XXVIII. THE EFFECT OF METHODS OF EXTRACTION ON THE COMPOSITION OF EXPRESSED APPLE JUICE, AND A DETERMINATION OF THE SAMPLING ERROR OF SUCH JUICES.

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(Received August 11th, 1919.)

A NUMBER of attempts have been made to follow the changes in chemical composition which fruits undergo during ripening and storage. The number of papers dealing with this subject is large, and many of them are published in obscure journals which are difficult of access. The investigations referred to below may however be regarded as typical in respect of methods, and an examination of these methods serves to show the necessity for the adoption of a standard procedure in the preparation of material for analysis, and for a careful estimate of the limits within which the results obtained are significant.

Most of the earlier work has been directed to following the changes of the fruit in respect of its content of sugar and of acid. In some cases acids and sugars have been estimated in a suspension of the pulp [Bigelow, Gore and Howard, 1905]. In others water or alcohol extracts have been made [Kulisch 1892, Browne 1901, Pfeifer 1876, 1877, 1879]. In the majority of cases, however, the juice has been directly expressed, either before or after killing the tissues [Dixon and Atkins, 1913¹]. It is clear that the results obtained by methods so various as these cannot be directly comparable. The value of extraction by water or alcohol evidently depends upon the completeness with which it is carried out, and in most of the extractions which have been made by this method, no attempt appears to have been made to determine this point. Davis and Daish [1914] found that it was necessary to extract leaves in a large Soxhlet apparatus for 18—24 hours with boiling alcohol before all the sugars were removed. No corresponding examination appears to have been made in the case of water extracts, but the extraction by cold water of pulp which has been merely cut up or grated must evidently be slow.

There can be no doubt that for most purposes the most satisfactory method of procedure is to press out the sap from pulp which has previously been

¹ See this paper for further references.

frozen. The advantages of this method have been demonstrated by Dixon and Atkins [1913], and though some doubt exists as to certain of their conclusions [Haynes 1919], yet they show clearly that the sap from untreated tissues is more dilute than that from tissues which have previously been exposed to low temperatures, indicating that some or all of the constituents of the sap are held back in some measure by the untreated pulp. It is possible however that tissues which have been frozen, although considerably more permeable than untreated tissues, still hold back some constituents of the juice. Dixon and Atkins made no attempt to ascertain whether this is the case.

The following experiments have therefore been undertaken with the object of determining how far the sap obtained from apples after freezing and pressing is uniform, *i.e.* whether the first runnings from the press are similar in composition to those obtained later, or whether the tissues still hold back certain of the constituents of the sap. Further, in most of the work hitherto published, the amount of divergence due to sampling error is entirely left out of account; indeed in some cases conclusions have been drawn from the analysis of single fruits. Experiments have therefore been made to ascertain the order of the error due to sampling in investigations of this kind.

A third series of experiments was undertaken with the object of ascertaining with what completeness chemical change in the sap was retarded by cold. It was found more convenient to use an ordinary freezing mixture than to freeze with liquid air, but in the first case the process was necessarily slower, and the apple pulp was usually left in the cold for at least 16 hours.

The following chemical and physical properties were investigated, and some or all were used as a basis of comparison in each case.

- (1) Value of P_H .
- (2) Value of Δ .
- (3) Time of fall in viscometer.

(These were all carried out by ordinary standard methods.)

(4) Electrical conductivity (using a Wheatstone Bridge and a Fitzpatrick commutator).

(5) Acidity (titration), using an iodometric method to avoid difficulties arising from the colour of the juice.

(6) Sugars (reducing sugars and cane sugar by Bertrand's method [1906]; the cane sugar was inverted by 10 per cent. citric acid, as recommended by Davis, Daish and Sawyer [1913]).

METHODS OF EXTRACTION.

The method adopted for extraction was to press out the juice after the tissues had been killed by freezing in an ordinary freezing mixture of ice and salt. A small handpress, worked on the lemon squeezer principle, and provided with a screw, was used. A thin filter cloth was found to be necessary,

and this was saturated with the juice before collection of the sample. The apples were peeled, cut up, and frozen in stoppered jars, and were usually left in the cold for about 16 hours. To ensure uniformity, the frozen pulp was thoroughly mixed before pressing. It was found that the sap obtained by this method from similar samples of pulp gave closely concordant results.

The following points were investigated:

I. *Whether rapid freezing by the use of liquid air produced any alteration in the character of the sample.* It is possible, as has already been stated, that chemical changes may occur during the somewhat prolonged process of freezing in an ordinary freezing mixture. It is also possible that further disruption of the cell membrane may take place when liquid air is used, and that this may affect the character of the sap. For the purpose of comparison, a uniform sample of pulp was divided into two portions, one of which was immediately frozen in liquid air, and pressed out as soon as it had warmed up to the temperature of the laboratory. The other portion was left overnight in a freezing mixture, and then treated in the same way. The chemical and physical properties of the two samples of sap thus obtained were compared.

Table I. *Comparative tests on freezing with liquid air and with a freezing mixture of ice and salt.*

	Liquid air	Freezing mixture
Freezing point depression (Δ)	1.260° C.	1.253° C.
Titration. 10 cc. juice = $N/10$ $\text{Na}_2\text{S}_2\text{O}_3$	9.15 } 9.20 cc. 9.25 }	9.25 } 9.27 cc. 9.30 }
Conductivity. $c \times 10^6$	245	229
Time of fall in viscometer	41.2"	41.2"
Total sugar (after inversion)	8.63 %	8.57 %

It will be seen that the differences obtained by using liquid air instead of a freezing mixture are very small. It is only in the case of electrical conductivity that the difference rises above the limits of experimental error. The reason for this difference has not at present been investigated.

II. *Whether the tissues after freezing are freely permeable to all those constituents of the cell sap present in the expressed juice.* For this purpose the juice was pressed out in several fractions, and these were examined separately. The results are shown in Table II.

In this case again the greater number of the results obtained were uniform. The concentration of acids and of sugars was found to be the same in the first fraction of juice from the press as in the last. The viscosity however varied greatly, indicating that the colloidal constituents of the sap are held back by the tissues. A corresponding difference was also observed in the amount of the precipitate obtained from the two fractions by the addition of alcohol. This precipitate is mainly pectin, which is the principal colloidal constituent of apple-juice.

Table II. *Results of fractional pressing.*

	Fraction	P _H	Δ °C.	Time of fall (viscometer)	Reducing sugars %	Total sugars %
Series I (unripe)	1	3.83	1.44	5' 24"	—	—
	2	3.84	1.42	0' 55"	—	—
	1	3.84	1.43	3' 34"	10.10	11.40
	2	3.85	1.42	1' 35"	9.97	11.35
Series II (ripe)	1	3.87	1.60	3' 43"	10.9	11.75
	2	3.82	1.60	1' 59"	11.05	11.65
		Fraction	Time of fall (viscometer)			
		1	4' 17"			
		2	0' 54"			
		Series III (unripe)	1	5' 29"		
			2	2' 59"		
			3	1' 20"		
	Fraction	Δ °C.	Titration	Conductivity (c × 10 ⁵)	Reducing sugars	Total sugars
Series IV (cold store)	1	1.110	11.3	241	6.20	6.99
	3	1.112	11.15	238	6.25	7.08
Weight of alcohol-precipitate from 10 cc. juice						
		1	0.0292 g.			
		3	0.0090 g.			
	Fraction	Δ °C.	Titration	Conductivity (c × 10 ⁵)		
Series V (ripe)	1	1.377	10.7	237		
	3	1.380	10.7	239		

III. *The probable error due to individual variability in the apples used.*
This was estimated in the usual way by the comparison of a number of samples of sap obtained from individual apples.

The apples on which these experiments were carried out had been kept in cold store for some months, and it is possible that they varied among themselves more than would have been the case had they been more recently picked. Several sets of experiments were made for this purpose. As the results obtained were very similar, it has been thought sufficient to give one set of results, the probable errors being calculated from ten observations¹.

These have been calculated for the purpose of attaining a definite standard of accuracy defined as the probability that the error of any observation may lie within certain limits. This is determined by the number of samples taken.

The standard probability will be taken here as 0.957 (22 to 1), *i.e.* three times the probable error, and the limit below which differences cease to be significant as 5 per cent.

¹ A non-mathematical treatment of the use of probable errors applied to biological work is given by Wood [1911].

Table III. *Probable error attaching to various measurements.*

No. of sample	Freezing point depression (Δ) °C.	Titration	Conductivity ($c \times 10^6$)
1	1.265	9.85	223
2	1.173	9.65	223
3	1.143	12.7	287
4	1.245	10.05	233
5	1.150	10.55	250
6	1.290	8.3	240
7	1.323	10.3	217
8	1.243	10.45	234
9	1.225	10.95	252
10	1.238	9.2	208
Mean = 1.230 ± 0.0127		Mean = 10.20 ± 0.25	Mean = 236.4 ± 4.8

The probable errors of a single observation are:

	Δ	Titration	Conductivity
%	0.0412 = 3.2	0.78 = 7.7	15.2 = 6.4

Since the probable error of the difference of the observations is equal to $\sqrt{2p}$, where p is the probable error of either observation, the above conditions are satisfied by the equation

$$\frac{3 \times \sqrt{2p}}{\sqrt{n}} = 5,$$

where n is the number of observations. The number of samples which must be taken in order that there may be a probability of 0.957 that a 5 per cent. difference is significant is therefore given by the equation

$$n = 2 \left(\frac{3p}{5} \right)^2.$$

The following are the values of n obtained from this equation:

For Δ	7.4
For titration	42.6
For conductivity measurements	29.4

The least number of samples required for these three estimations is therefore 8, 43, and 30 respectively.

These are large numbers and it is to be remembered that the standard of probability assumed is somewhat low. It is more usual to require a probability of not less than 0.969 (30 to 1), *i.e.* a difference of 3.2 times the probable error, in which case the necessary number of samples would be 9, 49, and 34 respectively.

The value of the probable error deduced from such a small number as ten samples is, of course, only approximate but it shows clearly how important is the sampling error. The use of more samples would probably give a somewhat larger probable error but the labour involved in working even with ten samples is considerable.

*The importance of the sampling error in measurements of this kind does not appear to have been fully realised by previous workers. It is possible

that the apples used in these determinations differed among themselves more than would have been the case had they been subjected to different treatment, and the error would probably have been considerably reduced if all the apples had been obtained from a single tree, since Kulisch [1892] has shown that apples from different trees may exhibit marked and constant differences; but whether this be so or not it is clear that the sampling error may be very large and from the nature of the material must be far from negligible in any experiments of this kind. Where experiments have been made on two or three fruits only, there can therefore be little doubt that the differences observed are not significant unless they are very large.

SUMMARY.

The juice obtained from apples after freezing has similar properties, whether the tissue is frozen rapidly by means of liquid air, or slowly by a freezing mixture.

The sugars and acids present in the tissues can be readily pressed out from the frozen and thawed pulp; colloidal substances, however, of which pectin is the most important, are held back to a large extent by the pulp, so that the amount of these substances present in the expressed juice is no measure of the quantity in which they are present in the tissues.

The samples investigated varied considerably, especially in acid content. These large fluctuations cause the probable error to be very large, and the number of samples required to give results significant within 5 per cent. for lowering of freezing point, for titration, and for conductivity, was 8, 43, and 30 respectively, when the standard of probability assumed was 0.957 (*i.e.* a probability of 22 to 1). The neglect of sampling errors in previous estimations of this nature detracts very seriously from the value of the results obtained and renders many of them worthless.

The work described above has been undertaken in connection with investigations of Cold Storage problems which have been carried out for the Food Investigation Board of the Department of Scientific and Industrial Research. We are indebted to Professor V. H. Blackman for suggestions on which this work has been largely based, and also for much help and advice.

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