

The Composition of Honey. II. Lactone Content

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The reversion of neutralized honey or honey solutions toward their original acid condition has been previously noted by Osborn (quoted by Walton (1)). It is a source of difficulty in determining the acidity of honey and has been encountered in attempts to provide a partly neutralized honey for commercial baking use (2). Cocker (3) has ascribed this drifting of pH to production of acid in honey by enzyme action, though his observations on the absence of the phenomenon in boiled honey solutions have not been substantiated in our laboratory.

We have noted the probable presence of lactone(s) in honey during ion exchange isolation of the acids of honey. The ion exchange columns apparently leaked acidity even though their capacity was far from ex-

ceeded. By repeated treatment, a truly neutral fraction was obtained. The acid fraction removed in the second pass showed the neutralization behavior previously noted in honey. Its identity is being investigated.

Determination of titratable acidity is a standard procedure in honey analysis (4). Considerable difficulty has been experienced in obtaining stable end points (4, 5); the present official procedure requires maintenance of phenolphthalein pink for ten seconds; titration must be relatively rapid to duplicate results.

In connection with an analytical survey of all important types of honey produced in the United States, the determination of acidity in honey has been re-examined. The official procedure has been modified to make it more reproducible and a determination of lactone content has been added. The procedure includes a direct titration for free acidity followed by addition of excess alkali

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and back-titration to the end point. Possible effect of the brief alkalinity in producing acidity from the honey sugars has been considered. Application of the procedure to 225 honey samples has shown that lactone is a general constituent of honey, and the frequency distribution of various levels of lactone content, free acidity, and their total is shown.

Experimental

Effect of Neutralization on pH of Honey Solutions.—Two solutions, each of about 13 g clover honey in 100 ml water, were titrated to pH 6.8–7.0 and let stand. One solution had been boiled for 10 minutes before neutralization to inactivate any enzyme that might be present. Figure 1 shows the course of pH with time. Each was brought back to neutrality after about 3 hours.

It is evident that the drop in pH value is not primarily due to enzymic production of acid. The possibility that there is an enzymic production of acid is not excluded, since the amount of alkali needed to restore the pH to 7.0 was 0.25 ml less in the boiled sample. The unboiled sample required 2.30 ml for the initial neutralization, while the boiled and cooled sample required 2.20 ml initially. This difference may have been due to loss of volatile acids.

An experiment was carried out exactly as described by Cocker (3). Again the pH drift was substantially identical in the heated and unheated samples. This is in contrast to Cocker's result in which the pH of only the unheated sample drifted.

Ion Exchange Treatment of Honey.—A solution (20% solids) of a clover honey was passed through a column of Dowex 50 and immediately through a column of Duolite A-4. The total acidity in the sample, including that from splitting the salt content, was less than 60% of the capacity of the Duolite A-4. After 150 ml (of a total sample of 3640 ml) had passed through, it was noted that the pH of the effluent had fallen to 4.5. This effect was observed in varying degree on each of three runs. A sample of the effluent was titrated with dilute alkali and showed the unstable end point described for honey, dropping from pH 8.25 to 6.9 in 38 minutes.

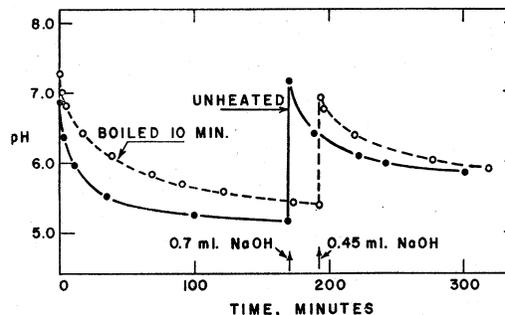


Fig. 1. pH drift in neutralized honey solutions.

The remaining "neutral" effluent fraction was preserved by concentration to 80% solids. Later 675 g of it was diluted to 20% solids and passed slowly through a column of Duolite A-4 (75% of calcd. capacity = 100 meq). The effluent varied in pH from 7.53 to 7.29 and showed no titratable acidity. It (neutral fraction) was evaporated *in vacuo* to 82% solids. The column was washed with water to a negative reducing sugar test and then stripped with 500 ml 1N NaOH. This was combined with 100 ml wash water and passed through a 350 ml column of Dowex 50 (75% capacity = 580 meq). The effluent was exactly neutralized, requiring 6.94 meq, and freeze-dried. The sodium salt weighed 1.4813 g, giving a calculated neutral equivalent of 213. The identity of the material is under investigation.

Titration of Lactone in Honey.—To determine whether the procedure ordinarily used to titrate lactones could be applied to honey, a solution of 49.5 g honey was diluted to 500 ml and 100 ml aliquots were titrated as follows:

- (1) Titrated to pH 8.30 by official A.O.A.C. procedure requiring 3.52 ml 0.1000N alkali. The pH then drifted downward.
- (2) Ten ml alkali was added to another 100 ml aliquot, giving a pH value of 9.80, and the solution was immediately brought to pH 8.30 with standard HCl. No subsequent drift was noted. Net alkali consumption was 4.15 ml.
- (3) Procedure 2 was repeated, except that the solution remained alkaline

(pH 9.8) for 15 minutes before back-titration. Net NaOH required, 4.43 ml.

- (4) Procedure 1 was repeated, requiring 3.32 ml.

In order to determine the possible contribution to acidity by the alkaline decomposition of sugars during the period when the solution is above pH 8.3, 52 g of the acid- and lactone-free neutral fraction prepared above was diluted to 500 ml and 100 ml aliquots were titrated as follows:

- (5) A.O.A.C. titration. Required 0.25 ml 0.1N alkali to pH 8.3. No drifting was observed.
- (6) To solution from (5), 5 ml alkali was added and it was immediately titrated to pH 8.3 with HCl. Net additional alkali consumption, 0.03 ml.
- (7) An aliquot was titrated to pH 8.3 (0.23 ml) and 5.00 ml alkali was added. After 15 minutes, it was back-titrated. Net additional alkali consumption was 0.15 ml.

Production of acid during the alkaline phase is thus negligible if the back-titration is carried out immediately.

Completeness of Lactone Titration.—The effect of several factors on the completeness of the titration of lactone by this procedure was investigated by using a sugar-free gluconolactone solution. The following treatment gave the same titer as the immediate back-titration described above: letting stand 15 minutes while alkaline; boiling while alkaline; and titrating boiling solution directly with alkali rather than adding excess alkali and back-titrating.

Determination of Free Acidity and Lactone Content of Honey.—The following titration is carried out with a pH meter and 10-ml microburets with extended tips delivering 0.05000N HCl and 0.05000N alkali into the 250 ml beaker used to contain the sample:

To a 10 g sample of honey contained in a 250 ml beaker is added 75 ml CO₂-free distilled water. The honey is dissolved and the solution is stirred with a magnetic stirrer. The electrodes of a pH meter are placed in the solution and the initial pH is recorded. The solution is then titrated with 0.05N

NaOH. The NaOH is added at such a rate that individual drops must tend to merge into a steady stream (5.0 ml/min.). The addition of NaOH is stopped when the pH reaches 8.5. Immediately 10 ml 0.05N NaOH is added by means of a 10 ml pipet and without delay the pH is brought back to pH 8.3 by the rapid addition of 0.05N HCl from a 10 ml buret. The amount of NaOH added from the buret, minus the "blank" correction, is considered the measure of the free acid present, and the amount of HCl used subtracted from 10 ml is a measure of the lactone content. The sum of free acid and lactone is the total acidity. All values are calculated to ml 0.1N alkali per 100 g sample, or milliequivalents per kilogram. The titration rate given is as rapid as found consistent with acceptable reproducibility. It was found that titration to pH 8.5 was equivalent to maintenance of phenolphthalein pink for 10 seconds, since the pH falls to 8.3 in that time.

This procedure has been applied to several hundred samples of honey. For 30 consecutive samples (in triplicate) the standard deviation of the free acidity titration was 0.20, that of the lactone determination 0.17, and that of total acidity, 0.15 meq/kg. The distribution of lactone content, free acidity, and their sum (total acidity) is shown in Fig. 2 for 225 samples, including 218 honeys and 7 honeydews. Detailed results

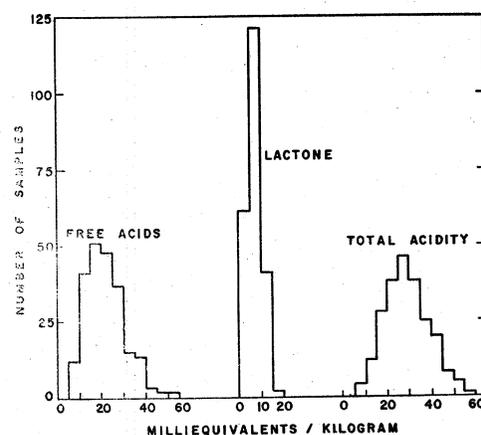


Fig. 2. Distribution of free acidity, lactone content, and total acidity in 225 honey samples.

will be presented later, together with other information on these and additional samples.

Summary

Honey contains lactone(s) as a general component. The official A.O.A.C. acidity titration for honey is unsatisfactory because of drifting end point caused by slow hydrolysis of the lactone content. A procedure is described to determine free acidity, lactone content, and total acidity in honey,

and the distribution of results of 225 analyses is given.

References

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