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## **Tyrosine Content of Sugar Beet Processing Products**

by

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# Tyrosine Content of Sugar Beet Processing Products

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The reaction of 1-nitroso-2-naphthol with tyrosine was used to study the tyrosine content of sugar beet during its storage and in its products after processing. In order to investigate the influence of different components which are present in sugar beets, the reaction was first applied to molasses (containing many impurities). The results obtained were constant and reproducible indicating that this rapid and accurate method is applicable to the determination of the tyrosine present in sugar beets.

## INTRODUCTION

The dark colour in the diffusion juices of sugar beets has been investigated by numerous investigators.

It has been reported that the coloured substances which have to be removed to obtain white sugar are mainly the oxidation products of tyrosine, dihydroxyphenylalanine and pyrocatechol [1,2]. Substances which are composed of amino acids combined with reducing sugars also play an important role in the blackening of thick juice [3]. Accordingly, tyrosine is responsible for the dark colour in the juices both as a phenol and as an amino acid.

This work was undertaken for the purpose of adding to ones knowledge of the tyrosine content of sugar beet both during its

storage and also in its products after processing. Earlier investigators have determined the amino acid contents of sugar beets by electrophoresis and paper chromatography [4]. In the present paper the colour reaction between 1-nitroso-2-naphthol and tyrosine was used. This reaction, which is specific for p-substituted free phenols, was first discovered by Gerngross, Voss and Herfeld [5]. To stabilize the red colour obtained during the reaction, a modified method was proposed by Ceriotte and Spandrio [6]. It was also shown by these investigators that the method can be used for the determination of tyrosine in proteins.

Before using this method to determine the tyrosine content of the different sugar products obtained in sugar beet processing, it was first necessary to study the influence upon the colour reaction of the various compounds present in sugar beet. Thus the colour reaction was first investigated with different molasses which contained more impurities than the other products of sugar beet processing. The tyrosine content of different juices was then determined.

#### RESULTS AND DISCUSSION

Solutions of molasses whose concentration ranged between 0.25 and 0.5 % were used. A colour-free solution of molasses prepared by treatment with decolourizing resin (Asmit 261) gave good results with the 1-nitroso-2-naphthol colour reaction.

Colour reactions carried out with different molasses containing various amounts of sulfur dioxide and invert sugars indicate that the reducing substances present in the molasses do not effect the reaction.

A recovery test was carried out to check the possibility that some of the tyrosine might have become bound to the resin during the decolourizing of the molasses solution. Known amounts of tyrosine were added to the solution of molasses which was then decolourised by Asmit 261 at room temperature. The results show that the effluent from the resin contained practically all the tyrosine (Table 1).

TABLE 1  
Recovery tests with 0.25 % molasses solution

Tyrosine added to 1 ml molasses solution ( $\mu\text{g}$ )	Total tyrosine content ( $\mu\text{g}$ )	Recovery (%)
0	4.5	100.0
1	5.5	"
2.5	7.0	"
4	8.5	"
5	9.5	"

Since the colour reaction takes place so long as the phenolic hydroxyl group and the ortho-and meta-positions of tyrosine are free, it was possible to determine both free tyrosine as well as tyrosine which was bound in proteins (uncoloured tyrosine) or in the coloured substances (coloured tyrosine) present in the different juices and molasses. This was confirmed by the results obtained from the colour reactions which were carried out with molasses solutions and with hydrolysed molasses solutions. The tyrosine content of the solutions was exactly the same in both cases.

The tyrosine content of molasses from different sugar plants in Turkey ranged between 0.12 % and 0.26%. The tyrosine content of different sugar beets, juices and molasses obtained in sugar beet factories are presented in Table 2.

TABLE 2  
Tyrosine Content of Different Sugar Beet Juices and Molasses Obtained During Sugar Beet Processing

	Uncoloured Tyrosine Content (% of dry matter)
Juice from mature beet .....	0.015 - 0.038
Juice from defecated beet .....	0.062
Raw diffusion juice .....	0.70 - 0.129
First liming juice .....	0.050 - 0.070
Second liming juice .....	0.045 - 0.065
First Carbonation juice .....	0.045 - 0.065
Second Carbonation juice .....	0.045 - 0.065
Thin juice .....	0.045 - 0.065
Molasses .....	0.260

As can be seen from Table 2, treatment of the raw diffusion juice with milk of lime eliminates the undesirable coloured impurities as well as free tyrosine and the proteins containing tyrosine. The tyrosine content of the thin juices becomes 0.050 to 0.070 depending on the conditions prevailing in the factory.

If it is assumed that the tyrosine present in the thin juice does not undergo changes during further processing, then the uncoloured tyrosine content of the molasses should be equivalent to that of the thin juice. i e, 0.28 - 0.40 However as can be seen from Table 2, the results obtained are 0.260. This result indicates that a part of the uncoloured tyrosine present in thin juice reacted with the reducing sugars to form black-coloured melanoidins.

This was also indicated by the determination of the coloured tyrosine (tyrosine which was bound in the coloured substances) content of molasses and the results obtained are presented in Table 3.

TABLE 3

Coloured Tyrosine (tyrosine which was bound in the coloured substances) and Uncoloured Tyrosine (free tyrosine and tyrosine which was bound in proteins) Content of Some of the Sugar Beet Processing Products

	Uncoloured tyrosine % of dry matter	Coloured tyrosine % of dry matter	Total % of dry matter
First liming juice	0.086	0.093	0.179 <sup>a</sup>
Thin juice	0.058	0.060	0.118 <sup>a</sup>
Molasses	0.274	0.200	0.468 <sup>b</sup>
Molasses	0.176	0.294	0.470 <sup>b</sup>

a- Calculated

b- Experimental

The tyrosine content of the thin juices changes between the ranges of 0.050 - 0.070 % on the conditions prevailing during liming and carbonation processes.

Since it is possible to determine in a very short time the tyrosine content of different products, the conditions prevailing

during liming and carbonation processes can be controlled by this very rapid method.

#### EXPERIMENTAL

##### Reagents.

A solution of 0.1 % 1- nitroso- 2- naphthol (recrystallised from ethyl alcohol) in 0.1 N sodium hydroxide was used.

2.5 N nitric acid solution was prepared by diluting chemically pure nitric acid (sp. gr. 1.42) with distilled water.

Concentrated hydrochloric acid (sp. gr. 1.19) was used.

Tyrosine was dried to constant weight and dissolved in water by slight acidification with hydrochloric acid. Solutions whose concentrations ranged from 1 to 20  $\mu\text{g}/\text{ml}$  were prepared for the colour reaction.

##### Procedure for the colour reaction.

The colour reaction was carried out according to the procedure described in the literature [6]. 5 ml of solution containing tyrosine (or the solution of molasses) were placed in a pyrex test tube whilst 5 ml of distilled water were placed in a second tube (for the blank). To each tube 1.0 ml of 1- nitroso - 2- naphthol solution, 0.5 ml nitric acid solution and 1.5 ml hydrochloric acid were added. The contents of the tubes were well mixed by shaking and then heated in boiling water for exactly 2 min. The reaction mixture was then cooled in a ice-water bath. The absorbance of the red colour obtained was measured with a Beckmann Model DU quartz spectrophotometer using 1 cm cells at 510 nm against blank treated in an identical manner ( $\epsilon_{510} = 19.000$ ).

#### RECOVERY EXPERIMENTS

To 1000 ml of 0.25 % molasses different amounts of tyrosine were added. The solutions were treated with the decolourising resin at room temperature and the colour reaction was then performed with 5 ml samples.

**Determination of the uncoloured tyrosine content (free tyrosine and tyrosine bound in proteins) of sugar beet juice and of raw diffusion juice.**

To 50 g of sugar beet juice whose dry matter content was known 200 ml of 96 % (v/v) ethyl alcohol were added. The resulting mixture was left to stand in the refrigerator for 24 hrs. The precipitate was removed by filtration and the filtrate was distilled under vacuum to remove alcohol. The pH of the remaining solution was adjusted to 4.8 with N/10 HCl and the mixture was allowed to stand for 12 hrs. The mixture was filtered and the filtrate passed through a column of Aberlite IR 120. The column was eluted with 3 N HCl and the volume of the effluent was made up to 400 ml. The tyrosine content was then determined according to the usual procedure.

**Determination of coloured tyrosine content of first liming juice, second liming juice, and of thin juice.**

50 g of juice was diluted to about 200 ml with distilled water. The resulting solution was passed through a column of Asmit 224/261. The coloured substances accumulated in Asmit were removed by 5 % NaCl solution. The resulting solution was diluted to 400 ml and its tyrosine content was determined.

**Determination of the uncoloured tyrosine content of the first and second liming juice and of thin juice.**

50 g of sample were diluted to 200 ml and the solution was passed through beds of cation exchange resin to remove the amino acids present in the juices. Then amino acids were eluted with 3 N HCl solution. The tyrosine content of the resulting solution, which was practically colourless, was then determined.

**Determination of the coloured, uncoloured and total tyrosine contents of molasses.**

2.5 - 5 g of molasses were dissolved in about 200 ml of water. The coloured substances were removed by filtration through a bed of decolourising resin Asmit 224/261 at room temperature at



a rate of 1 litre / 2 hrs. The effluent volume was made up to 1000 ml. The tyrosine content was then determined.

The coloured substance accumulated in the resin bed was removed by regeneration with 5 % NaCl solution. The amount of the coloured tyrosine in the effluent was determined.

The total tyrosine content of molasses was determined, using very dilute molasses solutions (0.25%) without previous treatment.

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