THE RÔLE OF NITROGEN COMPOUNDS IN THE FERMENTATION OF FRUIT JUICES.

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It is well known that nitrogen compounds are essential for the growth and activity of the yeast cells, *Saccharomyces ellipsoideus* (1, 2). During the fermentation of fruit and berry juices, any deficiency in the nitrogen content has often to be made good by the addition of compounds which contain nitrogen in an easily available form (3, 4, 5). Grape juice rarely needs such artificial additions as it contains sufficient nitrogen compounds for the primary fermentation and the subsequent enzymic changes. Contrary to Nolte (4) the present author finds that the fresh juice of various varieties of sweet orange (*Citrus aurantium*) also contains sufficient nitrogen compounds for the primary fermentation by yeast. It has been found possible to produce fermented juice containing as high as 17-18% of alcohol by volume from sweetened orange juice with suitable pure strains of *Saccharomyces ellipsoideus* and without the addition of any nitrogen compound. Further, fresh orange juice was found to contain nitrogen compounds in an amount equal to or even greater than the minimum requirements as stated by Lindner (6) for optimum fermentation.

It was noticed, however, that when sweetened orange juice was sterilized under pressure by heat, it lost much of its organic and also a considerable portion of its ammoniacal nitrogen. Grape juice similarly treated also lost some of its organic nitrogen, but the loss in this case was much less than in the case of orange juice. This may be due to greater heat stability of the organic nitrogen compounds contained in grape juice. This loss of nitrogen in orange juice in no way interferes with the primary stage of fermentation induced by yeast cells, as here again alcohol percentages of 17-18% by volume could be obtained on fermentation. But the subsequent stages of the life cycle of the yeast cells seemed in this case to be very greatly deranged. This was very clearly noticed when the so-called 'sherry' yeasts were employed. These yeasts after completion of the main fermentation pass into the film-forming and oxidative stage (7, 8), which is characterized by the tendency to form a film on the surface and a mild oxidation of the substrate by the yeast cells. The shape and arrangement of the yeast cells as seen under the microscope also change to a certain extent. This is particularly noticeable in the case of 'sherry' yeasts and is also met with, to a lesser degree, in the case of other varieties of *Saccharomyces ellipsoideus*. The 'film-forming' and

' oxidative ' stages were not noticed when sweetened orange juice, heat-sterilized under pressure, was employed and this may be due to the lack of sufficient nitrogen in a suitable form. Since ' heat sterilization ' processes are sometimes employed in the fermentation of fruit juices, it was thought desirable to investigate how far the nitrogen content of sweetened orange juice is lost during ' heat sterilization ' under different conditions.

The juice of Valencia and Washington Naval variety of oranges was employed as raw material. The sugar used for sweetening was pure crystal cane sugar whose organic and ammoniacal nitrogen contents were determined separately and found to be insignificant. The increase in volume of the juice due to the sweetening has been allowed for in compiling the results. This increase in volume was experimentally found to be about 12% when 20 gms. of cane sugar were dissolved in 100 c.c. of the juice of density of 8·5° Bx. Analytical procedures laid down by the Association of Official Agricultural Chemists of America (1935) have been followed throughout the analysis.

As was expected, preservation of orange juice, raw or sweetened, by processes not involving the application of heat, had no effect on the nitrogen content of the juice (Table I).

TABLE I.

	Freshly expressed Juice I.	Freshly expressed Juice II.	Preserved Juice I.	Preserved Juice II.	Preserved Juice III.
Ammoniacal and organic nitrogen % ..	0·077	0·081	0·071	0·078	0·081
Ammoniacal nitrogen %	0·014	0·012	0·010	0·014	0·010
Organic nitrogen % (by difference) ..	0·063	0·069	0·061	0·064	0·071
Proteins % (organic nitrogen × 6·25) ..	0·394	0·431	0·381	0·400	0·444

For determining the effect of heat, both fresh and chemically preserved orange juice was sweetened with 20% pure cane sugar and ' heat sterilized ' in different ways. Pure juice from ripe ' Sultana ' grapes was treated similarly. The results are recorded in Tables II, III and IV.

TABLE II.

(Fresh Orange Juice.)

	Original.	Sweetened and sterilized at 15 lbs./20 mts.	Sweetened and sterilized at 15 lbs./20 mts.	Sweetened and sterilized at 20 lbs./20 mts.
Ammoniacal and organic nitrogen %	0·081	0·009	0·005	0·002
Ammoniacal nitrogen % ..	0·012	0·007	0·004	0·002
Organic nitrogen % (by difference)	0·069	0·002	0·001	0·00
Proteins % (organic nitrogen × 6·25)	0·431	0·012	0·006	0·00

TABLE III.

(*Chemically Preserved Orange Juice.*)

	Original.	Sweetened and sterilized at 15 lbs./20 mts.	Sweetened and sterilized at 15 lbs./20 mts.
Ammoniacal and organic nitrogen % ..	0·077	0·004	0·005
Ammoniacal nitrogen %	0·014	0·002	0·003
Organic nitrogen % (by difference) ..	0·063	0·002	0·002
Proteins % (organic nitrogen × 6·25) ..	0·394	0·012	0·012

TABLE IV.

(*Pure Grape Juice.*)

	Original.	Sweetened and sterilized at 15 lbs./20 mts.
Ammoniacal and organic nitrogen % ..	0·070	0·055
Ammoniacal nitrogen %	0·010	0·007
Organic nitrogen % (by difference) ..	0·060	0·048
Proteins % (organic nitrogen × 6·25) ..	0·375	0·300

Sterilization of sweetened orange juice at 15 lbs. therefore destroys more than 80% of the organic nitrogen and also a considerable portion of the ammoniacal nitrogen. In pure grape juice the loss is not so great, only 20% of the organic nitrogen being lost.

In a further series of experiments sweetened orange juice was 'heat sterilized' in four different ways and the analytical data are given in Table V.

TABLE V.

	Original.	Sweetened and pasteurized at 92–95°C. for 20 minutes.	Sweetened and steamed at 0 lbs. for 15 minutes.	Sweetened and sterilized at 10 lbs. for 10 minutes.	Sweetened and sterilized at 10 lbs. for 15 minutes.
Ammoniacal and organic nitrogen % ..	0·077	0·070	0·063	0·040	0·030
Ammoniacal nitrogen % ..	0·014	0·010	0·008	0·007	0·007
Organic nitrogen % (by difference) ..	0·063	0·060	0·055	0·033	0·023
Proteins % (organic nitrogen × 6·25) ..	0·394	0·375	0·344	0·206	0·144

It is therefore obvious that the destructive action of heat on the nitrogen compounds contained in the sweetened orange juice depends upon the severity of this heat treatment. Pasteurization alone does not alter the nitrogen content very much, whilst autoclaving at 10 lbs. pressure decreases it by about 50% and at 15 lbs. pressure by over 80%. The duration of the 'heat sterilization' also has a significant influence. The loss of organic nitrogen is more

marked than the loss of ammoniacal nitrogen though both losses are considerable.

When sweetened orange juice which had lost much of its organic nitrogen through ' heat sterilization ' under pressure was fermented, its nitrogen content was found to increase once again. This increase is practically entirely due to the increase in organic nitrogen, the ammoniacal nitrogen being hardly affected. Sweetened orange juice was autoclaved in a cotton plugged flask at 15 lbs. pressure for twenty minutes. A portion was aseptically withdrawn for analysis. The rest was inoculated with a pure strain of *Saccharomyces ellipsoideus* and fermented at 25°C. Portions were aseptically withdrawn at various intervals for analysis (Table VI).

TABLE VI.

	Sweetened and autoclaved juice original.	After 1 day's fermentation.	After 4 days of active fermentation.	After 8 days of active fermentation.	After fermentation was nearly complete (12 days).
Ammoniacal and organic nitrogen % ..	0·004	0·006	0·045	0·067	0·070
Ammoniacal nitrogen %	0·003	0·003	0·004	0·004	0·004
Organic nitrogen % (by difference) ..	0·001	0·003	0·041	0·063	0·066
Proteins % (organic nitrogen × 6·25) ..	0·006	0·018	0·256	0·394	0·412

That the above apparent increase in organic nitrogen is due to soluble organic compounds and is not merely due to suspended yeast cells is apparent from the analysis of clarified fermented orange juices (Table VII). After completion of the active fermentation the fermented juice was settled and decanted off, and repeatedly filtered through paper on which a siliceous filter-aid like ' Hyflo super-cell ' had been deposited, till it was perfectly clear. The slight increase in the ammoniacal nitrogen in these clarified samples is probably due to the uptake of soluble ammoniacal compounds from the filter-aid.

TABLE VII.

(*Clarified Fermented Orange Juices.*)

	I.	II.
Ammoniacal and organic nitrogen % ..	0·069	0·060
Ammoniacal nitrogen % ..	0·011	0·012
Organic nitrogen % (by difference) ..	0·058	0·048
Proteins % (organic nitrogen × 6·25) ..	0·362	0·300

The increase in organic nitrogen during fermentation of orange juice is therefore due to some synthesis of soluble organic compounds by the yeast cells. Since the yeast cells in such fermented orange juices still failed to show any films or any signs of oxidation, it would appear that organic nitrogen, at least the organic nitrogen compounds synthesized by the yeast, are not available for its own metabolic activity in the ' after-fermentation ' stage. The addition of ammoniacal nitrogen would therefore seem to be desirable

at this stage. This is in accordance with the observation of Müller-Thurgau and Hopkins (9, 10), who found that those nitrogen compounds are most easily assimilated by the yeast cells which easily split off nitrogen in the form of ammonia. This does not prove that lack of ammoniacal nitrogen is responsible for the absence of the ' film-forming ' and ' oxidative ' stage in the above case. There may be some specific inhibiting substance produced during the ' heat sterilization ' under pressure. But this is doubtful because grape juice retains much of its original nitrogenous matter on similar ' heat sterilization ' and in the ' after-fermentation stage ' of such a juice, the film is easily formed and oxidation changes take place. Lack of nitrogen in an easily assimilable form may at least be a contributory factor.

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Summary.

1. Fresh orange juice contains sufficient nitrogen in an easily assimilable form for the primary fermentation by *Saccharomyces ellipsoideus*.

2. ' Heat sterilization ' under pressure destroys most of the organic and much of the ammoniacal nitrogen compounds in sweetened orange juice, the amount lost depending upon the pressure employed and the duration of the treatment. Pure grape juice under similar conditions suffers this loss to a much less degree.

3. The primary fermentation is not appreciably hindered by the above loss in nitrogen compounds, but the ' after-fermentation stages ', namely those of film-formation and oxidation, are in this case inhibited.

4. During fermentation the organic nitrogen content of the juice again increases, probably as a result of synthesis by the yeast of soluble organic nitrogen compounds. But these compounds do not seem to be available for the secondary changes, during the ' after-fermentation stage ', induced by the life cycle of yeast.

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