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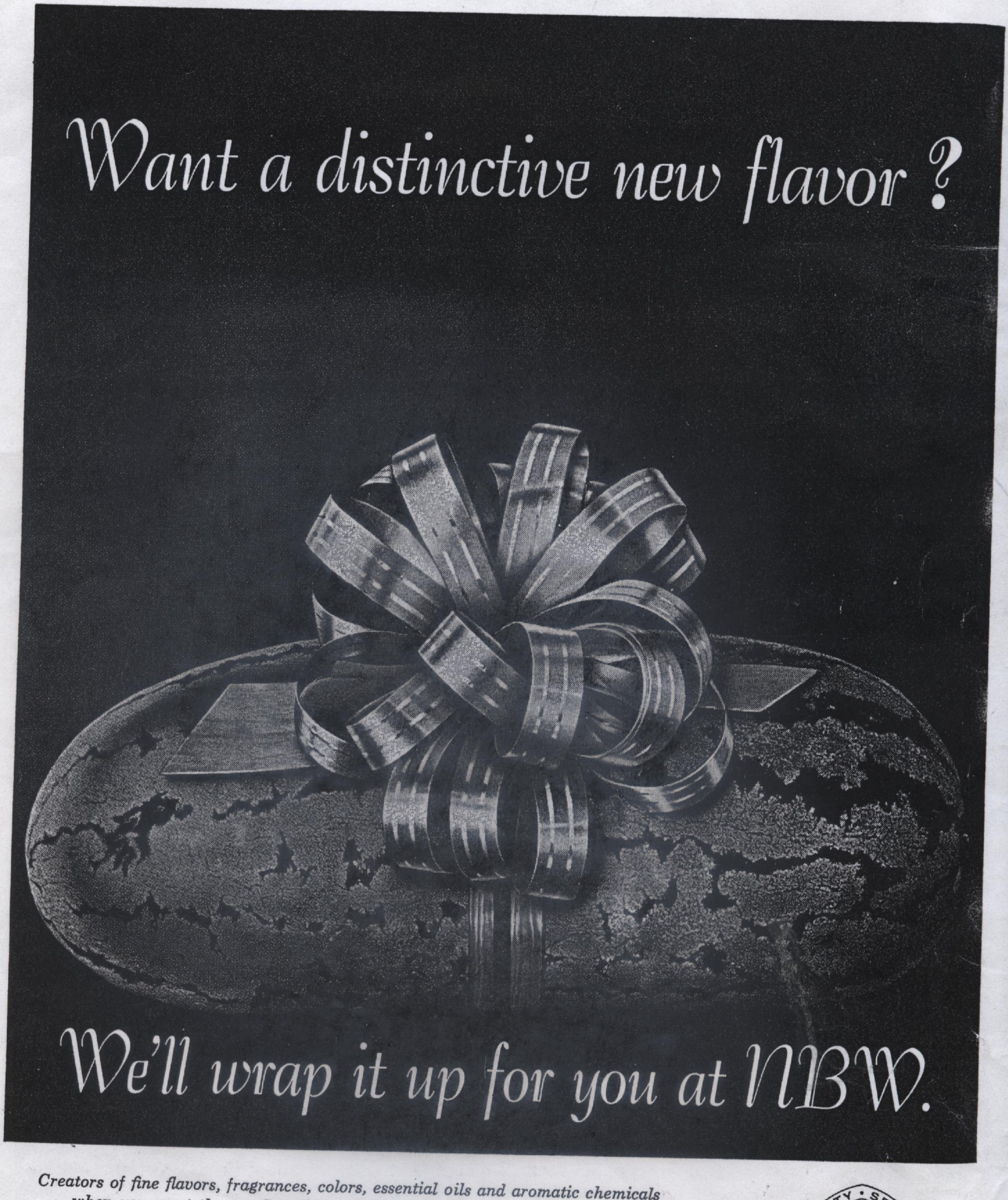

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Heat Processing Effect on Starch, Sugars, Proteins, Amino Acids, and Organic Acids of Tomato Juice

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SUMMARY

The variety of tomato used in this investigation was Heinz 1370. The juice was extracted, and half of it was frozen; the other half was canned and sterilized in the can at 220°F for 20 min.

Analyses of starch and total sugars, using the anthrone method, showed a loss due to processing of 40% and 19.4%, respectively. Gas liquid chromatography was used to investigate free sugar content, and isomers of tomato sugars. The loss of total fructose was found to be 17.15%, as compared to 19.09% in total glucose.

Densitometric tracings of protein electrophoretic patterns of acetone powders of both fresh and processed tomato showed differences in their patterns, probably due to the denaturation of protein and partial hydrolysis by processing.

Amino acids were qualitatively and quantitatively analyzed, using an automated amino acids analyzer. Nineteen amino acids were separated. Processing resulted in increased concentrations of all amino acids, and appearance of an unknown ninhydrin-positive compound, and the disappearance of glutamine and asparagine.

Organic acids were analyzed using silicic acid columns and a fraction collector. Paper chromatography was used to confirm their identification. Eight organic acids were separated. An increase in total acid was found after processing. Acetic, lactic, pyrrolidone carboxylic, malic, citric and an unknown organic acid were found to increase, whereas succinic and alpha-ketoglutaric acids were found to decrease.

INTRODUCTION

The free sugars of commercial varieties of tomato are predominantly reducing sugars, with sucrose rarely exceeding 0.1% on a fresh weight basis. Davies (1957) reported that, in general, more fructose than glucose was present in tomato. Amino acids were studied by Hamdy *et al.* (1962), and Kim and Seo (1962). They all agreed that the major amino acid in tomato juice is glutamic acid. Bradley (1960) and Bulen, *et al.* (1952) reported that the major organic acids in tomato were citric and malic. Other organic acids were also reported, such as acetic, lactic, succinic, alpha-ketoglutaric and pyrrolidone carboxylic acids.

The objective of this investigation was to study the effects of heat processing on sugars, amino acids and organic acids of tomato juice. Since heat of sterilization causes denaturation of protein and partial hydrolysis of starch to glucose, these constituents were also investigated.

EXPERIMENTAL

The tomatoes used in this study were Heinz 1370 variety produced by the Ohio Agricultural Research & Development Center, Northwestern Branch at Hoytville, Ohio. Samples of fresh juice were extracted after the tomatoes were washed and steamed, and the juice was sealed in cans. One-half of the tomato juice was transferred to a freezer at −20°C until needed for analysis. The other half was processed in a retort at 220°F for 20 min.

Starch and total sugars. Ten-gram samples of tomato juice solids were extracted 4 hr in Soxhlet apparatus with 200 ml of 80% ethanol, and the extract was made up to 250 ml. The residue was dried under vacuum and used for starch determination.

Starch and total sugar were determined according to McCready, *et al.* (1950).

Free sugars determination. Twenty-five ml of the alcohol extract was pipetted into a 50-ml round flask, and the sample was concentrated under vacuum to approximately 5 ml. Five ml of water was added and the sample was transferred to a vial and subjected to freeze drying. Ten mg of inositol (internal standard) was added.

The dried ethanol extract was methylsilated by adding 2.0 ml of anhydrous pyridine, 0.4 ml hexamethyldisilazone (HMDS) and 0.2 ml of trimethylchlorosilane (TMCS). The mixture was then allowed to stand overnight at room temperature. Samples (7.4 ml) were analyzed by gas-liquid chromatography, utilizing 3% silicone rubber (SE-52) on Gas Chrom Q80 support packed in a stainless steel column (1/8 in. × 10 ft).

Protein separation by disc electrophoresis. *Sample preparation:* Two hundred and fifty g of tomato juice (fresh and processed) was added to 250 ml acetone reagent, and the mixture was stirred and filtered. The filtered residue was taken up, washed twice with acetone and dried.

Protein extraction: Acetone powder (500 mg) was weighed into a centrifuge tube, 5-ml buffer (0.1 *M* phosphate, potassium salt, pH 8, containing 25% sucrose and 5×10^{-5} *M* EDTA, sodium salt) was added, and the mixture was stirred. After one hour at 0°C, the preparation was centrifuged 30 min at 20,000 × G in a refrigerated centrifuge.

Electrophoresis was performed on polyacrylamide according to the method of Ornstein *et al.* (1962).

Amino acid determination. The method used was described by Spackman, *et al.* (1958).

Organic acid determinations. Juices of fresh and processed tomatoes were expressed by squeezing through cheesecloth, and 10 ml of the clear syrup was freeze-dried. The procedure of Bulen, *et al.* (1952) and modified by Clements (1964) was used for analysis. The acids were eluted from the silicic acid column with mixtures of normal butanol in chloroform equilibrated against 0.5 *N* sulfuric acid according to the following schedule: 75 ml each of 2.5, 5, 10, 15, 20 and 25%, followed by 300 ml of 35% butanol in chloroform (v/v). Fractions (5 ml) were collected, and titrated with 0.01 *N* sodium hydroxide, with 0.1% phenol red as indicator. Peaks were tentatively identified by comparison of elution thresholds with known compounds.

For further identification, fractions representing individual peaks were combined, and the organic solvent was removed, using a separatory funnel. The aqueous phase was partially evaporated under a hood overnight, then was passed through $AGW-X_4$ (200 to 400-mesh) cation exchange resin in the acid form to remove the salt. The

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organic acids were chromatographed on S & S 589 White Ribbon acid-washed paper. Two dissimilar solvent systems were used: (1) iso-amyl alcohol equilibrated against 5 *M* formic acid, and (2) pyridine-ethyl acetate-acetic acid-water (5:5:1:3, v/v/v/v). Spots were detected with ammoniacal silver nitrate and with bromphenol blue.

RESULTS

Polysaccharide. The concentration of hydrolyzed polysaccharide calculated as starch in the alcohol-insoluble solid fraction was found to be 160 and 96 mg per 100 g of fresh and processed tomato juice, respectively. It is assumed that the major polysaccharide present in tomato is starch, but other polysaccharides (araban-galactan, xylan and x-cellulose) may be present.

Total sugars determination. Using the anthrone method, the total sugars of fresh and processed tomatoes were found to be 3.6 and 2.9%, respectively. Starch and sugar content in fresh and processed tomato juice, and percent loss due to heat of processing are shown in Table 1. Total sugar assayed by the anthrone method did not agree with the data obtained by gas-liquid chromatography. This was probably because sugars other than glucose and fructose were present in tomato juice, and these would also react with anthrone. The preliminary studies with sugars using gas-liquid chromatography also showed very low concentrations of arabinose and xylose. Sucrose was not found in the two samples. Paper chromatographic techniques showed the presence of glucose and fructose, and the absence of sucrose.

The advantage of using gas-liquid chromatography over analytical methods was that it permitted estimation of alpha and beta isomers of monosaccharides present in tomato juice (Table 2). When pure monosaccharides were dissolved in water, mutarotation also resulted in the distribution of isomers shown in the table.

Protein. Data were collected from densitometric tracings of disc electrophoresis pattern of the two samples. Each tracing was prepared from a single specimen, but the specimens were selected as typically representative of many gels obtained from each sample.

Amino acids. Amino acids in fresh and processed tomato juice are shown in Table 3. DL-Norlucine was used as an internal standard for calculation of each amino acid. Table 3 shows the amount and the percent distribution of amino acids in fresh and processed tomato juice and their increase during heat processing.

Organic acids. Table 4 shows meq acid per L. of organic acids in fresh and processed tomato juice. Silicic acid separation of the various juices indicated that acids in addition to malate and citrate were present in the effluent. All of the analyses described above were conducted in triplicate.

Starch. It was observed that starch content decreased 40% during processing. This loss was apparently due to the partial hydrolysis of starch to dextrins, maltose or glucose. The hydrolysis would be catalyzed by heat and the low pH of the tomato juice. The glucose that is formed may remain as a free sugar, or form compounds such as hydroxymethyl furfural.

Total sugar. The sugar content, using the method of McCready, *et al.* (1950), in fresh and processed tomatoes, was found to be 3.6 and 2.9%, respectively. These data agreed with the result obtained by Scott *et al.* (1947), who found a decrease in sugar concentration due to processing. The percent loss in total sugar due to processing was found to be 19.4%. In spite of the hydrolysis of starch during processing, the sugar content did not increase. This was probably due to the reaction of sugar with other constituents such as amino and organic acids (Andreotti, 1957; Andreotti, 1958). The complexes formed were decomposed to new compounds (Meyer, 1960). In acid media, the product formed can lose water and form a ring compound of the Schiff's base of hydroxymethyl furfural, and then eliminate the amide to form the free hydroxymethyl furfural (Hodges, 1953). Sugars can also undergo caramelization, a reaction which does not require an amine.

Free sugar. The total sugar of fresh and processed tomato juice, as determined by the gas-liquid chromatographic method, was found to be 3.26 and 2.73 g in 100 g of juice, respectively. Total fructose was found to constitute 1.70 and 1.41% of fresh and processed juices, respectively, as

Table 1. Starch and sugars content in fresh and processed tomato juice.

	Starch		Sugars	
Juice	mg in 100 g	Percent loss	grams in 100 g	Percent loss
Fresh tomato	160		3.6	
Processed tomato	96	40	2.9	19.4

Table 2. Free sugars in grams per 100 g of fresh and processed tomato juice.

Sugar	Fresh tomato juice	Processed tomato juice	Percent loss
Alpha fructose	1.46	1.24	15.1
Beta fructose	0.24	0.18	25.0
Alpha glucose	0.70	0.61	12.9
Beta glucose	0.87	0.70	19.6
Total	3.27	2.73	

Table 3. Amino acid content of fresh and processed tomato juice.

	Mg Amino acids in 100 g tomato		Percent distribution of amino acids		
Amino acids	Fresh	Processed	Fresh	Processed	Fold increase
Aspartic	5.5	51.6	12	16	9
Threonine	1.0	9.0	<1	<1	9
Serine	2.3	12.7	5	4	6
Unkown	......	0.6	0	<1	....
Asparagine and glutamine	7.8	......	17	0	....
Glutamic	21.9	212.5	48	64	10
Proline	0.1	0.4	<1	<1	4
Glycine	0.3	1.2	1	<1	4
Alanine	1.0	9.0	2	3	9
Valine	0.4	1.7	1	1	4
Methionine	0.2	0.9	<1	<1	5
Isoleucine	0.6	3.8	1	1	6
Leucine	0.6	3.0	1	1	5
Tyrosine	0.5	3.4	1	1	7
Phenylalanine	1.4	10.8	3	3	7
Lysine	0.9	5.1	2	2	6
Histidine	0.9	7.5	2	2	8
Arginine	0.7	4.4	2	1	6
Total	45.1	337.6			

Table 4. Organic acids in milliequivalent in fresh and processed tomato juice.

	Milliequivalent/liter	
Acids	Fresh	Processed
Acetic	1.06	1.56
Lactic	1.37	1.46
Succinic	0.60	0.49
Alpha-ketoglutaric	1.10	0.53
Pyrrolidone-carboxylic	0.81	8.10
Unkown	0.17	0.28
Malic	3.72	5.39
Citric	60.92	66.92

compared to glucose, which was found to constitute 1.51 and 1.32% of the juices. These data agreed with the results obtained by Davies (1957), who found more fructose than glucose in the fruit. Using the gas-liquid chromatograph method, it was possible to detect alpha and beta isomers of the sugars. Alpha sugar was found to be higher in concentration than beta forms.

The loss in total sugar due to processing was found to be 16.30%. The loss in glucose was found to be 19.09%, as compared to 17.15% for fructose. The greatest decrease in sugar was found to be in beta fructose (25%), whereas the lowest decrease was found in alpha-glucose (12.9%).

Amino acids. Nineteen amino acids were detected (see Table 3) a greater number than previously reported. Kim *et al.* (1962), using paper chromatography, reported the presence of 12 amino acids. Hamdy *et al.* (1962) identified 14 amino acids in their qualitative studies.

Seventeen free amino acids were found in processed tomato juice. The amino acid in highest concentration was glutamic acid, and this agrees with the data of Saravacos, *et al.* (1958) and Hamdy *et al.* (1962).

Glutamic acid comprises up to 48.45% of the total weight of amino acids and up to 64.39% in the processed juice. Second highest in concentration was aspartic acid. The amino acid which appeared in the lowest measurable concentration was proline. Processing resulted in a substantial increase in the free amino acids, probably as a result of denaturation and partial hydrolysis of protein.

The greatest increases occurred in glutamic and aspartic acids, alanine and threonine. Also a new unidentified amino acid was observed after processing. Asparagine and glutamine disappeared during processing. This could be due to the loss of amide ammonia (NH_3) to form glutamic and aspartic acids, which would partially account for the increase in ammonia in the canned juice. It could also be due to glutamine and asparagine deamination, and formation of pyrrolidone carboxylic acid. An unidentified ninhydrin-positive compound appeared in the processed tomato juice.

Organic acids. Eight organic acids were separated. Seven were identified by comparison of elution thresholds with known compounds. For further identification, fractions representing individual peaks were combined, and chromatographed on paper. This method was found to yield good results as compared to other methods used by previous investigators. Hamdy *et al.* (1962), using paper chromatography, separated four organic acids in fresh tomato juice. Bradley (1960) had shown the presence of six organic acids, three of which were not separated. Bulen, *et al.* (1952), also showed the presence of six organic acids in tomato juice.

In the present study, citric acid was found to be the major organic acid in fresh and processed tomato juice. Malic acid was found to be the second major organic acid in fresh juice, whereas pyrrolidone carboxylic acid was found to be the second major organic acid in the processed juice.

The ratio between citric, pyrrolidone carboxylic and malic acids before processing was found to be 15.5:0.4:1.0, as compared to 11.0:2.6:1.0 after processing. An increase in total acid after processing was observed, and this agrees with the data obtained by Scott *et al.* (1947). Acetic acid was found to increase by 32.1%, apparently due to oxidation of aldehydes and alcohols during processings, and deamination of amino acids, such as alanine to pyruvic (which is heat-labile and decarboxylates to acetic acid).

A slight increase in lactic acid was noted, whereas succinic acid was found to decrease. An increase in citric and malic acids after processing was noted. Heat may cause disruption and destruction of the cells, which could result in the liberation of free organic acids. This could occur generally with most of the acids determined.

Paper chromatography was used in order to show whether the increase in these acids was due to the formation of new acids such as glyoxylic or to others, which could have the same elution thresholds as the natural acids. Results did not show new acids, and the technique was a confirmation of the tentative identifications.

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