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Effect of sorghum based resistant starch rich product supplementation on lipid profile of healthy individuals.

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Abstract: *A sorghum based designer rawawas produced containing 36 percent resistant starch (RS) with cyclic cooling and heating processing combined with de-branching enzymes. The effect of consumption of the designer rawaon fasting glucose, PP glucose, total cholesterol, triglycerides, HDL-C and LDL-C was studied using rice rawaas control food. A total of 14 healthy subjects (male -7, female -7) with age group ranging from 18-22 yrs were selected for the study. Single blinded cross over design was used, where the subjects were unaware about the food. Four types of most commonly used recipes were prepared using 50 g (18g of resistant starch) of either designer rawaor control rawa(5 g of Resistant starch) using standards procedures and supplemented for 21 days and after one week wash out period control food was supplemented for 21 days, during the study period the subjects were allowed to consume their regular foods. The studies on lipid profile revealed that there was a significant effect of RS food supplementation on TC, TG, HDL-C, LDL-C (P =0.001, 0.004, 0.00, 0.002). However the difference was not uniform in both the sexes. The study demonstrated that consumption of designer rawaalong with regular diet is beneficial in terms of lipid profile.*

I. Introduction

Just like the fat in body, cholesterol is also a fat or lipid that is produced in the liver and is necessary for the proper functioning of the body. When cholesterol levels are in the normal range, in a healthy body, the blood flows freely through the veins and arteries. When the cholesterol levels are high, it starts forming clots (plaques) in the blood vessels causinghypertension (high BP), angina (chest pain), heart attacks, strokes andperipheral vascular diseases. As cholesterol is a fat that is insoluble in blood, it needs something to carry it or transport it in our body. LDL, HDL, VLDL are small molecules called lipoproteins that help with the same.**Good' cholesterol** is good because they help get rid of excess cholesterol by transporting them from the blood vessels to the liver for excretion. HDL (High Density Lipoprotein) is a good cholesterol.

'Bad' cholesterol is bad because it adds cholesterol to your blood by transporting it from the liver. Apart from that they also lead to the formation of plaque which puts you at risk of suffering from high BP,chest pain, heart problems etc. Formed from VLDL (Very Low Density Lipoprotein), it is this compound that helps mobilise cholesterol from the liver depositing it in your blood vessels.

Triglycerides are another type of fat or lipid that combine with cholesterol to form a compound called plasma lipids, that then get deposited in your blood vessels. Your body stores excess fat (that you get from food) in the form of triglycerides so that during times when you do not eat (like between meals) it can use these fat stores as a form of energy.

Grain sorghum is a rich source of phytochemicals that could potentially benefit human health. Sorghum is considered the world's fifth most important cereal after wheat, rice, maize, and barley. Worldwide, over 35% of sorghum is grown directly for human consumption. Sorghum and millets are gaining popularity due to their potential health benefits. Research has been carried out on the development of resistant starch in rice, maize, beans etc., and few studies were conducted on testing the physiological benefits. Since there were no studies on the physiological benefits of resistant starch produced from sorghum, the present investigation was undertaken. The process technology of improving the resistant starch content in sorghum using the combination of thermal and enzymatic methods was developed under the National Agricultural Innovative project on millet value chain of the university. The product was named as Designer Rawa, which has superior cooking and sensory quality over the regular rawa.

Park et al. (2004) this study was examined the effect of resistant starch on hypolipidemic actions, blood glucose, insulin levels and humoral immune responses in healthy overweight subjects. Healthy overweight subjects (over 120% of their ideal body weights) were fed either 24 g/d of resistant corn starch (RS) or regular corn starch (CS) for 21 d with their regular meals. Although this double-blind feeding regiment resulted in no significant changes in their weights or other physical parameters for the relatively acute period of intakes, there were significant lowering effects of serum total cholesterol ($p < 0.05$) in subjects supplemented RS, Compared with the control starch group.

Youneset al., 1995 studied the effect of supplementation of RS (25% raw potato starch), of a steroid sequestrant (0.8% cholestyramine), or both were compared on bile acid excretion and lipid metabolism in rats fed semipurified diets. RS diets led to a marked rise in cecal size and the cecal pool of short-chain fatty acids (SCFA), as well as SCFA absorption; cholestyramine did not noticeably affect cecal fermentation. Whereas cholestyramine was particularly effective at enhancing bile acid excretion, RS was more effective in lowering plasma triglycerides (29%).

II. Materials And Methods

Preparation of Designer rawa(resistant starch rich rawa) from sorghum

The preparation method for designer rawa was adopted from National Agricultural Innovative project (NAIP) of ANGRAU. Sorghum grain was used for the preparation of designer rawa. The grain was subjected to thermal and enzymatic treatments to enhance the RS content. De branching enzyme of 200 IU (the original name of the enzyme is not disclosed due as this process expected to patent) was used in this processing. The process results in to de- branching of branched amylopectin and then rearranging into linear chain and thus resisting the digestion. The resistant starch formation in the grain is due to starch retrogradation and termed as RS3. The grain was then converted into rawain an impact mill.

Sample

It includes Healthy men and women, between 18-25 years, Non-smoking, Not taking medication, non dieting (weight stable in prior 3 months), BMI 18-27, English literacy. Fourteen subjects in the age range of 18 - 25 years were selected for the 64 days study. After screening test subjects were finalized to conduct the study.

Study Design

Single blind cross over study was used means the subjects unaware of kind of food they are eating. After the selection of subjects, they were asked to take normal diet for fifteen days. On the 16th day RS food was supplemented for 21 days and the 7 days were left as washout period and again control food was supplemented for 21 days.

Supplementation

Every day freshly prepared foods, either RS food or control food which was prepared from 50g of rawawas provided to the subjects as breakfast. All the foods were kept in hot packs until supplied to the subjects.

Collection of blood samples

Blood samples were drawn from the subjects with the help of a trained laboratory technician at diagnostic center. Two ml of venous blood specimen was collected after a 12 hour overnight fast into vacutainer collection tubes. Serum was separated immediately after collection by centrifuging at 2000rpm for 10- 15 minutes and transferred to plastic storage vials. The vials were immediately stored at -20°C till further analysis.

Estimation of Lipid Profile

The serum samples were analyzed for lipid profile (Total cholesterol, Triglycerides, HDL-C and LDL-C) using the enzymatic kit method.

III. Results And Discussion

Resistant starch and Nutritional composition of the control and RS rich rawa.

Resistant starch rich rawa (RS food) was prepared using dehulled sorghum by cooling, cooking, drying recycling in combination with de-branching enzyme (the name is not mentioned as this method is under patent processing). The resistant starch food and the control food were analysed for the proximate and the resistant starch content. The results are presented in Table 1. The energy content of both the products is almost nearer. Major difference between RS food and control food is dietary fibre and RS content. The RS food has 36% and 8.5% against 10% and 3.9 % of RS and dietary fibre in control food.

Table 1 Resistant starch and Nutritional composition of the RS rawa and Control rawa

Nutrients	RS rawa	Control rawa
Energy (k cal)	352	345
Protein (g)	5.0	6.8
Fat (g)	1.9	0.05
Carbohydrates (g)	77.7	78.2
Dietary Fiber (g)	8.5	3.9
Resistant Starch(g)	36.0	10.0

Sorghum contains 8-10 g of protein, dehulling results in the loss of protein content depending on the degree of dehulling. The lower content of protein can be ascribed to dehulled grain used for the RS food. Similar results were reported by Charlotte AtsangoSerren (2010) where the protein content of sorghum and rice was reported as 5g and 6.8g. Resistant starch content of cooked sorghum flour ranged from 2.30 to 2.80 g (Platel and Shurpalekar, 1994; Knudsen and Munck, 1988). Among the processed cereals, popped sorghum had the highest RS content of 5.51 g (Platel and Shurpalekar, 1994). The dietary fibre content ranged from 7.5 to 10. 9 (Knudsen and Munck, 1988).

Effect of RS food VS control Food on Lipid levels of healthy subjects

The lipid profile data obtained before and after supplementation of the RS food for 21days and normal food for another 21 days in the crossover study was subjected to paired t-test to find out if there are any mean changes from base line values due to supplementation.

Table 2 Mean lipid values of the RS food VS normal food supplementation

Subjects	RS Food				Control Food			
	TC* (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL* (mg/dl)	TC* (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL* (mg/dl)
Overall	102.0 (1.5)	107.5 (4.9)	41.01 (0.82)	72.33 (1.8)	102.1 (1.5)	107.61 (4.9)	41.04 (0.81)	72.35 (1.79)
Male (n=7)	106.04 (2.19)	99.78 (6.9)	41.78 (1.14)	77.7 (2.54)	106.0 (2.19)	99.78 (6.9)	41.78 (1.14)	77.7 (2.5)
Female (n=7)	98.33 (2.19)	115.4 (6.9)	40.3 (1.14)	67.00 (2.54)	98.3 (2.19)	115.4 (6.9)	40.3 (1.14)	67.0 (2.5)

Figures in parenthesis are SE; *P<0.05

The mean values are shown in Table 4.6 and differences and the results of the significance test are presented in above Table. There was no significant difference among the total subjects as well as between the male and female subjects in the means of all the parameters except total cholesterol (p<0.02) and LDL

It was observed from the results that there was a significant effect of RS food supplementation on TC, TG, HDL-C, LDL-C (P<0.001, 0.004, 0.00, 0.002). However the significant difference was not uniform in both the sexes. For instance the mean TC, TG (P=0.57, 0.73) were not reduced significantly in males, while those were reduced significantly in females. Similarly significant reduction in LDL-C was observed in males and not in females (P=0.069). Only HDL-C was significantly reduced in both males and females (P<0.05), which indicates that better effect was on HDL-C (P=0.00) followed by TC (P=0.001), LDL-C (P=0.002) and TG (P=0.004).The results of the t- test between baseline and the normal food supplemented levels showed that over all there was a statistically significant difference between the baseline and the control food supplemented lipid components of TC, TG, HDL-C and LDL-C (P=0.003,0.007,0.00 and 0.002). Here unlike in the case of RS food lipid values the significant differences were not observed in TC, TG and LDL-C (P= 0.093, 0.10 and 0.06) in females except in HDL-C (0.008).

Table 3 Effect of RS food VS control food on lipid levels of healthy subjects

		Cholesterol			Triglycerides			HDL-C			LDL-C		
Details of pairing		Mean difference	S.D	Sig. (2-tailed)	Mean difference	S.D	Sig. (2-tailed)	Mean difference	S.D	Sig. (2-tailed)	Mean difference	S.D	Sig. (2-tailed)
B-RS	Over all	1.36429	1.24222	.001*	0.6321	0.68546	.004*	-0.5500	0.4238	.000*	1.39786	1.38031	.002*
	Female	1.34714	1.51745	.057	0.5857	0.71281	.073	-0.686	0.501	.011*	1.476	1.766	.069
	Male	1.38143	1.01984	.012*	0.6785	0.71055	.045*	-0.414	0.308	.012*	1.320	0.998	.013*
B-C	Over all	1.25000	1.26744	.003*	0.6035	0.70832	.007	-0.5785	0.4370	.000*	1.37643	1.36784	.002*
	Female	1.23286	1.63503	.093	0.54286	0.75467	.106	-0.743	0.506	.008*	1.476	1.766	.069
	Male	1.26714	0.89809	.010*	0.014	0.038	.049*	37.191	9.522	.000*	1.277	0.956	.012*
RS-C	Over all	-0.11429	0.29051	.165	-0.0285	0.08254	.218	-0.0285	0.0726	.165	-0.021	0.05789	.189
	Female	-0.114	0.302	.356	-0.043	0.113	.356	-0.057	0.098	.172	1.476	1.766	.069
	Male	-0.114	0.302	.356	0.66429	0.71339	.049*	1.320	0.998	.013*	-0.043	0.079	.200

B- RS- Baseline resistant starch food B-C- Baseline control RS-C- Resistant starch food- control food * Significant (P< 0.05) ** significant (P<0.01)

The t-test between RS food and control food supplemented levels revealed that there was no statistically significant difference between them except in HDL-C of males. This might be due to the small amount of RS content (not RS3) residual effect of the RS foods which was supplied initially or insufficient wash out period or might also be due to the good dietary practices followed during the study period. Similar kind of results obtained by Achour et al. (1997) who concluded that retrograded high-amylose corn starch (RS3) leads to a reduction in lipolysis in the post-absorptive period.

Jenkins et al. (1998) reported conflicting data as RS2 and RS3 had no effect on serum lipid profiles. While using the same type of RS, subjects were only tested for two weeks. They opined that RS supplementation requires a longer period of time to promote an effect. Significant lowering effects of serum total cholesterol ($p < 0.05$) and serum LDL-cholesterol ($p < 0.05$) in subjects supplemented RS were observed when 24g/day RS was for 21 days than 24g/g of regular corn starch in healthy over weight subjects(over 120% of their ideal body weights) Park et al. (2004). This gives an idea that RS in any kind of grain could favorably affect the blood lipid levels.

IV. Conclusion

The studies on lipid profile revealed that there was a significant effect of RS food supplementation on TC, TG, HDL-C, LDL-C (P =0.001, 0.004, 0.00, 0.002). However the difference was not uniform in both the sexes. For instance the mean TC, TG (P=0.57, 0.73) were not reduced significantly in males, while those were reduced significantly in females. Similarly significant reduction in LDL-C was observed in males and not in females (P=0.069). Consumption of RS food resulted in the reduction of TC, TG, HDL-C and LDL-C were 1.35, 0.64, 1.48 and 1.76 percent respectively. When sex was considered, only HDL-C was significantly reduced in both male and female (P<0.05), which indicates that better effect was on HDL-C (P=0.00) followed by TC (P=0.001), LDL-C (P=0.002) and TG (P=0.004).

With regards to control food supplementation, over all there was a significant effect (P<0.05) on lipid components of TC, TG, HDL-C and LDL-C (P=0.003, 0.007, 0.00 and 0.002). Here unlike in the case of RS food lipid values the significant differences were not observed in TC, TG and LDL-C (P= 0.093, 0.10 and 0.06) in females except in HDL-C (0.008). The results reveled that there is no significant change from RS food to control food supplementation. This can be attributed to the carryover effect of initial RS food supplementation. This can be a beneficial effect of RS food consumption which indicates that regular inclusion of RS in the diet positively controls the lipid levels.

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