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The Vitamin B-Complex Content of Bottled Swiss Grape Juices

by

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Introduction

In recent years numerous investigations have been undertaken in various countries, notably Germany, France and the United States, regarding the vitamin B-complex content of grapes, musts and wines. However, relatively little information has been published on the vitamin content of commercially produced grape juice. DANIAL and MUNSELL (1932) established that two varieties of grapes contained fair amounts of vitamin B, although practically none was found in commercial grape juices. Frozen grape juice as well as other frozen fruits, juices and vegetables were examined for their vitamin content by Marie BURGER et al (1956).

In the following experiments, four juices, made from four varieties of grapes, and bottled in 1957, have been assayed by microbiological methods for ten vitamins of the B-complex. These were as follows: nicotinic acid, biotin, thiamine, the folic acid complex, riboflavin, the vitamin B_6 complex, inositol, p-aminobenzoic acid, choline and pantothenic acid. Microbiological methods were selected over chemical methods because (a) of simplicity and accuracy, (b) interfering substances can be kept to a minimum and (c) chemical methods for some of the vitamins assayed do not exist.

Until the end of World War II, the production of grape juice in Switzerland has been almost non-existant, being limited to experimental and home production. To-day, due to a decrease over the past few years of Swiss white wine consumption, over 6 million liters of grape juice are produced annually in both large and small bottling plants throughout Switzerland. Since the vitamin content of these commercially produced Swiss grape juices has been unknown, this investigation was initiated. In consideration of the need for information of this type, the "Schweizerische Weinbaufond" has sponsored this work.

Experimental Procedure

Bottled Grape juices (two red and two white) were selected at random from four producers. These juices, which had been made from the 1956 grape crop consisted of the following types: Blauburgunder, Räuschling, Riesling X Sylvaner, and Veltliner.

The bottles containing the juices were stored at 10°C. After samples had been withdrawn, the remaining contents of the bottles were stored under toluene at 2°C.

Assay methods

The vitamins used in the preparation of standard solutions were supplied by Hoffmann-La-Roche, Basel, and stored in dessicators over phosphorous pentoxide at room temperature.

The technique described by NYMON and GORTNER (1946) for *Lactobacillus arabinosus* and *L. helveticus* was adopted for the maintenance of stock cultures of the bacterial assay organisms. All were carried as stab cultures on liver-tryptone agar with the exception of *Streptococcus faecalis* which was carried on liver-tryptone agar slants.

Thiamine at the rate of 10 μg per tube (10 ml) was added to the medium carrying cultures of *Lactobacillus fermenti* (BARTON-WRIGHT 1952). With an increase in storage time of *Leuconostoc mesenteroides* 10100, employed in the determination of riboflavin, the assay blanks exhibited a progressively higher growth. The addition of riboflavin at the rate of 10 μg per tube to the liver-tryptone medium carrying *L. mesenteroides* eliminated this tendency with the result that normal blanks were again obtained.

Stock cultures of *Neurospora* mutants were carried as slopes on Difco *Neurospora* culture agar and renewed at fortnightly intervals.

The vitamin content was estimated by the methods cited in Table 1.

Table 1

Assay Methods and Organisms Utilized

Vitamin	Organism	Reference
Nicotinic acid	*L. arabinosus* 17—5 ATCC Nr. 8014	BARTON-WRIGHT (1952)
Biotin	*L. arabinosus* 17—5 ATCC Nr. 8014	LYNES and MORRIS (1948)
Thiamin	*L. fermenti* 36 ATCC Nr. 9338	FITZGERALD and HUGHES (1949)
The folic acid	*Streptococcus faecalis* NCTC Nr. 6459	JONES and MORRIS (1949)
Riboflavin	*Leuconostoc mesenteroides* ATCC Nr. 10100	KORNBERG et al (1948)
Vitamin B_6 complex	*Neurospora sitophila* ATCC Nr. 9276	STOKES et al (1943)
Inositol	*Neurospora crassa* (mutant 37401) ATCC Nr. 9683	BEADLE (1944)
p-Aminobenzoic acid	*Neurospora crassa* (mutant 1633) ATCC Nr. 9278	AGARWALA and PETERSON (1950)
Choline	*Neurospora crassa* (mutant 34486) ATCC Nr. 9277	HOROWITZ and BEADLE (1943) AOAC VIII. Ed. (1955)
Pantothenic acid	*L. arabinosus* 17—5 ATCC Nr. 8014	ANTENER (1958)

Nicotinic acid was determined according to the method described by BARTON-WRIGHT (1952). Difco niacin assay medium was substituted for that recommended by BARTON-WRIGHT.

Biotin was determined by the method of LYNES and NORRIS (1948) with Difco biotin assay medium being substituted as the basal medium.

Thiamine was determined essentially by an improved method of FITZGERALD and HUGHES (1949). At first considerable difficulty was experienced with the use of this method in that the blanks (containing the sulphite treated extract) were far too high. By diluting the inoculum to 20 ml with 0.9 per cent saline solution, instead of to 10 ml as recommended by FITZGERALD and HUGHES, low blanks were obtained.

The method finally adopted for the preparation of the inoculum involved the transfer from a stock culture of a small amount of inoculum to tubes containing 10 ml of waterdiluted (1 : 1) Difco thiamine assay medium enriched with 10 μg of thiamine. After an incubation period of 16 — 18 hours, the culture was centrifuged aseptically, and the cells washed three times by repeated additions of 0.9 per cent saline solution followed by centrifugalization. The washed cells were suspended in 10 ml of saline solution and one drop of the suspension diluted to 25 ml with saline solution. One drop of the diluted suspension was then added to each tube.

Extraction of thiamine was accomplished by hydrolysis with 0.1 N hydrochloric acid according to MÜCKE (1957) instead of by digestion with papain and takadiastase as recommended by FITZGERALD and HUGHES (1949). Difco thiamine assay medium was utilized as the basal substrate.

The folic acid was determined, with some modification, according to JONES and MORRIS (1949) based on the method of TEPLEY and ELVEHJEM (1945). Samples were prepared for assay by extraction with Difco desiccated chicken pancreas as recommended by Methods of Analysis A.O.A.C. (1950). Of the total folic acid content of grapes, juices and wines, HALL et al (1956) found that 60 — 70 per cent was found to be "free" or determinable without enzymatic extraction. KLAUSHOFER (1958) found no difference in the folic acid content of beer which had not been extracted and beer extracts prepared enzymatically from hog's kidneys and/or chicken pancreas. In the work reported here, comparisons were made between the folic acid found in samples which had been extracted with the chicken pancreas enzyme and those which had not been extracted. No significant difference was noted. Therefore, the samples were prepared for assay as follows: The pH of 50 ml aliquots of the juices was adjusted to 7 with potassium phosphate and diluted with water so that one ml contained approximately 0.002 gamma of folic acid. The range of folic acid required to establish a standard curve was 0.001 — 0.005 gamma. The content of each assay tube was inoculated with one drop of a twice washed *Streptococcus faecalis* culture suspended in 50 ml of a 0.9 per cent saline solution instead of 20 ml as recommended by JONES and MORRIS. The basal substrate consisted of Difco folic acid assay medium.

The use of *Leuconostoc mesenteroides* (KORNBERG et al 1948) as the test organism for riboflavin was necessitated by the small ribonflavin content of the juices. The organism was found to respond to 0.0005 gamma of riboflavin, thus allowing for a greater dilution of the test extract than would otherwise have been possible with the use of *Lactobacillus helveticus*. The method was found to be simple and straightforward. Response was measured turbidimetrically at a wave length of 5400 Å. The basal substrate was Difco riboflavin assay medium, which may be utilized equally well with *Leuconostoc mesenteroides* or *Lactobacillus helveticus*.

The vitamin B_6 complex (pyridoxine, pyridoxamine, pyridoxal) was determined by the method of STOKES et al (1943). It has been stated by MORRIS et al (1949) that average losses of 35 per cent of pyridoxine solutions in water occur when submitted to the sulphite-peroxide treatment suggested by STOKES et al. As an alternative, MORRIS et al recommended alkaline extraction of the assay material with 1 N sodium hydroxide. However, the destruction of thiamine by means of alkali was not practical due to the breakdown of the reducing sugars, which in turn lowered the pH of the extract to less than 7 during the period of heating, thus destroying a portion of the B_6 content. TATUM et al (1946) reported that the addition of thiamine to the basal substrate increased the sensitivity of *Neurospora sitophila* to pyridoxine. On the other hand MORRIS et al have shown, that on occasion, the presence of thiamine markedly interferes with the assay. By addition of thiamine at the rate of 0.03 gamma per ml of basal substrate, low blanks and consistant assay values were

encountered. Moreover, it was found possible to harvest the mycelium in three days as against five without the addition of thiamine. Difco choline assay medium was found to be a satisfactory basal substrate.

Other than the use of Difco choline assay medium as the basal substrate, inositol was determined according to the method of BEADLE (1944).

p-Aminobenzoic acid was determined by the method of AGARWALA and PETERSON (1950). The basal substrate consisted of 57 grams of Difco choline assay medium, 4 grams of Difco vitamin-free acid-hydrolyzed casein, 5 grams of asparagine and water to 1 000 ml. For extraction, the juices were diluted 1 : 1 with water, autoclaved one hour at one atmosphere, cooled, and the pH was adjusted to 7 with 3 per cent potassium phosphate before being made to a suitable volume with water.

With the exception of the extraction method, pantothenic acid was determined according to the Official Methods of A.O.A.C. (1955). Takadiastase was utilized for the extraction of the juices, with some modification, according to ANTENER (1958). The method of extraction finally adopted was as follows: To 5 ml of juice, 0.5 per cent acetate buffer (pH 4.5) and 0.02 grams of takadiastase were added. The whole was incubated under toluene for 24 hours at 37° C. After incubation, the pH of the extract was adjusted to 7 with NaOH and steamed for 15 minutes to inactivate the enzyme and drive off the toluene. Upon cooling, the volume of the extract was made to 100 ml with double distilled water.

Choline was determined by the method of Horowitz and BEADLE (1943).

Table 2

The Vitamin Content of Musts and Juices
Given by Various Authors and Compared with Swiss Bottled Juices
(Gamma per 100 ml)

Vitamin	Bottled Swiss Juices				RADLER (1957) Musts	BURGER et al (1956) [1]	CASTOR (1953) Musts	HALL et al (1956) Musts	PERLMAN and MORGAN (1945) Musts
	1	2	3	4					
Nicotinic acid	165	141	213	143	180-880	140-260	80-370	160-240	-
Biotin	1.60	0.62	1.06	1.01	0.1-6.0	-	2	-	-
Thiamine	15.1	trace	7.20	25.0	-	17-23	-	34-50	70-120
Folic acid	0.34	0.19	0.30	trace	-	2-5	-	1-5	-
Riboflavin	4.27	trace	4.13	4.69	-	20-35	6-26	9-16	20-145
B_6 Complex	17	trace	13.5	15.0	30-290	14-16	50	60-100	70-145
Inositol [2]	458	476	482	340	-	-	388	-	-
PABA	nil	nil	nil	nil	-	-	3.8	-	-
Choline [3]		to trace		to trace					
Pantothenic	1.0	1.2	2.3	0.5	-	-	4	-	-
acid	74	48	46	38	40-350	25-48	50-140	50-100	40-1050

[1]) Commercially prepared frozen grape juices
[2]) gamma per ml
[3]) mg per 100 ml
1. Blauburgunder
2. Räuschling
3. R × S (Riesling × Sylvaner)
4. Veltliner

Results and Discussion

Table 2 compares the results reported in this paper with those of some previous authors.

Nicotinic acid was found in all samples in a fairly high and uniform concentration, with the values agreeing with those found by other authors. Whereas HALL et al (1956) noted that the musts from red grapes were better endowed than those from white, in the work reported here little difference was noted between the nicotinic acid content of the red and white varieties of bottled grape juices.

With the exception of Räuschling, which contained about one half as much as the other three juices tested, the biotin content of the juices was almost the same. These values (0.006 — 0.016 gamma per ml) correspond approximately to those reported in musts by PEYNAUD and LAFOURCADE (1956) and CASTOR (1953). RADLER (1957) found that the difference in biotin concentration between a large number of musts produced from different varieties of grapes was considerable.

The thiamine content varied greatly among the four juices tested (trace — 0.25 gamma per ml). From Table 2 it is clear that previous authors reported little variation in thiamine content between the musts tested. Furthermore, these values for musts were found to be higher than those of the processed grape juices. It should be noted (Table 2, p. 60) that the only report found regarding the thiamine content of a processed juice (frozen) yielded values closer to those of the Swiss bottled juices.

The folic acid content was extremely low as compared with that found previously in musts and frozen juices, the highest value being about 10 times lower than those reported for the frozen juices and musts. This low folic acid content is difficult to attribute to any one cause. As folic acid breaks down rapidly when heated with acid, losses may have occurred during the pasteurization of the juices at a low pH. Large losses may also have taken place due to the action of light. This is discussed under riboflavin.

Riboflavin can be protected from decomposition only when the juices are stored and distributed to dealers in dark glass bottles or tins. Three of the juices tested (two red and one white) contained similar amounts of riboflavin, while the fourth (white) contained only a trace. The values of the former were lower than any previously reported for musts and frozen grape juices. The bottles containing the juices were stored in the laboratory in the dark at or near 0^0C, but losses of the vitamin may have occurred during the storage period at the bottling plant and later in the laboratory. PERLMAN and MORGAN (1944) reported that some samples of riboflavin-reinforced juices which had been stored at a lower temperature, and only partially protected from light, showed somewhat greater loss of riboflavin than did samples which had been stored in dark bottles at higher temperatures.

Pasteurization of grape juices usually takes place in a well lit room with the bottles emerging from the pasteurizer at a temperature somewhat higher than the room. The bottles in which the juices are sold are of a light green glass which affords little protection from light during processing and distribution.

With the exception of the juice prepared from Räuschling grapes, there was little variation of the vitamin B_6 complex content among the four juices. The vitamin B_6 content was similar to that reported by BURGER et al (1956) for

frozen grape juices. However, the assay values for this vitamin in Swiss processed juices are considerably below those previously reported for musts. Pyridoxine, pyridoxal and pyridoxamine are light labile, the destruction being most rapid in the ultraviolet region of the spectrum in neutral or alkaline solutions. Pyridoxine, however, is slightly sensitive to light even at pH 1. Again it would seem, as in the case of folic acid and riboflavin, that a larger loss of light labile vitamins will occur with an increase in handling.

As was expected, large amounts of inositol were found in the bottled juices. The inositol values found were in line with those given for fresh grape musts by CASTOR (1953).

p-Aminobenzoic acid was found to be absent from the juices. Even though minimal values are obtained by heating at one atmosphere for one hour, this method was preferred over alkaline or acid treatment due to the possibility of pteroylglutamic acid break down, which would in turn allow an additional amount of p-aminobenzoic acid to be available to the test organism. AGARWALA and PETERSON (1950) pointed out, that with alkaline treatment, liberation of p-aminobenzoic acid continued at almost a steady rate for an 8 hour period, whilst with acid treatment, the p-aminobenzoic acid released during the first hour of heating was approximately ten fold over that obtained by heating with water. However, after one hour, a loss occurred due to either the destruction of p-aminobenzoic acid, or its conversion into a form unavailable to the organism. As reported elsewhere (MATTHEWS and LÜTHI 1959) relatively large amounts of p-aminobenzoic acid have been found in apple juice concentrates and apple pomace after treatment with water.

ROBINSON (1951) indicated that choline is not present in grape products. The apparent choline activity, as reported here, was less than that found by CASTOR (1953) in fresh grape musts. However, CASTOR pointed out that monomethylaminoethanol can replace choline for the growth of *Neurospora* mutant (strain 34486) and was not certain that the choline activity found by him in musts and wines was due entirely or in part to the effect of monomethylaminoethanol.

The handling of the grape juices at the bottling plants in so far as storage and pasteurization are concerned, was not the same at each plant. All but the Blauburgunder were flash pasteurized at 90 °C before storage and directly after pressing. The Blauburgunder juice was stored under CO_2 followed by flash pasteurization directly before bottling. This involved heating at the following temperatures: 20 minutes to reach 68 °C, 20 minutes over 68 °C with maximum temperatures of 70—72 °C, and 15 minutes to cool to 30 °C. Cooling from 30 °C to storage temperature took place outside of the pasteurizer. Which of the bottling stages, if any, effect the loss of light labile vitamins could only be determined by assaying the juice for these vitamins at various steps of the processing. Filters may also be a factor influencing, to some extent, the loss of vitamins in some bottling plants. It is interesting to note that the juice made from Räuschling grapes was less endowed with most of the B vitamins than were the other juices tested. It is felt however, that this may be due to the type of juice rather than the handling at the plant. It has been noticed in this laboratory, that very frequently, a malolactic fermentation by *"Bacterium gracile"* will not take place, or proceeds very slowly, with wines prepared from Räuschling grapes. This would suggest that one or more growth factors are absent or are not present in sufficient amounts to support the growth of *"B. gracile"*.

Summary

Ten vitamins of the B-complex were determined by microbiological methods in four commercially prepared Swiss grape juices.

The amounts found are reported and compared with those found by some previous authors in grape musts and frozen grape juices.

A majority of the juices tested contained measurable amounts of the vitamin B-complex. The nicotinic acid, biotin, thiamine, inositol and pantothenic acid content of the juices compared favorably with that found previously in grape musts and frozen grape juices. The light-labile vitamins, folic acid, riboflavin, and the vitamin B_6 complex as well as p-amino-benzoic acid and choline were found to be present in lesser quantities then those previously reported for grape musts and frozen grape juices.

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