A Review of Recent Developments in the Chemistry of Sugar Beet

A. Carruthers[1]

Mr. President, I was deeply honored when I received the invitation, from Mr. G. Rorabaugh, to speak before your general assembly and I count it a special privilege to be able to do so here in Denver, where many distinguished members of your Society have lived and worked. The honor which has been afforded to me is, I believe, a tribute to the work of our research group at Nottingham and in this connection I should like to mention especially Mr. J. F. T. Oldfield who has been actively concerned in all of the work which our group has carried out.

It can justifiably be stated that our knowledge of the composition of sugar beet juice and of the chemical changes which occur during processing of the beet has advanced very considerably during the past two decades. Prior to this time quantitative data on juice analysis referred to groups of components classifying them as sugars, nitrogen-containing organic substances, non-nitrogenous organic substances and ash. Certainly many of the individual substances within the groups had been recognized for a long time, but now that they can be separated and their concentrations can be measured by methods which are specific and precise we are in a much better position to evaluate their significance in relation to factory performance.

With these advances in knowledge of the chemical composition of beet, and of the chemical reactions in the factory process, it is possible to define the extent to which the technologist is irrevocably limited by the composition of his beet material and the extent to which he is ultimately capable of modifying the juice composition for maximum operating efficiency.

The composition of raw juice is basically determined by the composition of beet juice but it has been clearly demonstrated that the conditions operating in the diffuser can have a profound effect on the final composition. For instance the content of the pectin complex may be increased at least tenfold if the water used in diffusion is even mildly alkaline, as it may be if ammoniacal condensates are used for make-up. The extraction of excessive amounts of pectin not only represents a loss of valuable feeding stuff but it may also be detrimental to the process. The polygalacturonide fraction of the pectin is removed in clarifica-

[1] Director of Research, British Sugar Corporation, Limited, The Grange, Bramcote, Nottingham, England. Presented at the Twelfth General Meeting, American Society of Sugar Beet Technologists, Denver, Colorado, February 7, 1962.

tion but some of the araban portion is liberated during the liming stage and remains in the clarified liquors. When unfavorable diffusion conditions prevailed, amounts of araban of the order of 300 mg/100S were found in molasses and the pectin extracted was equivalent to a loss of as much as 7% of the beet marc.

It is now realized that even when temperatures throughout the diffusion system are adequate to suppress the activity of mesophilic bacteria, nevertheless, marked effects on juice composition can arise through the activity of thermophilic organisms. Strains of *Bacillus stearothermophilus* which can flourish at temperatures up to 80°C have been found in factory juices and if the diffusion conditions allow these organisms to attain the logarithmic phase they will rapidly produce lactic acid and corresponding losses of sucrose will ensue. Following the demonstration that beet juice contains negligible amounts of lactic acid and that raw juice might at times contain as much as 0.6 g/100S, factories in general have adopted more stringent measures, either by maintaining a high level of temperature throughout the diffusion system or by a greater use of bactericidal agents, to suppress bacterial activity.

The thermophilic bacteria also attack nitrate which is derived from the beet and convert it to nitrite. At a later stage in the process sulphur dioxide is introduced into the juice and, by a complex reaction with the nitrite, yields imidodisulphonic acid, the potassium salt of which is sparingly soluble. If the concentration in the final syrups exceeds the saturation level, the imidodisulphonate may crystallize out with the sugar. Even where this does not occur it is still important to note that, to the extent that nitrite reacts with sulphur dioxide, this reduces the value of the latter since the real purpose of adding it is to minimize color formation.

When raw juice is produced under more or less sterile conditions its pH is about 6.3 and the aim of the clarification process is to prepare second carbonatation juice, containing less calcium than the raw juice, but with a pH of about 9. To bring about this change in pH without adding any bases, it is necessary that the acidic radicals, principally phosphate, oxalate, citrate, and to a less extent sulphate and malate, removed during clarification should appreciably exceed the removal of the basic magnesium and calcium ions. Fortunately the acid removal normally amounts to some 25 - 40 meq. per 100 sugar while the base removal only ranges from about 10 - 15 meq. per 100 sugar. The situation is still finely balanced, however, because some 4 - 6 meq. of excess base remaining in the juice is associated at the higher pH with

the amino acids and residual citrate which buffer the juice, while the acids produced by fermentation during diffusion, and by degradation of invert during clarification, neutralize a further 5 - 15 meq. of excess base. Only the remaining fraction of the base excess is then available to permit absorption of carbon dioxide to the point in the carbonate : bicarbonate equilibrium corresponding to minimum residual lime salts.

Now that analytical techniques have made it feasible to measure the changes which occur during clarification it is possible to avoid empirical assessments such as 'effective' or 'natural alkalinity' and, in terms of precise chemical constituents, to ascribe unfavorable lime salts and lack of juice stability either to deficiencies in juice composition or to inadequate operating technique.

It is not always practicable or desirable to attempt to measure all of the constituents of juice but considerable insight into the processing features of juice, or of beet, can be obtained by determination of four main components which together are responsible for some 80 - 90% of the refractometric nonsugars in second carbonatation juice.

These four main components, potassium, sodium, amino acid nitrogen and betaine, are present in the same proportions relative to sucrose in raw juice, or aqueous extracts of brei and they are not significantly eliminated in preparation of second carbonatation juice.

Potassium and sodium can be readily measured by flame-photometry and together these ions are responsible for virtually all of the ash components in second carbonatation juice. The associated anions in second carbonatation juice differ from those in raw juice but the average equivalent weight is known so that it is possible to calculate the weight contribution of these components.

In the past, the Stanek Pavlas copper reagent has been used extensively to estimate amino acid nitrogen in beet and it has also been applied to process juices. It has, however, been demonstrated that this value gives only a rough indication and is generally much higher than the true content of amino acid nitrogen. A direct determination using the Moore and Stein ninhydrin-hydrindantin reagent has now been developed to give a value which is far more closely correlated with the sum of the individual amino acids than is the Stanek Pavlas value.

About half of the nitrogen in clarified juice originates as amino acid in beet and most of this nitrogen is present in beet as glutamine which contains both an amide and an amino nitrogen group. The conversion of this amino acid to pyrrolidone car-

boxylic acid and ammonia causes particular problems during processing as the ammonia is volatilized in the evaporators, leaving the acid residue to contribute to juice instability.

The other main component, betaine, is a particularly stable compound and does not apparently associate in the specially undesirable processing difficulties but, since it is quantitatively the most prominent single organic nonsugar in beet juice, it obviously has a considerable influence on purity.

The main difficulty in estimating betaine has been that no specific reagent is known and hence the removal of interfering ions has hitherto been tedious. The discovery that betaine is not absorbed by mixtures of strong anion and weak cation exchangers, while all known interfering ions can be absorbed on the same resin mixture, now permits the simple but precise colorimetric determination of betaine after precipitation as betaine reineckate.

Particular emphasis has been given to these four determinations because they are part of our aim to give the factory technologist and the seed breeder simple methods of analysis which will yield results capable of precise interpretation. In this respect, individual measures of potassium and sodium provide more information than conductivity or ash, Moore and Stein nitrogen replaces noxious nitrogen, and we can also include betaine as the principal remaining nitrogen compound.

If the quality of beet is to be assessed by determination of individual nonsugars it is essential to have some method of compounding the individual results so that, for example, we can discriminate between a sample having a high potassium and a low amino acid content and another sample low in potassium but high in amino acid content.

The mean equivalent weight of the anions in second carbonatation juice is about 58 so that the potassium and sodium salts are respectively equal to 2½ and 3½ times the weight of potassium and sodium per 100 sugar. The effective weight of the amino acids and their degradation products in second carbonatation juice is assumed to be 10 times the Moore and Stein nitrogen per 100 sugar in raw juice or brei extract. The factor of 10 is slightly larger than the average factor required to convert amino acid nitrogen to weight of amino acid, but the ultimate contribution of the amino acids to the nonsugars will be greater than their actual weight if the juice stability is sufficiently reduced to make addition of soda ash necessary. Betaine passes unchanged through the factory process and the weight contribution of this

compound to the second carbonatation juice nonsugars is equal to the concentration per 100 sugar in raw juice or brei extract.

The contributions of the principal nonsugars in terms of values measured per 100 sugar in raw juice or brei extract are therefore summed to give an impurity value of 2.5 potassium + 3.5 sodium + 10 amino acid nitrogen + betaine. By this summation it is possible not only to obtain a measure of the total nonsugars which should be present in second carbonatation juice but also to assess the relative importance of each of the principal constituent groups.

The seed breeder may choose to concentrate his selection on one particular component but, since these components may vary independently, the ultimate criterion of quality is the purity of second carbonatation juice. Although second carbonatation juice can be prepared in the laboratory for such an assessment, the procedure is not generally suitable for treatment of large numbers of samples, particularly when the quantities of beet material are small.

At the 8th Meeting of the American Society of Sugar Beet Technologists, Brown and Serro reported a new method for clarifying pressed juice from individual beets to yield a clear juice for purity determination. The pressed juice was treated with lime and clarified in two stages with saturated oxalic acid solution. Data were presented to show that the purities obtained by the new method, called oxalation, and by standard carbonatation were essentially identical and the procedure was recommended for the assessment of beet quality. Subsequent analysis of oxalated juice, however, showed that the inorganic constituents were present in rather different proportions from those in carbonatated juice and the residual calcium level was some 10 to 20 times greater than normal. Since the solubility of calcium oxalate in water is very low, it is surprising that oxalic acid is not a more efficient agent in the juice system, but we also know that about 3% of the oxalate in raw juice is not precipitated in the factory clarification process, even though the residual calcium and oxalate far exceed the aqueous solubility product.

The oxalate treatment also eliminates about one-fifth to one-quarter of the potassium and sodium ions and, though these two effects are to some extent compensating in effect on purity, it is obviously desirable that the clarified extract should be as similar as possible to real second carbonatation juice. We have therefore used an adaption of the Brown and Serro method using 3 M phosphoric acid instead of saturated oxalic acid for deliming. The residual calcium, potassium and sodium in the phosphated juice

are very similar to the factory levels and the phosphate treatment is also superior in that less dilution is caused by 3 M phosphoric acid than by saturated oxalic acid, which is only 0.8 M. The purity of the phosphated juice is not distinguishable from that of standard second carbonatation juice and if further information on composition is required, the brilliantly clear juice can be employed for determination of potassium, sodium and betaine.

Amino acid nitrogen can also be assessed since the slight cyclisation of glutamine under the clarification conditions is largely balanced by the liberated ammonia which is determined at equivalent color yield by the Moore and Stein reagent. It is probably preferable, however, to measure the amino acid nitrogen directly in the pressed juice or in the lead extract used for determination of sugar in beet, since the dilution required is such that the color of the initial juice is unimportant.

An automated process has been installed at the Central Laboratory of the British Sugar Corporation to prepare phosphated juice from all of the thousands of samples of beet from the seed variety, fertilizer and other trials organized by the Corporation. Electronically controlled apparatus dilutes the pressed juice to a standard brix, adds the milk of lime and titrates the mixture with pH controlled burettes to the two end points. The heating and cooling stages are thermostatically controlled and the samples are processed at a rate of 12 per hour. The entire process, including the polarization of the clarified juice and estimation of solids with a fifth place dipping refractometer, is operated by one person.

In addition to sucrose, invert sugar and raffinose, de Whalley and Gross showed chromatographically that beet syrups contained kestose, one of a series of trisaccharides composed of two molecules of fructose and one of glucose. Three such fructosyl-sucrose compounds can be formed by the transfructosylase activity of yeast or mold invertase and the detection of the trisaccharide as in sugar beet products was originally attributed to an action of yeasts or other micoorganisms. However, it has since been confirmed that the trisaccharide occurs naturally in beet and apparently may be present in greater amounts in beets which have been grown under drought conditions. Two of the trisaccharides can be produced from sucrose by an enzyme preparation from the leaves of sugar beet and these are apparently similar to those formed by mold invertase while yeast invertase additionally produces a preponderance of the third trisaccharide which does not generally occur in significant concentration in beet products.

It is fitting to recall that Brown and Serro revealed that myo-inositol and galactinol are normal constituents of beet and in some areas the inositol content may equal that of raffinose. The detection of these oligosaccharides and glycosides illustrates the superiority of the chromatographic over the chemical or enzymatic methods for the determination of raffinose.

Although white sugar is produced to extraordinary standards of purity, the improved analytical techniques now permit the estimation of some of the minute traces of impurities still remaining in the sugar and, from examination of the amounts of these constituents, it is apparent that some components are present in relatively higher proportions in sugar than in the standard liquor from which the sugar was crystallized. It is therefore clear that the impurities did not arise simply from the presence of a film of mother liquor on the crystals. This finding was to be expected if co-crystallization of raffinose and sucrose occur, or if the mother liquor becomes saturated with any compound such as potassium imidodisulphonate, but apparently this phenomena also arises with floc constituents which may be present in sugar at a concentration of more than 10 times that which could be attributed to a mother liquor film.

Floc and foaming present related, but not identical, problems in the production of high quality sugar. About 10 years ago, Eis and his collaborators showed that raw juice floc was largely composed of oleanolic acid and its glycosides. This group of compounds is commonly called saponin and Walker and Owens later demonstrated that white sugar floc contained many other constituents. They considered that the acid insoluble saponin was the prime cause of floc and that, as the saponin coagulated, it scavenged other impurities from the solution. Since floc is manifested in acidified beverages, methods have been evolved for measuring the floc produced in acidified white sugar solutions either visually or gravimetrically. The principal disadvantage of these methods is that the floc coagulates only slowly so that there is necessarily a considerable delay between production of the sugar and the determination of the floc characteristics. Moreover the gravimetric method normally measures only the methanol soluble portion, or alternatively the saponin fraction of the floc.

This latter was found to represent only about 20% or less of the total floc in British beet sugars and consequently a more general estimate of surface-active trace impurities seemed desirable as an assessment of the suitability of sugar for bottling purposes. The depressive effect of surface-active impurities on the polarographic oygen maxima of sugar solutions proved to be

a suitable basis for a general estimation of this type. The pioneer work of Vavruch in evaluating sugars on the basis of polarographic behavior had led to a simple technique and, by using a recording polarograph to determine the polarographic peak height from sugar samples collected at the commencement of each strike, it is possible to assess the quality of the sugar sufficiently rapidly to decide whether or not the sugar is suitable for bagging as bottlers' sugar.

That it has been possible to resolve many of the problems associated with sugar beet chemistry is due to the development of new analytical techniques such as paper chromatography, high voltage electrophoresis and ion exchange fractionation. These methods have permitted the detection and determination of constituents at concentrations far below the limits of the classical approach. Even with very precise methods of analysis, however, it is tedious, and sometimes impossible, to elucidate the order of events in a dynamic chemical or biological process but, with the advent of the atomic pile, cheap radioactive isotopes have become available so that it is now commonplace to employ radioactive labelling of specific components in order to detect any reaction products unequivocably. Moreover since the labelling is quantitative the ratio of each product to the precursor can readily be determined.

Carbon-14, one of the radio isotopes most suited for sugar investigations, has a very long half life so that the diminution in activity is of no significance in the period of any normal experiment and, as the emission is pure β radiation of fairly low energy, only relatively simple screening and health precautions are required in the laboratory. The low energy of the emission presents some counting difficulties but, with very thin end-window counters, it is feasible to make direct G.M. counts of labelled sugars very rapidly and with a precision, as measurer by the count-rate : background ratio, similar to that obtained by the more time-consuming scintillation count techniques.

As an illustration, the detection and determination of residual oxalate in second carbonatation juice was achieved by clarification of juice containing a negligible weight concentration of radioactive oxalic acid. Traces of oxalic acid in clarified juice were identified chemically but, if calcium oxalate precipitation during defecation and carbonatation were incomplete, the measurement of residual oxalate in clarified juice by conventional calcium precipitation would require considerable verification because this latter precipitation might also be incomplete. In contrast only a few hours work were required to demonstrate that 3% of the

radioactivity, and hence 3% of the total oxalate in raw juice, was not removed by clarification. Experiments involving alkaline degradation of fructose-C14 also showed that the quantities of oxalate which could be produced by destruction of monosaccharides in evaporation were very small in comparison with the residual oxalate in thin juice and it was therefore possible to conclude that the prime cause of oxalate scaling in evaporators was incomplete oxalate elimination in clarification and not decomposition of oxalogenic substances.

The range of products which may be produced by the alkaline degradation of reducing sugars under different conditions is so vast that it would be a formidable task to attempt chemical separations for determination of the yield of any but the major degradation products in clarified beet juice. In this field also the load of work is significantly lessened by labelling the monosaccarides before clarification. In one typical experiment raw juice containing added glucose-14 was defecated with lime and carbonatated in the laboratory. Of the fructose 95% was degraded and, of the degraded material, none was absorbed on a strong cation exchanger while 93% was separated and recovered by absorption on a strong anion exchanger and elution with ammonium carbonate. This material was concentrated and separated into nine bands by high voltage electrophoresis. The bands were detected by autoradiography and the relative amounts of each were determined by elution and direct G.M. counting. About 5% and 40% of the activity was present in the two most mobile bands corresponding to glycollic and lactic acid respectively.

The four principal remaining bands contained saccharinic acids of increasing chain length as the mobility decreased. Alone responsible for one band was 2-4-Dihydroxybutyric acid, but as the chain length increased each band contained an increasing number of the isomeric saccharinic acids so that on lactonization of the hexosaccharinic acid band it was possible to identify five isomeric glucosaccharinc-lactones. At this stage the proportion of individual saccharinic acids in each band has not been established but it is known that the relative proportions of the acidic products can be varied by changing the clarification conditions and a range of saccharinic acids can be separated from molasses by ion exchange fractionation.

As an example of the more complex radiochemical applications in the field of photosynthesis, the work of Calvin and his collaborators is well known. On exposing photosynthetic materials to carbon-14 dioxide these workers found that, although

phosphates of glucose and fructose were rapidly produced, the first detectable free carbohydrate was sucrose and not a monosaccharide. Detailed examination of the changes in concentration of the radioactive compounds demonstrated that UDPG was labelled at an early stage of the process. UDPG was also being investigated by Leloir and Cardini and the characterization of this compound as a possible intermediary provided an important link in the chain of events leading to the present knowledge of the vital role of UDPG as a co-enzyme in the synthesis of sucrose.

Buchanan employed carbon-14 dioxide in photosynthetic experiments and demonstrated the formation of sucrose phosphate in the leaves of sugar beet as well as of other plants. He postulated that the sucrose phosphate was formed according to:

UDPG + fructose 1 phosphate $\rightleftarrows$ sucrose phosphate + UDP (1)

Leloir and his associates later demonstrated that an enzyme could be obtained from certain plant materials which would synthesise sucrose according to the following reaction:

UDPG + fructose $\rightleftarrows$ sucrose + UDP (2)

Extracts of sugar beet leaves or roots gave negative or non-reproducible results. However, workers in the Western Regional Laboratories of the USDA have recently demonstrated the presence of enzymes in young sugar beet leaves which will accomplish both of the reactions 1 and 2. In our laboratories at Bramcote it has been established that the root of the sugar beet contains an enzyme which will effect the synthesis of sucrose according to reaction 2. It was also shown that the enzymes and substrates necessary to provide a supply of the co-enzyme UDPG are present in the root and it is, therefore, not axiomatic that all of the sucrose is synthesized in the leaf system.

It is unnecessary to stress the importance of polarimetry, both financially and in process control in the sugar factory, and it has long been realized that automatic polarization is desirable, not only to minimize human errors, but also because the operation may be combined with automatic recording.

Many of the earliest attempts to avoid visual balancing of the polarimeter employed a single-field polarizer with a single photocell, and the 'crossed' position of the analyser was detected by the minimum in the photocell output. While the accuracy was similar to that of visual instruments, the photoelectric measurement was much more time consuming.

Photoelectric polarimeters were later designed using the conventional double-field polarizer and the two half-fields were fed separately to two photocells, the outputs of which were balanced

by rotating the analyzer. To eliminate differences in characteristics between the two photocells an additional blank balancing operation was required.

This additional balancing stage was avoided in an automatic polarimeter developed by the Spreckels Sugar Company. The two half-fields were fed to a single photocell but the light was interrupted by a rotating semicircular shutter so that, when the fields were unbalanced, the light intensity varied between a maximum and a minimum during each rotation of the shutter. The resulting alternating current output from the photocell was amplified to operate a balancing motor to equalize the field automatically; the phase difference between the mainline alternating current and the photocell output was employed to drive the quartz compensator in the correct direction to the balance point at which the alternating current from the amplifier fell to zero. As far as is known this instrument, which was described to the ASSBT in 1948, represented the first successful fully-automatic polarimeter. The polarization of the sample was printed directly from the quartz compensator and the results, estimated to 0.01%, could be obtained and printed at the rate of 400 samples per hour.

The principle of scanning the fields from a double-field polarizer to produce alternating current from a single photocell, in conjunction with various forms of time-base to indicate the correct direction of adjustment to the balance point, has been used in several subsequent automatic saccharimeters and polarimeters and some of these instruments have been employed commercially.

A major advance in practical automatic polarimetry has however occurred more recently in the development at the National Physical Laboratory of an instrument having no moving parts. Both the cyclic modulation of the incident polarized light and the balancing of the optical rotation of the sample are accomplished by means of the magneto-optic or Faraday effect, that is the use of a controlled electromagnetic field to render a glass block optically active. An adaptation of this instrument, the ETL-NPL automatic polarimeter, has been installed in control laboratories and in the tare laboratories at many sugar factories. Interference and Polaroid filters are used to produce a narrow waveband of plane polarized light which is passed through a glass rod forming the core of an electromagnet carrying a 60 cycle a-c supply. The alternating magnetic field induces alternating optical activity in the glass so that the plane of polarization is modulated over an angle of 3° either side of the unmodulated direction. The mod-

ulated beam then passes successively through the sugar solution, through a second Faraday cell to a Polaroid analyzer set in the crossed position relative to the polarizer and thence to a photomultiplier. The photomultiplier output is rectified to provide negative feedback inducing optical activity in the second Faraday cell equal and opposite to the rotation of the sample. The amplifier gain is so arranged that the instrument remains balanced automatically and the current flowing in the second Faraday cell is proportional to the optical rotation of the sample. This current can be used to operate a precision indicator or to display the polarization on an illuminated digital converter and the polarization can also be recorded automatically on a printing unit or punch card machine. The basic range of the instrument is only $\pm$ 0.5 angular degrees and the tube length employed is much less than in visual polarimetry. High relative precision at low rotation is advantageous because the absorption of the sample usually decreases exponentially while the rotation decreases linearly with decreasing cell length. It is therefore possible to measure optical activity in solutions which are far too dark for precise visual polarimetry. The polarizer can also be offset to examine angular rotations within its range of $\pm$ 0.5° anywhere in the total range of $-$ 90° to $+$ 90°.

For solutions containing more than 13% sucrose the ultimate precision of the polarimeter is about twice that of visual instruments while for solutions of low optical activity the automatic polarimeter is considerably the more precise since, with no offset, it is possible to obtain a full-scale reading for a 1.6% solution of sucrose in a 4 cm cell. The polarization of this dilute solution can be determined to 1 part in 2,500.

Various other automatic polarimeters have been developed using the Faraday effect either for modulation, compensation or both and these instruments have raised interesting problems in connection with the International Sugar Scale. There is a growing tendency to employ green light sources because their photoelectric characteristics and ease of reproduction are superior and photoelectric instruments also permit the use of shorter tube lengths. The International Sugar Scale at the moment is strictly only applicable to the dichromate filtered white light source and the 20 cm tube length and consequently, I.C.U.M.S.A. is endeavoring to define a sugar scale for modern instruments. We hope to reach some measure of agreement on the new scale at the 1962 Session.