape marc in this study is justified for its high nutritional content of them (YI et al., 2009; ELIAS; OLIVEIRA and SCHIAVON, 2010), associated with your phenolic composition, presenting an important antioxidant property, and consequently, benefit to the human health (HEINEMANN et al., 2005; VENDANA et al., 2008).

Rice stands out for being a basic food for the majority of the population in various parts of the world and the most produced and consumed cereal in the world (WALTER et al., 2008). As for nutritional value, rice provides 20% of the world's dietary energy, while wheat supplies 19% and corn 5% (FAO, 2004). In addition to being a carbohydrate-rich food (WALTER et al., 2005), it can also be an important source of digestible proteins, minerals (mainly phosphorus, iron, and calcium), and B complex vitamins, including B1 (thiamine), B2 (riboflavin), B3 (niacin) and B9 (folic acid). However, its functional characteristics are still little known (MONKS, 2010). Being a product of plant origin, rice is a cholesterol-free food with a low lipid content (WALTER et al., 2008), although oryzanol and tocotrienol are present. The main fatty acids in rice are palmitic, oleic, and linoleic, corresponding to approximately 95% of the fatty acids present in the total lipid content (MONKS, 2010).

Grapes are rich in phenolic compounds, with the highest content found in organic grapes (MULERO et al., 2010). The main compounds are flavonoids (anthocyanins, flavonols, and flavonols), stilbenes (resveratrol), phenolic acids (derivatives of cinnamic and benzoic acids) and a wide variety of tannins (MALACRIDA and MOTTA, 2005). Anthocyanins, a group of natural pigments with various phenolic structures, and glycosides of flavonols, present in higher concentrations in the bark (DOWNHAMAND COLLINS, 2000), are among the most studied phenolic compounds in grapes, for their outstanding antioxidant activity and anti-inflammatory and anticancer properties (NEGRO et al., 2003). The species *Cabernet sauvignon* is highlighted as the red grapes most used in winemaking, resulting in delicate and fine wines (JUBILEU et al., 2010). Some studies focus on the antioxidant potential in marc, consisting of seeds and skins (ALONSO et al., 2002; ROCKENBACH et al., 2008; NEVES et al., 2011) rejected during the winemaking process, and the stalks, which receive far less attention, although they contain a significant amount of polyphenols (SOUQUET et al., 2003).

Based on the great importance of these foods in nutrition and the need to understand their potential of beneficial interference with human health, the main objectives of this study were to identify phenolic compounds present in the parboiled rice (*Oryza sativa*) and grape marc and investigate their biological effects in *Wistar* rats submitted to a hypercholesterolemic diet.

2 MATERIAL AND METHODS

2.1 EXPERIMENTAL ANIMALS

The use of animals for research promotes an accurate assessment of the control of the diet, and metabolic and biochemistry parameters influenced by the foods (ANGELOVA and BOYADJIEV, 2013). Small rodents like rats are ideal models for the research of cardiovascular pathologies, induced or not (RUSSELL and PROCTOR, 2006).

The Ethics and Animal Experimentation Commission - EAEC at the Federal University of Pelotas, under Protocol 23110.007731/2012-86, approved this study. The experiments with animals were conducted at the Central Animal Laboratory of the Federal University of Pelotas, from July to September 2013. Male Wistar rats (*Rattus norvegicus*, Albinus variety, class Rodentia) were used (n = 24) with an average age of 60 days. We used Wistar rats due to their low cost, high resistance to infection, availability of a suitable site for experimentation, and above all, for being the model most frequently used for research on the nutritional aspects (SHANAIDER and SILVA, 2004)

2.2 FEEDSTOCK

Grains of polished parboiled rice (*Oryza sativa*) from the 2010 harvest were used and maintained under a controlled temperature of 15ºC in the grain storage house of the Grain Laboratory/UFPEL. Grape marc was also used, resulting from the pressing of red grapes from the species *Vitis vinifera*, *Cabernet sauvignon* variety, 2010 harvest.

2.3 SAMPLE PREPARATION

The polished parboiled rice grains were crushed and ground at room temperature in a cutting mill. The milled rice grains were sifted in an 80 mesh (British standard screen) nylon sieve and the marc in a 60 mesh (GERMANI et al., 1997).

The grape marc with 63% moisture was subjected to dehydration in a circulating air oven at a temperature of 40 °C for 16 hours. Food mixtures were prepared with the products of the screening of parboiled rice grains and grape marc. Mix 1 contained 40% rice and 60% grape marc, whereas Mix 2 was composed of 60% rice and 40% grape marc. The opposing concentrations were defined whit the aim of evaluating the biological response of the increment of this raw material on the diet. The pellets were prepared in the electric mixer by adding 10ml of soybean oil per 1 kg of diet and water qsp.

2.4 DIET

The experimental diets were formulated according to the recommendations of the American Institute of Nutrition (AIN-93M) (REEVES et al., 1993), all isocaloric and isoproteic (MAHAN and SCOTT-STUMP, 2010). Diets were made according to the recommendations of AOAC (2006). Soybean oil doesn't contain isoflavone, then, doesn't express the estrogenic and antiestrogenic influence on health (JOOYANDEH, 2011).

In the hypercholesterolemic diets, commercial cholesterol of 1% was added to induce hypercholesterolemia in animals (ROSSI et al., 2000). After formatting the pellets, they were dried in an air-forced circulation stove at 40°C for 6 hours and stored in polyethylene bags in a freezer at -18°C.

2.5 DETERMINATION OF TOTAL PHENOLS AND ANTHOCYANINS

The total phenol content of Mix 1 and Mix 2 was determined through the method proposed by Singleton and Rossi (1965). Anthocyanin analysis of Mix 1 and Mix 2 was performed according to Vanini et al. (2009).

2.6 BIOLOGICAL ASSAY

The biological assay was conducted under controlled conditions of temperature and humidity (23±1ºC and 50 to 60%, respectively) and a photoperiod of 12 hours. The experiment was divided into two periods: adaptation and treatment. Diets were administered in the form of pellets containing Mix 1 and Mix 2. The animals were weighed and divided into four groups of six animals each. The biological assay lasted 57 days, with the first seven days corresponding to the period of adaptation, in which the animals were given water and diet from the Central Animal Laboratory-UFPEL "*ad libitum*". During the following 50 days of treatment, they were fed experimental diets. At the end of the experiment, after fasting for 12 hours, the animals were anesthetized with dextroketamine hydrochloride and euthanized. Their blood was collected by cardiac puncture using a heparinized syringe and placed in test tubes. The epididymal fat and the liver of the rats were weighed after removal. The epididymal fat index and hepatosomatic relationship were obtained according to Melo et al. (2007). For monitoring the weight gain, the animals were weighed three times a week on alternate days. Weight gain was calculated by the difference between weights at the beginning and end of the experiment. Feed intake was daily observed and the amount of feed offered was restored daily or as needed. The feed efficiency coefficient (FEC) was calculated according to Sgarbieri (1996).

2.7 BIOCHEMICAL EVALUATION

Plasma analyses of glucose, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), triacylglycerols, urea, creatinine, glutamic oxaloacetic transaminase (SGOT), glutamic pyruvic transaminase (GPT) and alkaline phosphatase were quantified by the enzymatic methods of Labtest Diagnóstica® (2011).

2.8 STATISTICS

The data were analyzed for normality with the Shapiro-Wilk test; homoscedasticity with the Hartley test; and independence of residuals with graphical analysis. Data were then subjected to analysis of variance by F test ($p \leq 0.05$). If a statistical significance was observed, the effects of the mix were evaluated by t-test ($p \leq 0.05$); the effects of the diets compared to the control (standard) by Dunnett test ($p \leq 0.05$); and between diets by Duncan test ($p \leq 0.05$) to choose the best diet on nutritional parameters.

3 RESULTS AND DISCUSSION

3.1 TOTAL PHENOLS AND ANTHOCYANINS

Table 1. Total phenol content and total anthocyanins of Mix 1 and Mix 2.

Samples	Total phenols*	Total anthocyanins**
Mix 1	484.67±28.00 A[1/2/]	8.26±0.87 [1/]
Mix 2	151.67±11.72 b	7.00±0.35
Overall average	318.17	7.76
CV (%)	6.7	9.5

[1/] Means of three determinations ± standard deviation. [2/] Means followed by the same letter in the column do not differ by t-test ($p \leq 0.05$). CV: coefficient of variation. Mix 1: 40% of parboiled rice and 60% of grape marc. Mix 2: 60% of parboiled rice and 40% of grape marc. * Expressed as: mg GAE/g; ** expressed as: mg 100/g.

As shown in Table 1, Mix 1 had the highest concentration of grape marc and showed higher levels of total phenols (484.67 mg GAE/g) compared to Mix 2 (151.6 mg GAE/g).

Table 1 also shows the anthocyanin concentration in Mix 1 is 18% greater than in Mix 2. Similar data were found by Soares et al. (2008) in Isabel and Niagara grape marc and by Rockenbach et al. (2008). In vitro and in vivo studies show that anthocyanins can mitigate the oxidative stress involved in the atherosclerotic process. Several mechanisms may be involved in this process, such as the ability of anthocyanins to inhibit the oxidation of LDL (CHANG et al., 2006) and reduce the oxidative injury of vascular endothelial cells (YI et al., 2010).

3.2 FEED INTAKE, WEIGHT GAIN, AND FEED EFFICIENCY COEFFICIENT (FEC %)

Table 2. Feed intake, weight gain, and FEC of male *Wistar* rats fed experimental diets based on Mix 1 and Mix 2.

Diet	Feed intake (grams)	Weight gain (grams)	Feed efficiency coefficient
Standard diet (AIN 93M)	1137.97±0.91	94.30±12.57[2]	7.66±0.34
Diet + Cholesterol (1%)	1253.60±15.09b [1/]*	118.38±12.97	10.14±0.91
Diet + Chol. + Mix 1	1285.39±25.84a*	124.00±14.81	8.37±0.96
Diet + Chol. + Mix 2	1223.37±15.55c*	116.72±11.83	9.31±0.91
Overall average	1225.08	114.6	8.98

CV (%)	1.4	11.5	9.6

*, ns Significant and not significant, respectively, compared to the control (standard diet) by Dunnett test (p≤0.05). 1/ Means followed by the same letter in the column do not differ by the Duncan test (p≤0.05) when diets are compared. 2/ Means of six measurements ± standard deviation. CV: coefficient of variation. NF: not significant by F test (p≤0.05). Chol.: Cholesterol. Mix 1: 40% of parboiled rice and 60% of grape marc. Mix 2: 60% of parboiled rice and 40% of grape marc. Feed efficiency coefficient: Weight gain/diet consumption x 100.

The Mix 1 diet had the highest consumption (Table 2) but did not differ from the cholesterol group (1%), so the animals fed a diet with the greatest grape marc concentration showed higher consumption (Table 2). This result observed in the treatments with the highest proportion of grape marc is probably promoted by the concentration of carbohydrates, fats, and alcohols present in this alternative ingredient, which have palatalizing and flavoring effects (FERREIRA et al., 2007).

Regarding animal weight gain, there was no significant difference between treatments because diets were isoproteic, isocaloric, and isolipidic, except for the treatment Mix 1 where the rats gained 31.5% more weight than the standard diet, due to the highest consumption of diets in that treatment. The FEC did not differ significantly among the experimental diets. Similar findings occurred in a study by Ishimoto (2008), which examined healthy and hypercholesterolemic hamsters fed with pomace extracts and grape marc flour from winemaking and juice production and observed FEC of 12.0% and 12.2% respectively, without significant differences between groups. It was observed that the cholesterol diet had an FEC above 22.3% compared to the standard diet. The best response, i.e. the lowest rate between consumption and weight gain, was found in the treatment of the Mix 1 diet.

3.3 EPIDIDYMAL FAT MASS (G), EPIDIDYMAL FAT RATIO, LIVER WEIGHT (G), AND THE HEPATOSOMATIC RATIO OF MALE RATS

Table 3. Epididymal fat mass, epididymal fat ratio, liver mass, and the hepatosomatic ratio of male *Wistar* rats fed experimental diets with Mix 1 and Mix 2.

Diet	Epididymal fat mass (grams)	Epididymal fat ratio %	Liver mass (grams)	Hepatosomatic ratio (%)
Standard diet (AIN 93M)	5.14±0.87	1.43±0.22 2/	9.15±1.47	2.45±0.15 2/
Diet + Cholesterol (1%)	6.55±0.69 b 1/ns	1.66±0.07b ns	15.99±1.29a 1/*	3.68±0.24a*
Diet + Chol. + Mix 1	9.49±1.54a*	2.08±0.32a*	15.91±2.53a*	3.54±0.24a*
Diet + Chol. + Mix 2	6.45±1.19b ns	1.81±0.19ab*	14.21±1.73a*	3.90±0.17a*
Overall average	6.77	1.71	13.48	3.30
CV (%)	16.1	11.7	12.9	6.1

*, ns Significant and not significant, respectively, compared to the control (standard diet) by Dunnett test (p≤0.05). 1/ Means followed by the same letter in the column do not differ by the Duncan test (p≤0.05) when diets are compared. 2/ Means of six measurements ± standard deviation. CV: coefficient of variation. Chol.: Cholesterol. Mix 1: 40% of parboiled rice and 60% of grape marc. Mix 2: 60% of parboiled rice and 40% of grape marc. Epididymal fat ratio: epididymal fat weight/body weight x 100.
Source: Prepared by the authors.

According to Table 3, no significant difference was observed in epididymal fat mass between treatments about standard diet, except for Mix 1 due to the higher dietary consumption, as shown in Table 2. With the addition of cholesterol (1%) to the diet AIN 93M, there was an increase of 27.4% in epididymal fat to the standard diet. Rats fed with Mix 1 diet had an increased epididymal fat mass of 84.6% compared to rats fed the standard diet and 44.9% compared to the cholesterol diet (1%). Walter (2009), who assessed the physiological effect of rice grains with light brown, red, and black pericarp on rats, found different results, since animals that consumed feed containing rice grains presented significantly lower epididymal fat weight than those in the control group. Regarding the epididymal fat ratio, the Mix 1 and Mix 2 diets differed significantly ($p \leq 0.05$) compared to the standard diet.

Between diets, it was observed that the cholesterol diet increased epididymal fat content by 16% compared to the standard diet. The cholesterol diet did not differ among treatments, except from the Mix 1 diet, which is consistent with the results found in the analysis of epididymal fat.

It can be concluded that the hypercholesterolemic diet used in this study was sufficient to cause obesity in mice since the adiposity index of the animals was increased compared to their respective controls. Similar data were found in the study by Denardin et al. (2009), who observed that there was no effect of dietary intake with rice on the epididymal fat of animals.

Also in Table 3, all treatments showed significant differences in liver weight relative to the standard diet. With the addition of synthetic cholesterol (1%) in the diet AIN 93M, there was an increase of 75% in body weight, which also occurred in other treatments. The increased liver weight may indicate increased metabolic activity in the body, leading to liver overload due to cholesterol (RODRIGUES 2011). These results are in agreement with the data found by Macqueen et al. (2007), which showed an increase in body weight of non-obese rats ingesting a high-fat diet. All diets differed significantly ($p<0.05$) from the standard diet with the hepatosomatic ratio. The cholesterol addition to the standard diet increased the hepatosomatic ratio by 50%. There was no significant difference between diets concerning the cholesterol diet (1%). These findings are in agreement with Monks (2010), who studied the effect of different degrees of polishing rice in the percentage ratio between the liver mass and the final weight of the rats and found no significant differences between the experimental groups.

3.4 TOTAL CHOLESTEROL, HIGH-DENSITY LIPOPROTEIN (HDL), LOW-DENSITY LIPOPROTEIN (LDL), AND VERY LOW-DENSITY LIPOPROTEIN (VLDL)

Table 4. Profiles of total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) of male *Wistar* rats fed experimental diets with Mix 1 and Mix 2.

Diets	**Total cholesterol (mg d/L)**	**High density lipoprotein - HDL (mg d/L)**	**Low-density lipoprotein - LDL (mg d/L)**	**Very low density lipoprotein - VLDL (mg d/L)**
Standard diet (AIN 93M)	67.67±5.68	23.16±2.04[2/]	28.40±4.04	8.75±2.63
Diet + Cholesterol (1%)	83.50±7.09a[1/] *	15.33±1.37b*	50.00±4.73a*	12.50±1.29b*
Diet + Chol. + Mix 1	65.60±4.72b [ns]	18.75±3.40a*	35.50±3.83b*	15.67±1.53a*
Diet + Chol. + Mix 2	72.00±4.60b [ns]	19.50±2.43a*	40.67±4.63b*	13.00±1.73b*
Overall average	72.48	19.23	39.09	12.31
CV (%)	7.8	11.9	11.10	15.3

*, [ns] Significant and not significant, respectively, compared to the control (standard diet) by Dunnett test ($p \leq 0.05$). [1/] Means

followed by the same letter in the column do not differ by the Duncan test (p≤0.05) when diets are compared. [2/] Means of six measurements ± standard deviation. CV: coefficient of variation. Chol.: Cholesterol. Mix 1: 40% of parboiled rice and 60% of grape marc. Mix 2: 60% of parboiled rice and 40% of grape marc.

The diet supplemented with cholesterol (1%) showed statistical significance in total cholesterol. The other diets showed no differences compared to the control (standard diet - AIN 93M) (Table 4). Molena-Fernandes et al. (2010) compared the effects of golden and brown flax flour on lipid profile and weight gain in *Wistar* rats, and average total cholesterol levels did not differ significantly between groups. This behavior of cholesterol is also consistent with the study by Roberto (2012), who evaluated the effect of diets designed with guava residue (peel and seed) as a source of fiber on the parameters of biological response in *Wistar* rats. As observed in Table 4, there was a 23.2% increase in total blood cholesterol levels due to the presence of 1% synthetic cholesterol in the AIN 93M diet. It should be noted that Mix 1 diet promoted lower plasma cholesterol concentrations (21.4%) than the cholesterol diet. Regarding the analysis of lipoprotein HDL, only Mix 1 diet showed statistical significance (p≤0.05) when compared to the standard diet. According to Table 4, if hypercholesterolemia was induced, rats fed the cholesterol diet had decreased levels of blood HDL by 33.3% compared to the standard diet. Therefore, Mix 1 and Mix 2 diets, which were hypercholesterolemic, showed an increase of HDL in the blood of rats by 22.3% and 32.6%, respectively. In the study by Soares-Filho et al. (2011), rats consuming moderate amounts of red wine showed an increase of 6.63% in the serum levels of HDL, compared with rats consuming the control diet. Helbig (2007) found a rate of 38.29% in blood levels of HDL in rats fed parboiled rice. Our results show that plasma HDL increased when the concentrations of Mix 1 and 2 were elevated in the diets (Table 6). According to Lerma-Garcia et al. (2009), the oryzanol present in the lipid fraction of parboiled rice can raise levels of HDL. HDL is extremely important because they help remove cholesterol already deposited, reducing the risk of atherosclerosis and heart attack (Andrade, 2006). With plasma concentrations of LDL lipoprotein (Table 6), it was observed that the cholesterol diet (1%) increased blood levels of LDL by 78.6% compared to the diet AIN 93M. All diets were significantly different (p≤0.05) from the cholesterol diet (1%), especially the reduction of lipoprotein LDL by 29% in Mix 1. Pérez-Jiménez et al. (2008) conducted a study of diet supplementation in humans with grape antioxidant fiber, reducing the total cholesterol, LDL, and triglycerides in hypercholesterolemic individuals. The low-density lipoprotein (LDL) tends to be deposited in the arteries and is associated with the onset and acceleration of the atherosclerotic process. Oxidative modification of LDL is an important factor in the development of atherosclerotic lesions (GIEHL, 2007). As for the contents of very low-density lipoprotein (VLDL), all diets showed significant differences compared to the control (standard diet - AIN 93M). Rats fed diet and cholesterol and Mix 1 diet presented the greatest VLDL cholesterol levels.

3.5 PROFILES OF GLUCOSE, TRIACYLGLYCEROLS, UREA, AND CREATININE

Table 5. Profiles of glucose, triacylglycerols, urea, and creatinine in male *Wistar* rats fed experimental diets with Mix 1 and Mix 2.

Diets	Glucose (mg/dL)	Triacylglycerols (g/dL)	Urea (mg/dL)	Creatinine (mg/dL)
Standard diet (AIN 93M)	118.75±10.72	47.33±10.69[2/]	44.67±3.50	0.87±0.03[2/]
Diet + Cholesterol (1%)	123.40±14.64 ab[1/] ^{ns}	67.20±13.92a^{ns}	44.40±4.33a[1/]^{ns}	0.91±0.04a^{ns}
Diet + Chol. + Mix 1	117.75±11.12 b^{ns}	78.00±7.00a*	29.00±3.56c*	0.79±0.03b*
Diet + Chol. + Mix 2	138,00±6,00 a^{ns}	66.20±8.23a^{ns}	37.80±4.02b*	0.81±0.04b^{ns}
Overall average	125.17	65.19	39.75	0.84
CV (%)	8.8	16.4	9.7	4.4

*, ^{ns} Significant and not significant, respectively, compared to the control (standard diet) by Dunnett test (p≤0.05). [1/] Means followed by the same letter in the column do not differ by the Duncan test (p≤0.05) when diets are compared. [2/] Means of six measurements ± standard deviation. CV: coefficient of variation. Chol.: Cholesterol. Mix 1: 40% parboiled rice and 60% of grape marc. Mix 2: 60% to 40% parboiled rice and grape pomace.

As shown in Table 5, serum glucose concentrations did not differ between the study groups at the end of the experiment. Rats fed the cholesterol diet (1%) showed a 4.5% increase in fasting glycemia about the AIN 93M. The lowest glucose level was found in treatment Mix 1. Glycemic control preserves endothelial function and reduces the risk of developing cardiovascular problems. Positive effects on glucose homeostasis with polyphenols and plant extract rich in polyphenols, in vitro and animals, have been reported (HANHINEVA et al., 2010). In mice that received a high-fat diet, the addition of resveratrol increased their insulin sensitivity and the number of mitochondria in their liver (Baur et al. 2006). Moura et al. (2012) subjected rats to a high-fat and hypercaloric semi-purified diet with 35% fat: 31% of animal origin, 39% saturated fat, and 4% vegetable origin (soybean oil), and observed that serum glucose concentrations did not differ between the groups at the end of the experiment. According to Monks (2010), food digested slowly or with a low glycemic index has been associated with improved diabetes control and reduced blood lipids. As for triacylglycerol levels, Mix 1 diet showed a statistical difference from the diet AIN 93M, whereas other diets did not differ. There was no significant difference among diets, and rats fed Mix 1 diet showed the highest levels of triacylglycerols. In the study by Walter (2009), blood concentrations of glucose, total cholesterol, HDL cholesterol, and triacylglycerols during fasting did not differ between treatments. Mixed results were observed in other studies evaluating the consumption of concentrated fractions of rice with black pericarp on these parameters. In the work developed by Chiang et al. (2006), there was no difference in plasma levels of triacylglycerols and total cholesterol between mice consuming a control diet or containing extract of rice with black pericarp, but HDL cholesterol levels were significantly higher for the latter group.

Regarding urea analysis (Table 5), it was observed that the cholesterol diet did not differ statistically from the standard diet AIN 93M. In Mix 1 diet, with higher amounts of grape marc, the animals showed a reduction of 35% in blood levels of urea and of 15.4% in the Mix 2 diet. Bezerra et al. (2008) highlighted the protective effect of *Vitis vinifera* on renal function in rats submitted to renal ischemia by clamping for

40 minutes. The diet with greater grape marc concentration showed better plasma urea levels in rats. As for plasma creatinine levels, shown in Table 5, it was observed that Mix 1 diet showed a difference compared to the control (standard diet - AIN 93M), but there was no statistical difference between the diets.

3.6 ACTIVITIES OF THE ENZYMES GLUTAMIC OXALOACETIC TRANSAMINASE (SGOT), GLUTAMIC PYRUVIC TRANSAMINASE (GPT), AND ALKALINE PHOSPHATASE (U/L) IN RATS

Table 6. Activities of the enzymes glutamic oxaloacetic transaminase (SGOT), glutamic pyruvic transaminase (GPT), and alkaline phosphatase (U/ L) in male *Wistar* rats fed experimental diets with Mix 1 and Mix 2.

Diet	SGOT (U/L)			GPT (U/ L)			Alkaline phosphatase (U/L)		
Standard diet (AIN 93M)	156.00±14.53			56.00±4.08	[2/]		74.80±6.98		
Diet + Cholesterol (1%)	221.20±10.92	b[1/]	*	60.00±0.82	b[ns]		123.20±9.63	a	*
Diet + Chol. + Mix 1 30%	246.33±9.71	a	*	62.17±7.22	ab[ns]		114.80±10.73	ab	*
Diet + Chol. + Mix 2 30%	214.33±9.71	b	*	69.75±6.40	a	*	106.80±7.46	b	*
Overall average	211.14			62.00			104.9		
CV (%)	5.3			9.0			8.4		

*, ns Significant and not significant, respectively, compared to the control (standard diet) by Dunnett test (p≤0.05). [1/] Means followed by the same letter in the column do not differ by the Duncan test (p≤0.05) when diets are compared. [2/] Means of six measurements ± standard deviation. CV: coefficient of variation. Chol.: Cholesterol. Mix 1: 40% of parboiled rice and 60% of grape marc. Mix 2: 60% of parboiled rice and 40% of grape marc. SGOT - glutamic oxaloacetic transaminase. GPT - glutamic pyruvic transaminase.
Source: Prepared by the authors.

As shown in Table 6, all the diets were significantly different in SGOT (p≤0.05) when compared to the standard diet (control), i.e., there was increased enzyme activity in all groups. However, the values found for the enzyme SGOT did not differ from the reference values (81 to 180.0 U/L) according to Melo et al. (2012). There was an increase of 42% in SGOT enzyme in the cholesterol diet (1%) when compared to the standard diet. It was observed that Mix 1 diet caused an increase of the enzyme by 11.4% with the cholesterol diet (1%), indicating higher enzyme activity in these treatments. The results differed from those found by Monks (2010), who analyzed the effects on rats of rice polishing intensity on plasma levels of liver enzymes alkaline phosphatase (ALP) and alanine aminotransferase (ALT) and registered ALP values between 37.8 U /L to 39.8 U/L and ALT from 24.6 U/L to 28.8 U/L.

Regarding the GPT, most showed no significant difference when compared to the standard diet (control). The values of the enzymatic activity of GPT did not differ from the reference values (36 to 58.0 U/L) found in the study by Melo et al. (2012). Cholesterol diet treatment (1%) showed an increase in enzyme activity by 7.1% compared to the diet AIN 93M. Mix 2 diet showed higher enzymatic activity. According to Monks (2010), the SGOT and GPT enzymes serve as specific indicators to diagnose possible hepatobiliary and hepatocellular damage, as they are released into the bloodstream when the metabolic rate is increased in the liver.

All diets were different concerning the alkaline phosphatase when compared to the standard diet (control). The cholesterol diet (1%) presented the highest enzyme activity (64.3%) compared to the standard diet AIN 93M. However, there was no significant difference between the diets, and the values found showed no enzyme changes since the maximum reference value of enzyme activity is 79-196 U/L according to Melo et al. (2012).

The elevation of the hepatics enzymes shows directly proportional to the appearance of the symptoms of metabolic syndrome like hypertension and atherosclerosis, which is the fundament of this research (ARAÚJO et al., 2005).

4 CONCLUSIONS

The addition of parboiled rice and grape marc improved nutritional assessment parameters of hypercholesterolemic diets with increased concentrations of protective factors. The greatest proportion of grape marc in the diet caused an increase in the availability of antioxidants, reducing blood levels of LDL lipoprotein and cholesterol without compromising parameters related to renal function. The presence of parboiled rice in a hypercholesterolemic diet increased the plasma levels of HDL lipoprotein. This study indicates that the consumption of these food sources assists in the control of dyslipidemia. The data don't care enough to show the efficiency of the diets about atherosclerosis, but, the biochemical parameters indicate a significative lipidic reduction, which is predictive of cardiac protection.

ACKNOWLEDGEMENTS

We would like to thank the Higher Education Personnel Improvement Coordination (CAPES), the National Council for Scientific and Technological Development (CNPq), the Foundation for Research Support of the Rio Grande do Sul State (FAPERGS), the Department of Science and Technology of the State of Rio Grande do Sul (SCT-RS), Food Technological Innovation Pole of the Southern Region and Laboratory Dr. Rouget Perez (Pelotas, RS, Brazil).

REFERENCES

ALONSO, A.M., GUILLN, D.A., BARROSO, C.G., PUERTAS, B., AND GARCA, A. 2002. Determination of the antioxidant activity of wine byproducts and its correlation with polyphenolic content. Journal of Agricultural and Food Chemistry, 50(21), 5832-5836.

ANDRADE, A.C.M. 2006. Ação do vinho tinto sobre o sistema nervoso simpático e a função endotelial em pacientes hipertensos e hipercolesterolêmicos. PhD Thesis, University of São Paulo, São Paulo, Brazil.

ANGELOVA, P., & BOYADJIEV, N. 2013. A review on the models of obesity and metabolic syndrome in rats. Trakia J Sci, 11(5).

AOAC - ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 2006. Official methods of Analysis. (18 ed.). Washington, DC, USA.

ARAÚJO, L. M. B., LIMA, D. S., & BATISTA, L. M. 2005. Associação da gama-glutamil transferase e a síndrome metabólica em mulheres obesas. Brazilian Archives of Endocrinology and Metabolism. vol 49 nº 4

BAUR, J.A., PEARSON, K.J., PRICE, N.L., JAMIESON, H.A., LERIN, C., AND KALRA, A. 2006. Resveratrol improves health and survival of mice on a high-calorie diet. Nature, 444, 337-342.

BEZERRA, J. S., TEIXEIRA, W., AND VATTIMO, M.F.F. 2008. Efeito protetor da *Vitis vinifera* na lesão renal aguda isquêmica em ratos. Jornal Brasileiro de Nefrologia,30(2), 99-104.

CHANG, Y.C., HUANG, K.X., HUANG, A.C., HO, Y.C., AND WANG, C.J. 2006. Hibiscus anthocyanins-rich extract inhibited LDL oxidationand LDL-mediated macrophages apoptosis. Food and Chemical Toxicology, 44(7), 1015.

DENARDIN, C.C., BOUFLEUR, N., RECKZIEGEL, P., AND SILVA, L.P. 2009. Influência do consumo de arroz ou de macarrão no desempenho e resposta metabólica em ratos. Alimentos e Nutrição, 20(3), 441-449.

DOWNHAM, A., AND COLLINS, P. 2000. Colouring our foods in the last and next millenium. International Journal of Foods Science and Technology, 35, 5-22.

ELIAS, M.C.; SCHIAVON, R.A. ; OLIVEIRA, M. 2010. Aspectos científicos e operacionais na industrialização de arroz. In: ELIAS, M.C.; OLIVEIRA, M. ; SCHIAVON, R.A. Qualidade de Arroz na Pós-Colheita: Ciência, Tecnologia e Normas. 1. ed. Pelotas: Santa Cruz, v. 1, p. 353-8.

FAO - FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS. 2004.Rice and human nutrition - international year of rice. Retrieved May 6, 2012 from: <http://www.fao.org/rice2004/index_en.htm>.

FERREIRA, W.M., HERRERA, A.D.P.N., SCAPINELLO, C., FONTES, D.O., MACHADO,L.C., AND FERREIRA, S.R.A. 2007. Digestibilidade aparente dos nutrientes de dietas simplificadas baseadas em forrageiras para coelhos em crescimento. Arquivo Brasileiro de Medicina Veterinária e Zootecnia,59(2), 451-458.

GERMANI, R., BENASSI, V.T., AND CARVALHO, J.L.V. 1997. Documento n. 28 - Métodos de avaliação de grão e farinha de trigo. Rio de Janeiro, RJ, Brazil: Embrapa.

GIEHL, M.R., DAL BOSCO, S.M., LAFLOR, C.M., AND WEBER, B. 2007. Eficácia dos flavonóides da uva, vinho tinto e suco de uva tinto na prevenção e no tratamento secundário da aterosclerose. Scientia Medica, 17(3), 145-155.

HANHINEVA, K., TORRONEN, R., BONDIA-PONS, I., PEKKINEN, J., KOLEHMAINEN, M., MYKKANEN, H., AND POUTANEN, K. 2010. Impact of dietary polyphenols on carbohydrate metabolism. International Journal of Molecular Sciences, 11(4), 1365-1402.

HEINEMANN, R.J.B.; FAGUNDES, P.L.; PINTO, E.A.; PENTEADO, M.V.C.; LANFERMARQUEZ, U.M. 2005. Comparative study of nutrient composition of commercial brown, parboiled and milled rice from Brazil. Journal of Food Composition and Analysis, v.18, n.4, p.287-296.

HELBIG, E. 2007. Efeitos do teor de amilose e da parboilização do arroz na formação de amido resistente e nos níveis glicêmico e lipêmico de ratos W*istar*. PhD Thesis, Federal University of Pelotas, Brazil.

ISHIMOTO, E.Y. 2008. Efeito hipoglicemiante e antioxidante de subprodutos da uva em hamsters. São Paulo. 2008. PhD Thesis, University of São Paulo, São Paulo, Brazil.

JAEKEL, L.Z., AND RODRIGUES, R. 2011.The effect of a soybean and rice beverage on the lipid and glycemic metabolisms in hamsters. Ciência e Agrotecnologia, 35(6), 1211-1217.

JOOYANDEH, H. 2011. Soy products as healthy and functional foods. Middle-East Journal of Scientific Research, 7(1), 71-80.

JUBILEU, B.S., SATO, A.J., AND ROBERTO, S.R. 2010. Caracterização fenológica e produtiva das videiras 'Cabernet sauvignon' e 'Alicante' (Vitis vinifera L.) produzidas fora de época, no norte do Paraná. Revista Brasileira de Fruticultura,32(2), pp 451-462.

KOMATSU, T.R., BURITI, F.C.A., AND SAAD, S.M.I. 2008. Inovação, persistência e criatividade superando barreiras no desenvolvimento de alimentos probióticos. Revista Brasileira de Ciências Farmacêuticas, 44(3), pp 329-347.

LERMA-GARCIA, M.J., HERRERO-MARTINEZ, J.M., SIMÓ-ALONSO, E.F., MENDONÇA, C.R.B., AND RAMIS-RAMOS, G. 2009. Composition, industrial processing and applications of rice bran y-orizanol. Food Chemistry, 115, 389-404.

MACQUEEN, H. A., SADLER, D.A., MOORE, S.A., DAYA, S., BROWN, J.Y., SHUKER, D.E.G., SEAMAN, M., AND WASSIF, W.S. 2007. Deleterious effects of a cafeteria diet on the livers of non-obese rats. Nutrition Research, 27, 38-47.

MAHAN, L., AND SCOTT-STUMP, S. 2010. Alimentos, nutrição e dietoterapia. (12 ed.). Rio de Janeiro: Elsevier Editora Ltda.

MALACRIDA, C.R., AND MOTTA, S. 2005. Compostos fenólicos totais e antocianinas em suco de uva. Ciência e Tecnologia de Alimentos, 25(4), 659-664.

MELO, S.S., NUNES, N.S.I., BAUMGARTEN, C., TRESSOLDI, C., FACCIN, G., ZANUZO, K., MICHELS, M.K., CUNHA, N., SPECHT, S., SILVA, M.W. 2007. Efeito da erva-mate (*Ilex paraguariensis* A. St. Hil.) sobre o perfil metabólico em ratos alimentados com dietas hiperlipídicas. Alimentos e Nutrição, 18(4), 439-447.

MELO, D.S., CORRÊA, A.D., MARCOS, F.C.A., SOUSA, R.V., ABREU, C.M.P., AND SANTOS, C.D. 2008. Efeitos da farinha de folhas de mandioca sobre a atividade das enzimas AST, ALT, FA e lipídios hepáticos de ratos *Wistar*. Ciência e Tecnologia de Alimentos, 28(Supl.), 32-37.

MELO, M. G. D., DÓRIA, G. A. A., SERAFINI, M. R., AND ARAÚJO, A. A. S. 2012. Valores de referência hematológicos e bioquímicos de ratos (*Rattus novergicus* linhagem Wistar) provenientes do biotério central da Universidade Federal de Sergipe. Scientia Plena, 8(4). pp 1-6.

MOLENA-FERNANDES, C. A., SCHIMIDT, G., NETO-OLIVEIRA, E. R., BERSANI-AMADO, C.A., AND CUMAN, R.K.N. 2010. Avaliação dos efeitos da suplementação com farinha de linhaça (*Linum usitatissimum* L.) marrom e dourada sobre o perfil lipídico e a evolução ponderal em ratos *Wistar*. Revista Brasileira de Plantas Medicinais, 12(2), 201-207.

MONKS, J.L.F. 2010. Efeitos da intensidade do polimento sobre parâmetros de avaliação tecnológica e bioquímica, perfil lipídico e conteúdo de ácido fólico em grãos de arroz. PhD Thesis, Federal University of Pelotas, Pelotas, RS, Brazil.

MOURA L.P., DALIA, R.A., ARAÚJO, M.B., SPONTON, A.C., PAULI, J.R., MOURA, R.F., AND MELLO, M.A.R. 2012. Alterações bioquímicas e hepáticas em ratos submetidos à uma dieta hiperlipídica/hiperenergética. Revista de Nutrição, 25(6), 685-693.

MULERO, J., PARDO, F., AND ZAFRILLA, P. 2010. Antioxidant activity and phenolic composition of organic and conventional grapes and wines. Journal of Food Composition and Analysis, 23(6), 569-574.

NEGRO, C., TOMMASI, L., AND MICELI, A. 2003. Phenolic compounds and antioxidant activity from red grape marc extracts. Bioresource Technology, 87(1), 41-44.

NEVES, G.G., GIL, G., FAVRE, G., AND FERRER, M. 2011. **Potencial polifenólico de la uva: índices propuestos y posibles aplicaciones.**Comunicata Scientiae,2(2), 57-69.

PÉREZ-JIMÉNEZ, J., SERRANO, J., TABERNERO, M., ARRANZ, S., DÍAZ-RUBIO, E., GARCIA-DIZ, L. GOÑI, I., AND SAURA-CALIXTO. 2008. Effects of grape antioxidant dietary fiber in cardiovascular disease risk factors. Nutrition, 24, 646-653.

PINHO, C.P.S., DINIZ, A.S., ARRUDA, I.K.G., LIRA, P.I.C., CABRAL, P.C., SIQUEIRA, L.A.S., AND FILHO, M.B. 2012. Consumo de alimentos protetores e preditores do risco cardiovascular em adultos do estado de Pernambuco. Revista de Nutrição, 25(3), pp 341-351.

REEVES, P.G., NIELSEN, F. H., AND FAHEY, G.C. 1993. AIN-93 purified diets for laboratory rodents; final report of the American Institute of Nutrition ad hoc writing committee and the reformulation of the AIN-76A rodent diet. Journal of Nutrition, 123(11), 1939-1951.

ROBERTO, S.B. 2012. Resíduo de goiaba: metabolismo em ratos e aplicabilidade em barras de cereais. MSc Dissertation, Federal University of Santa Maria, Santa Maria, RS, Brazil.

ROCKENBACH, I.I., SILVA, G.L., RODRIGUES, E., KUSKOSKI, E. M., AND FETT, R. 2008. Influência do solvente no conteúdo total de polifenóis, antocianinas e atividade antioxidante de extratos de bagaço de uva (*Vitis vinifera*) variedades Tannat e ancelota. Ciência e Tecnologia de Alimentos, 28(Supl.), 238-244.

ROSSI, E.A., VENDRAMINI, R.C., CARLOS, I.Z., UEIJI, I.S., SQUINZARI, M.M., SILVA JUNIOR,S.I., AND VALDEZ, G.F. 2000. Effects of a novel fermented soy product on the serum lipids of hypercholesterolemic rabbits. Arquivos Brasileiros de Cardiologia, 74, 213-215.

RUSSELL, J. AND PROCTOR, S. 2006. Small animal models of cardiovascular disease: tools for the study of the roles of metabolic syndrome, dyslipidemia, and atherosclerosis. Cardiovascular Pathology, 15: 318-330.

SGARBIERI, V.C. 1996. Proteínas em alimentos proteicos: propriedades-degradações- modificações. São Paulo, Brazil: Livraria Varela.

SCHANAIDER, A., & SILVA, P. C. 2004. Uso de animais em cirurgia experimental. Acta Cir Bras, 19(4), 441-7.

SINGLETON, V.L., AND ROSSI JR, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungst acid reagents. American Journal of Enology and Viticulture, 16, 144-158.

SOARES, M., WELTER, L., KUSKOSKI, E. M., GONZAGA, L., AND FETT, R. 2008. Compostos fenólicos e atividade antioxidante da casca de uvas Niágara e Isabel. Revista Brasileira Fruticultura, 30(1), 59-64.

SOARES-FILHO, P.R., CASTRO, I., AND STAHLSCHMIDT, A. 2011. Efeito do vinho tinto associado ao exercício físico no sistema cardiovascular de ratos espontaneamente hipertensos. Arquivos Brasileiros de Cardiologia, 96(4), 277-283.

SOUQUET, J.M., LABARBE, B., LE GUERNEVÉ, C., CHEYNIER, V., AND MOUTOUNET, M.2003. Phenolic composition of grape stems. Journal of Agricultural and Food Chemistry, 48(4), 1076-1080.

SOUZA, T. A. C.; SOARES JÚNIOR, M.; CAMPOS, M. R. H.; SOUZA, T. S. C.; DIAS, T.; FIORDA, F. A. 2013. Bolos sem glúten a base de arroz quebrado e casca de mandioca Semina. Ciências Agrárias, Londrina, v. 34, n. 2, p. 717-728.

VANINI, L.S., KWIATKOWSKI, A., AND CLEMENTE, E.2009. Extraction and stability of anthocyanins from the Benitaka grape cultivar (*Vitis vinifera*L.). Brazilian Journal of Food Technology, 12(3), 213-219.

VENDANA, M. I. S.; ZIEMER, C.; MIGUEL, O. G.; PORTELLA, A.; CANDIDO, L. M. B. 2008. Efeito do processamento na atividade antioxidante de uva. Alim Nutr., v. 19 , n. 2, p. 159-165.

WALTER, M. 2009. Composição química e propriedades antioxidantes de grãos de arroz com pericarpo marrom-claro, vermelho e preto. PhD Thesis, Federal University of Santa Maria, RS, Brazil.

WALTER, M., SILVA, L. P., AND DENARDIN, C. C. 2005. Rice and resistant starch: different content depending on chosen methodology. Journal of Food Composition and Analysis, 18(4), 279-285.

WALTER, M., MARCHEZAN, E., AND ÁVILA, L.A. 2008. Arroz: composição e características nutricionais Ciência Rural, 38(4), 1184-1192.

YI, C.; SHI, J.; KRAMER, J.; XUE, S.; JIANG, Y.; ZHANG, M.; MA, Y.; POHORLY, J. 2009. Fatty acid composition and phenolic antioxidants of winemaking pomace powder. Food Chemistry, v.114, p.570-576.

YI, L., CHEN, C., JIN, X., MI, M., LING, W., AND ZHANG, T. 2010. Structural requirements of anthocyanins in relation to inhibition of endothelial injury induced by oxidized low-density lipoprotein and correlation with radical scavenging activity.FEBS Letters, 584(3), pp 583-590.