

NUTRITIONAL AND METABOLIC INDICES IN BEVERAGES
AFTER ENZYMATIC DEGRADATION OF STARCH

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ABSTRACT

In the present study the nutrient content of wines and beers was determined after the enzymatic degradation of starch through the metabolic effects of these beverages when supplied in the diet of rats. Control animals [Control, Control (1) and Control (2)] were given a beverage-free diet and experimental rats were fed purified diets supplemented with beverages equivalent to 3 l beer/day or 1 l wine/day for a 60 kg man. The beverage supplemented diets appeared to stimulate growth during the experimental period. Serum analysis showed some differences in the concentration of total serum cholesterol, high density lipoproteins (HDL) and low density lipoproteins (LDL), as well as in alkaline phosphatase levels and urea. These data indicate that beverages may have an influence on the nutritional process particularly with regard to protein and vitamin D metabolism.

INTRODUCTION

There are implications in the literature that beverages may be nutritionally beneficial (1-6). Beverages can form an important part of the energy supply of the diet. It is important to determine the effect of different nutrients present in beverages such as sugars, fatty acids and alcohol on metabolism, since the quantities of these compounds can be varied during the malting and fermentation processes by regulation of enzymes and hence improve the nutritional value of the final product (2,7,8). However, no major research has been reported on the enzymes in connection with the degradation of starch and the nutritional value of beverages after such enzymatic treatment(2,3).

In this study, fermentation and post-fermentation were conducted to estimate the relationship between polysaccharides and fatty acids with a view to it's significance for the nutritional and metabolic value of beverages. It was done through the indices of cholesterol levels, urea, amylase, alkaline phosphatase, mineral content and electrophoretic protein patterns in serum of rats fed beverage supplemented diets as well as through the measurements of food consumption and body weight gains(2,3).

MATERIALS AND METHODS

The nutrient content of Israeli beer samples after enzymatic reactions and South African wines (dry white, low alcohol dry white, dry red and

semi-sweet white) was determined and an assessment made of the nutritional and metabolic effects of these beverages when supplied in diet of rats.

The present investigation was carried out on four types of wine from grapes grown in the Cape Province, South Africa and were made by Stellenbosch Farmers Wineries. The experiments were conducted also on lager beer (10 °B) under processing conditions at the National Brewery Ltd. Netanya, Israel. Six thousand kilogram of raw materials, comprised of 50% malt and 50% sorghum in addition to 240 hl (hectoliter) of water were used in the mash. Enzymes were used to assist in the utilization of adjuncts (Termamyl 60L, Novo-Industria/s; Convertases 70SC & SA, Schwarz) and to supplement the malt quality (Fungamyl 800 L, Novo-Industria/s). Termamyl 60L, Convertases 70SC & SA. This α -amylase liquefies adjuncts. Fungamyl 800 L. This α -amylase hydrolyzes starch and dextrins to fermentable sugars (2,3).

The National Brewery Ltd. used a mash decoction system. One mash was 100% malt, the other was comprised of the sorghum and 10% of the total weight of the malt and contained the Termamyl, Convertases 70SC & SA respectively. Both were mashed at 50°C, and the all-malt mash was retained at this temperature during the liquefaction of the sorghum. The two mashes were then mixed and heated to 76°C after saccharification steps at 60-62°C and at 71°C (1).

Control I was this regular product of National Brewery Ltd. but without the enzymes added. Control II was the all-malt mash, used to obtain comparative data on nutritional and metabolic value. Four test samples were used. Test I, Test II and Test III contained each of 50% malt and 50% sorghum respectively with 0.1% Convertase 70SC, 0.1% Convertase SA; 0.1% Termamyl 60L based on the weight of the sorghum and 0.3g of Fungamyl 800L per hectoliter of wort. Test IV was made also from 50% malt and 50% sorghum plus 0.1% Termamyl 60L based on the weight of the sorghum and 0.4g of Fungamyl 800L per hectoliter of wort(1).

For all samples, the wort was boiled for 1.5 hr with the hop extract added in three portions (110g/hl). After being boiled, the wort was pumped into a settling tank and cooled by means of a plate heat exchanger to a pitching temperature of 10°C. It was pitched with *Saccharomyces Carlsbergensis* yeast, with 50% (v/v) solid content of yeast slurry in a proportion of 0.5 l/hl. At this point enzyme Fungamyl 800L was added to the test wort. The maximum temperature reached during 7-8 days of fermentation was 12°C (1).

Male wistar rats (120g) were housed individually in stainless steel metabolism cages and fed on diet consisting of 70.5% starch, 18.0 ovalbumin, 5.0% salt-mix, 5.0% sunflower oil, 1.0% cod liver oil, 0.3% choline chloride and 0.25% vitamins. Beer-supplemented animals were fed the same diet supplemented with lyophilized beer at a concentration corresponding to an intake of 6.0 ml original beer/day/rat. Wine

supplemented animals were fed also the same diet supplemented with lyophilized wine at a concentration corresponding to an intake of 2.0 ml wine/day/rat. Therefore one rat of 120 g in one day receives 6.0 ml of beer or 2.0 ml of wine. This corresponds approximately to 3 l of beer or 1 l of wine for a 60kg man. All animals received the alcohol equivalent of this beverage intake at a rate of 1.0 ml of 24% ethanol per day by stomach intubation. Food consumption and body weight were monitored. After four weeks on these diets, the animals were sacrificed and blood samples withdrawn from the heart. Control animals [Control, Control (1)] and Control (2)] were given wine-free or beer-free diets in two separate groups. Normal rat serum was taken from the serum bank as a standard for comparison of results received during the experiments (2,3).

Serum samples were taken at the end of the experiment period and analyzed for total protein, albumin, globulin, urea, alkaline phosphatase and amylase. Serum mineral concentrations were determined by atomic absorption spectroscopy. Total, HDL and LDL cholesterol were determined by the methods of Epstein (9) and Ononogbu and Lewis (10).

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) in 5, 7 and 10% (Poly) Acrylamide Gel [(P)AAG] for Protometer [Protein Monomer (subunit)] with Molecular Weight (apparent MW) in Kilo-Dalton, unit 10^3 of MW (kD) and Coomassie Brilliant Blue 250R(CBBR) for staining as well as conventional methods for wine and beer compositions were used in this study (11). The chloroform and acetone extracts of lyophilized samples were used for glycerol determination by IR spectroscopy (12).

RESULTS AND DISCUSSION

Analysis of the nutrient and chemical composition showed that the wines differed with regard to alcohol, reducing sugars, caloric content and glycerol in original wine samples as well as in lyophilized ones (Table IA) (3).

The beer samples after enzymatic reactions with α -amylases differed in some indices (Table IB). Wines with low-alcohol content had less amount of glycerol, which is due to the length of fermentation, as well as alcohol content (Table IA). With the regulation of attenuation limit of fermentation it is possible to produce noncarbohydrate beer, or a beer with a reduced or controlled carbohydrate content (13,14). These differences in the nutrient content of the various wines and beers may indicate a difference in nutritional value when forming part of the diet (Table IB).

Animals fed diets supplemented with dry and semi-sweet white wines particularly showed significantly more body weight gain than the control animals. This is probably the result of increased food consumption in the case of those animals fed dry white wine, but not in the case of those fed semi-sweet white wine (Table IIA) (3).

Table I. COMPOSITION OF WINES AND BEERS

IA

Indices	Types of wine			
	Dry white	Low alcohol dry white	Dry red	Semi-sweet
Lyophilized weight, g/100 ml	1.90	1.74	2.80	5.50
Alcohol, % volume	11.34	9.09	11.85	10.50
Reducing sugars, % weight	1.70	2.50	2.00	28.20
Glycerol, mg/100 ml	130.0	67.0	102.0	98.0
Reducing sugars, % lyophilized weight	8.50	18.00	7.00	45.00
Caloric content Kcal/l	45.65	39.67	48.04	83.65

IB

Indices	Control	Control	Test	Test	Test	Test
	I	II	I	II	III	IV
Lyophilized weight, g/100 ml	2.90	3.70	3.50	3.50	3.50	2.05
Attenuation limit of fermentation, %	74.0	84.9	75.0	74.9	88.5	89.8
Sugar spectrum, %						
Glucose	1	4	1	1	7	10
Maltose	41	50	46	42	55	58
Maltotriose	29	21	27	30	27	9
Dextrins	29	25	26	27	21	25
Glycerol, mg/100 ml	6.0	7.1	5.2	6.0	14.0	17.0
Alcohol, % volume	4.05	5.13	4.05	4.05	4.05	2.20
Caloric content, Kcal/l	16.73	23.90	17.45	18.16	21.27	9.32

NUTRITION REPORTS INTERNATIONAL

Table II BODY WEIGHT GAINS, FOOD CONSUMPTION, AND ANALYSIS OF SERUM OF RATS FED ON DIFFERENT TYPES OF WINE AND BEER

IIA

Wines Component	Normal	Control (1)	Dry white	Low alcohol dry white	Control (2)	Dry red	Semi-sweet white
Food consumption, (g)		357.6	405.4	366.1	345.7	372.2	357.3
Wt. gain, (g)		98.0	112.8*	106.6	87.5	90.2	100.4**
Total protein, g/l	65.0	62.6	61.5	60.6	64.2	59.5	61.3
Albumin, g/l	35.2	34.8	34.2	34.6	36.3	34.0	34.5
Globulin, g/l	32.3	28.5	27.3	26.0	30.2	28.0	28.5
Urea, m mol/l	5.50	5.30	4.40	5.20	5.45	5.40	5.25
Amylase, lu/l	6487.0	6837.0	6270.0	5818.0	6522.0	6214.0	6492.0
Alkaline phosphatase, lu/l	196.0	180.2	161.5	147.8	185.7	171.5	165.3

*p < 0.01

**p < 0.05

Growth of the rats fed beer diets was not significantly different from that of control animals, given a beer-free diet. Three of the beer-supplemented diets had little effect on body weight gain while the other two (Control I and Test I) resulted in a reduced body weight gain during the experimental period when compared with the Controls (Table IIB).

IIB

Beers Component	Control for rats	Control I	Control II	Test I	Test II	Test III	Test IV
Food consumption, (g)	354.4	339.2	351.5	335.3	381.0	354.4	300.1
Wt. gain, (g)	95.4	72.8*	100.7	76.6*	98.3	99.8	69.9
Total protein, g/l	67.2	57.3	69.2	57.3	59.3	60.3	56.5
Albumin, g/l	37.4	35.3	36.4	35.7	35.3	32.5	30.7
Globulin, g/l	31.3	29.3	28.9	32.2	32.4	30.6	28.7
Urea, m mol/l	5.39	5.43	5.40	5.50	5.54	5.10	5.00
Amylase, lu/l	6794.0	6384.0	6400.0	6328.0	6590.0	6240.0	6104.0
Alkaline phosphatase, lu/l	183.4	175.4	170.3	171.4	173.5	160.4	154.3

* p < 0.01

Therefore the beverages supplemented diets appeared to stimulate growth which was shown in the increased food consumption and in the differences of carbohydrate content in cereals for beer samples (Tests III, IV) and grapes (Low alcohol white wine).

The concentration of total serum cholesterol as well as HDL and LDL cholesterol were significantly different in groups fed different wine diets. The concentration of high density lipoproteins (HDL) was increased by feeding the rats dry white and low alcohol white wines, Tests I-IV and low alcohol white wine but dry red and semi-sweet white wines. Control I and II decreased the concentration. The concentration of low density lipoproteins (LDL) was increased with semi-sweet white wine and significantly increased with a low alcohol wine as well as with Test III and IV, while dry white and dry red wines, Controls I and II had no effect. (Fig. 1, pos. a,b).

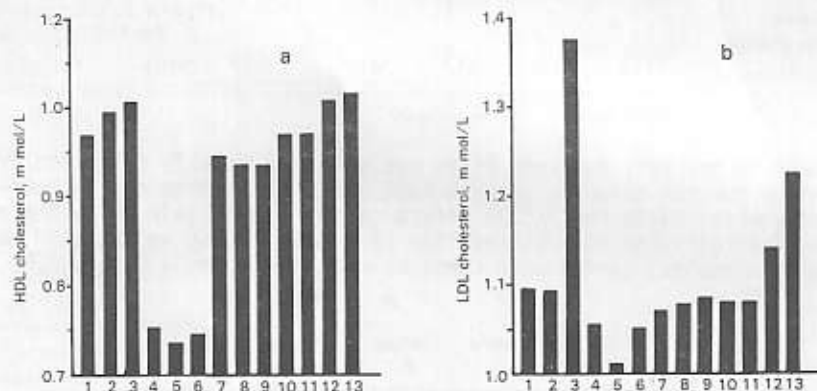


Fig. 1 The different beverage supplemented diets on the HDL (a) and LDL (b) levels in rat serum.
(1 - 13) respectively — Control (1) for rats; dry white; low alcohol dry white; Control (2) for rats; dry red, semi-sweet white wines; Control for rats; Control I; Control II; Test I; Test II; Test III; Test IV.

The total cholesterol concentration was significantly increased by low alcohol wine and Test IV. It was also increased by dry white and semi-sweet white wines and all beer samples, but decreased with dry red wine. The total triglyceride concentration was increased by dry white wine and Control II, but decreased by the other beverage diets, especially low alcohol white wine and Test IV. (Fig. 2, pos. a,b).

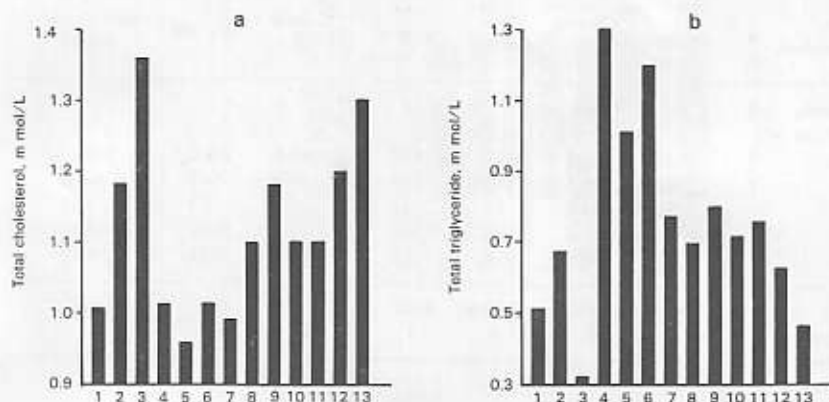


Fig 2 The different beverage supplemented diets on the total cholesterol (a) and total triglyceride (b) levels in rat serum. (1 - 13) respectively — Control (1) for rats; dry white, low alcohol dry white; Control (2) for rats; dry red and semi-sweet white wines; Control for rats; Control I, Control II, Test I, Test II, Test III and Test IV.

Total protein albumin and globulin concentrations showed very slight differences (Table IIB). Serum urea was significantly lower in animals fed the dry white and semi-sweet as well as for low alcohol dry white wine diets; Test III and Test IV indicating a beneficial effect of these beverage diets on protein catabolism (Table II).

Considerably elevated serum amylase is used as an indication of pancreatic related disorders. While the beverages supplemented diets result (Table IIB) in depressed, diminished amylase activity for dry white, semi-sweet and low alcohol dry white wines, Tests III and IV, the reduction is not large enough to indicate a protective effect on the pancreas.

Since alkaline phosphatase is an indication of vitamin D status the results of lowering in all groups fed beverage supplemented diets for different wines and for different beers point to a beneficial effect of beverages on bone metabolism and structure (vitamin D metabolism). Table IIA and B).

Consumption of beverages resulted in very little effect on the mineral balance for wines and for beers in the experimental animals (Table IIC).

III

Wines Component	Normal	Control I	Dry white	Low alcohol dry white	Control II	Dry red	Semi-sweet white
Minerals, mg/100 ml							
Ca	220.0	170.0	160.0	180.0	260.0	200.0	210.0
Mg	14.5	14.7	14.2	14.5	14.8	14.5	14.6
Fe	3.2	3.1	3.2	3.0	3.1	3.3	3.0
Zn	160.0	151.0	155.0	155.0	165.0	157.0	147.0
Cu	107.0	98.0	95.0	101.0	96.0	93.0	97.0
Beers							
Component	Control for rats	Control I	Control II	Test I	Test II	Test III	Test IV
Minerals, mg/100 ml							
Ca	147.0	154.7	147.4	140.4	141.3	149.5	160.5
Mg	16.3	15.3	15.0	17.4	15.9	16.4	16.9
Fe	4.5	5.4	4.0	5.8	4.3	5.9	4.1
Zn	159.4	164.5	160.4	160.3	162.1	161.4	154.5
Cu	106.4	108.5	107.4	107.9	114.3	109.2	110.8

Isoelectric focusing (PAGIF) and SDS-PAGE from whole serum of rats fed the beer samples with different enzymes revealed very slight differences and showed some major bands in the range of MW's (molecular weights) - 17.0 - 169.0 kD. The most prominent fraction was at 169.0 kD for all enzyme-treated samples, except Test IV (Fig. 3,I) and for wines (27.0 - 168.0 kD) (Fig. 3,II).

Received data of HDL and LDL, total cholesterol and triglyceride levels indicate that the beverage supplemented diets significantly effect the concentration of serum cholesterol in different blood fractions. LDL has a detrimental effect on heart disease by promoting arteriosclerosis, while HDL beneficially influences by helping to prevent arteriosclerosis (15).

Serum analysis showed no differences in total protein, albumin or globulin concentrations, but SDS-PAGE and PAGIF revealed slight differences in serum protein composition. Serum analysis also showed significantly lower alkaline phosphatase levels in rats fed beverage supplemented diets indicating a possible beneficial effect of beverages on vitamin D status in these animals. Serum urea was significantly lower in groups fed certain wine and beer diets indicating a possible influence of beverages on protein catabolism. Serum mineral concentrations were similar in all groups (2,3).

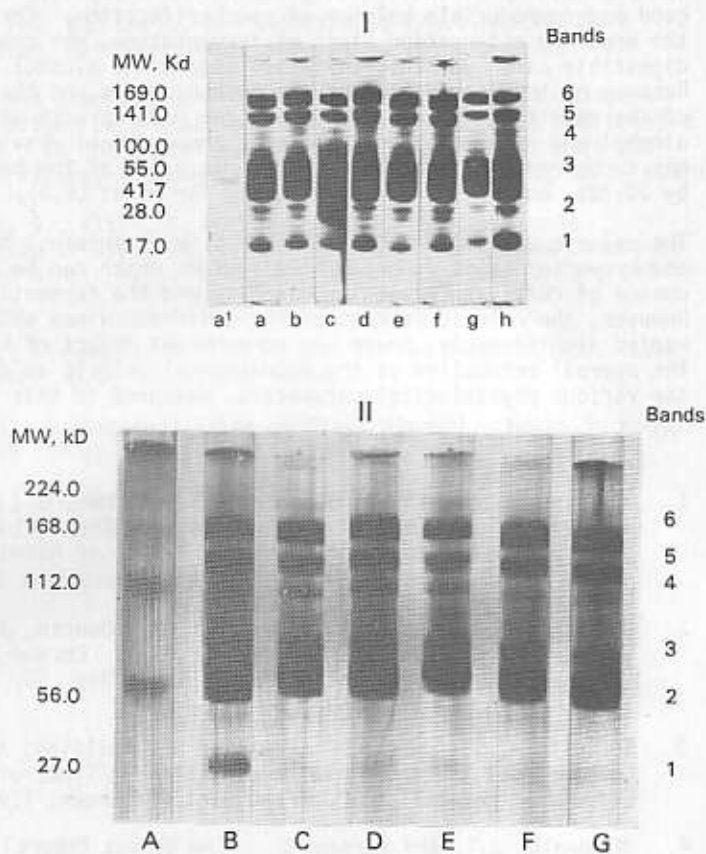


Fig. 3. SDS-PAGE in 5% (P)AAG at pH 7.0 of rat serum proteins (μg protein) from the following samples:

I (a - g) — fractions from Rat Control, Control I, Control II, Test I, Test II, Test III, Test IV; h — normal serum; a¹ — standard protein calibration mixture.

II A, B, C, D, E, F, G, respectively molecular marker, normal, semi-sweet white, dry red, low alcohol dry white, dry white wines and control (3).

The possibility of individually dosing the α -amylases will lead to a good and reproducible balance of saccharification. Corresponding to the apparent attenuation limit of fermentation, the content of digestible carbohydrates, dextrans, sugars and alcohol can be reduced. Because of the degradation of the carbohydrates and the fermentation of the resulting sugars, the final beer ends up with an increased alcohol and reduced caloric content, the original gravity of the wort has to be reduced, and thus the caloric value of the beer is reduced by 20-30%, as well as in the same way for wines (2,3).

The caloric content of wines and beers is of interest to the wineries and breweries as it is one of the factors which can be altered by the choice of cultivar (grapes or cereals) and the fermentation process. However, the caloric content of the different wines and beers studied varied significantly, there was no apparent effect of this factor on the overall metabolism of the experimental animals as determined by the various physiological parameters, measured in this study.

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