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Analytical Methods Section

Determination of ascorbic acid in fruit and vegetables using normal polarography

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The ascorbic acid content of fruit and vegetables was determined by normal polarography. It was found that oxalic acid and EDTA protected the vitamin during the sample preparation procedure but oxalic acid was preferred to be used for polarographic analysis. Citrate buffer was used as the supporting electrolyte since it was already present in fruit and vegetables and resisted the change in pH upon the addition of the fruit extract. The most suitable pH of the supporting electrolyte was determined to be 4·5. A procedure is suggested for the determination of ascorbic acid in fruit and vegetables. The standard deviation for the method based upon the pooled precision data was found to be 1·0 for tomatoes and 3·1 for oranges.

INTRODUCTION

In general, titrimetric and spectrometric methods have been used for the determination of ascorbic acid. But both methods have certain limitations due to the presence of interfering substances (Gilliam, 1945; Sebrell & Harris, 1967).

Polarographic methods can be used for ascorbic acid determinations (Diemair *et al.*, 1961; Lento *et al.*, 1963; Strohecker & Henning, 1966; Heyrovsky & Zuman, 1968; Adachi *et al.*, 1971; Mason *et al.*, 1972; Linquist, 1975; Linquist & Farroha, 1975; Pachla & Kissinger, 1976; Ratzkowski & Korol, 1977; Sontag & Kainz, 1978; Branca, 1980; Gerhardt & Windmüller, 1981; Falat & Cheng, 1982; Lechien *et al.*, 1982; Amin, 1983; Hoffmann, 1984; Roehle & Voight, 1984; Lau *et al.*, 1985; Kozar *et al.*, 1988) and have some advantages over colorimetric and titrimetric ones. In this method, the preparation of the sample is relatively simple and requires fewer steps. Because of comparative rapidity, the oxidation of the vitamin is prevented during the sample preparation and the measurement stages. In addition, analysis can be carried out with a few millilitres of the sample. The colouring, and especially the suspended material which show undesirable effects in the spectrophotometric measurements, do not necessarily interfere.

The main limitations of polarography are the relatively low sensitivity, the limited anodic voltage range of the mercury electrode (Gilliam, 1945; Strohecker & Henning, 1966; Heyrovsky & Zuman, 1968) and interferences caused by high concentrations of chlorides or thiols (Heyrovsky & Zuman, 1968). On the other hand, the method is specific for ascorbic acid and an inspection of the anodic current will indicate the presence of interfering materials. Since the ascorbic acid content of fruit and vegetables is at such a level that it can be determined by normal polarography with a simple preparation step, there was no need to use expensive instruments for its determination.

MATERIALS AND METHODS

Reagents

All the reagents used were of analytical-reagent grade. Triply distilled water was used for the preparation of all solutions and at all other stages of analysis.

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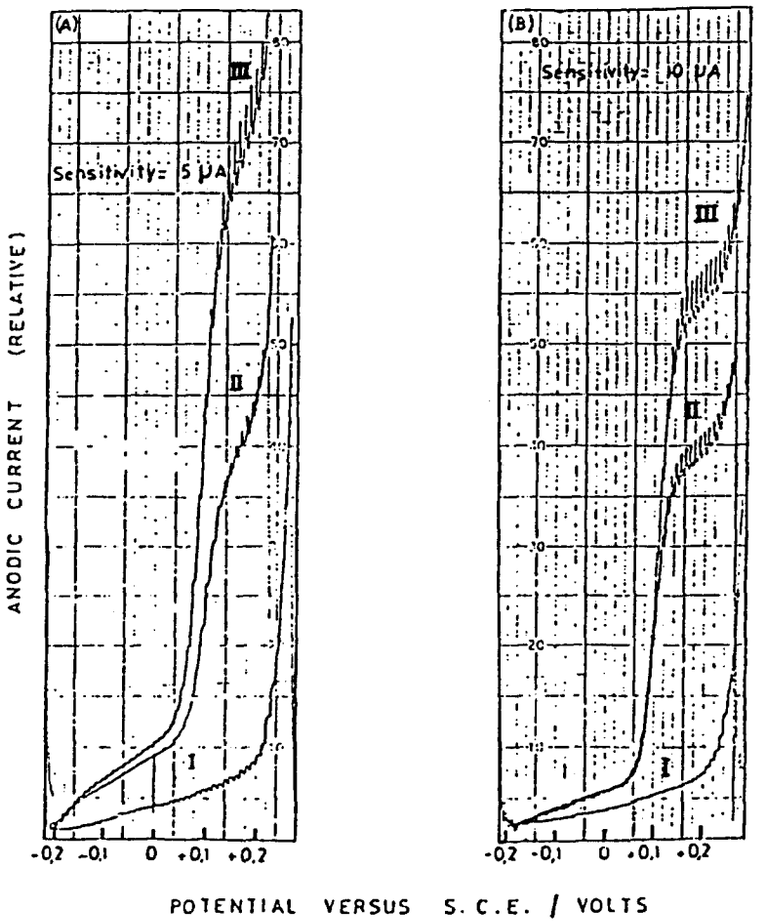

Fig. 1. Determination of ascorbic acid in (A) tomato and (B) orange samples. Scan rate = 0·2 V/min, chart speed = 1 in/min, t = 3·2 s. (I) = 5 ml of citrate buffer (pH = 4·5) + 1 drop of 0·5% gelation. (II) = (I) + 5 ml of sample. (III) = (II) + 0·1 ml of standard solution.

Standard ascorbic acid solution (5 mg/ml) was prepared daily by dissolving 250 mg ascorbic acid in 50 ml of water.

Citrate buffer (pH 4·5). The pH of 0·5 M citric acid was adjusted to 4·5 by dropwise addition of 0·5 M KOH, using a pH-meter. This solution was stable for 2 weeks.

Apparatus

The current–voltage curves were obtained by a polarography system constructed by the Physics Department of Hacettepe University (Ankara, Turkey), based on the diagram of Heath EUW–198, and the recorder was a strip chart recorder (SR–255B) built up by Heath Schlumberger. The polarographic cell was obtained from Princeton Applied Research and the saturated calomel electrode (Coleman) was used as the reference electrode. The dropping mercury electrode was of the Heath System and t values of the dropping capillary were between 3 and 4 s when the capillary tip dipped into the supporting electrolyte and no potential was applied to the mercury drop.

The scan rate and chart speed were 0·2 V/min and 1 in/min, respectively and the potential sweep was adjusted between the limits −0·2 V to +0·3 V.

Procedures

Synthetic samples

Citrate buffer (5 ml, pH 4·5) in the polarographic cell was de-aerated with nitrogen for 10 min and the current was recorded. Then 2 ml of ascorbic acid solution (0·40 mg/ml) was added and, after de-aerating the solution, the diffusion current was measured. Then 0·1 ml of standard solution (5 mg/ml) was added, the second diffusion current was recorded and the ascorbic acid content of the sample was calculated by the standard addition method.

Fruit and vegetable samples

The weighed sample was whipped in the blender with 50 ml of 0·2% oxalic acid for about 2–3 min. The extract was filtered through a cotton gauze and the volume was measured. Citrate buffer (5 ml, pH 4·5) in the polarographic cell was de-aerated for 10 min, the residual current was recorded and a known volume of the extract (1–5 ml) was added. After de-aerating the solution, the diffusion current was measured. Then 0·1 ml of standard solution (5 mg/ml) was added and the second diffusion current was recorded (Fig. 1). The ascorbic acid content of the extract was obtained by standard addition method from the following equation:

$$C_1 = \frac{i_1 c v}{(i_2 - i_1) V + i_2 v}$$

where C_1 = concentration of ascorbic acid per ml of extract
c = concentration of ascorbic acid per ml of standard solution
v = ml of standard solution of ascorbic acid added
V = ml of buffer plus extract solution
i_1 = wave height of the extract solution
i_2 = wave height of extract solution plus standard ascorbic acid

2,6-Dichlorophenolindophenol titrimetric method

The procedure followed for the determination of ascorbic acid was given by AOAC (1984).

RESULTS AND DISCUSSION

Stability of ascorbic acid

Ascorbic acid decomposes fairly quickly both during sample preparation step and storage period. The loss is mostly due to its oxidation and the reaction is catalysed by the copper ions (Khan & Martell, 1967; Ogata *et al.*, 1968; Shtamm & Skurlatov, 1974; Davidson & Grieger-Block, 1977). This reaction is also catalysed by an enzyme called ascorbic acid oxidase which contains copper ions and is present in natural materials (Sebrell & Harris, 1967; Birch & Parker, 1974). For this reason, many stabilizing acid solutions were examined during this work. EDTA was also included, considering that it would complex copper and thus inhibit the oxidation (Table 1).

Table 1. Stability of ascorbic acid in different acid solutions[a]

Medium	Decomposition (%)		
	Replicate 1	Replicate 2	Replicate 3
Water	53	24	41
0·2% $H_2C_2O_4$	0	0	11
1% Citric acid	9	9	14
0·07% HNO_3	14	6	15
0·07% HCl	20	22	27
0·07% $HClO_4$	7	12	14
EDTA	6	7	0
Water/Cu^{+2}	100	93	91
0·2% $H_2C_2O_4$/Cu^{+2}	10	8	7
1% Citric acid/Cu^{+2}	51	53	41
0·07% HNO_3/Cu^{+2}	50	44	52
0·07% HCl/Cu^{+2}	86	71	75
0·07% $HClO_4$/Cu^{+2}	65	45	47
EDTA/Cu^{+2}	9	5	6

[a]Initial concentration = 0·40 mg/ml.
Cu^{+2} = 2 ppm.
EDTA = 0·0012 M.

For this purpose, 14 stoppered 10-ml bottles containing ascorbic acid and the reagents were kept at room temperature for 24 h. Three replicate experiments were carried out but on different days. Since there was no temperature control during these experiments, the results were given as different runs instead of giving a mean value for the three replicate measurements (Table 1).

Only two solutions (oxalic acid and EDTA) were found to be reasonably satisfactory. Although EDTA afforded excellent stability for ascorbic acid, samples in this medium could not be polarographed easily because a second wave with the half-wave potential of +190 mV was also recorded besides the anodic wave of ascorbic acid ($E_{1/2}$ = +80 mV at pH 4·5). When only EDTA (10^{-4} M) was added to the citrate buffer, the same current ($E_{1/2}$ = +190 mV) was observed due to the oxidation of Hg to Hg $(EDTA)^{-2}$. The formation of this second wave limited the potential range for the ascorbic acid current. Thus, although there was no difference between the stability of ascorbic acid in EDTA and oxalic acid, the latter one was preferred to be used for the polarographic analysis.

Supporting electrolyte

Various supporting electrolytes, namely acetate buffer (pH 4·7) (Heyrovsky & Zuman, 1968), acetate buffer saturated with sodium oxalate (pH 5·5) (Strohecker & Henning, 1966), phosphate buffer (pH 8·0) and potassium biphthalate–sodium hydroxide buffer (pH 6·2) (Gilliam, 1945) have been used in the polarographic analysis of ascorbic acid in fruit and vegetables, but the citrate buffers have not been used up to now. Due to the similarity in the composition of fruit and vegetables, citrate buffers were chosen as the supporting electrolyte in this work, anticipating that they would resist the pH change upon the addition of fruit and vegetable extract. It is well known that the supporting electrolyte should have high buffer capacity for the precision of the measurement and it was observed that citrate buffers fulfilled these requirements.

Concentration range and sensitivity

According to the experiments, the lowest detectable quantity of ascorbic acid was found to be 25 μg/ml ($1{\cdot}42 \times 10^{-4}$ M) and the linearity of the current–concentration relationship was ascertained for the range of 25–250 μg/ml ($1{\cdot}42 \times 10^{-4}$ to $1{\cdot}42 \times 10^{-3}$ M). In this range, by using the calibration curve or the standard addition technique, ascorbic acid can be determined quantitatively. The sensitivity (i/c), defined as the diffusion current divided by the concentration, was obtained for the pH values of 2·5–6·0. Comparison of these values by factorial variance analysis revealed significant

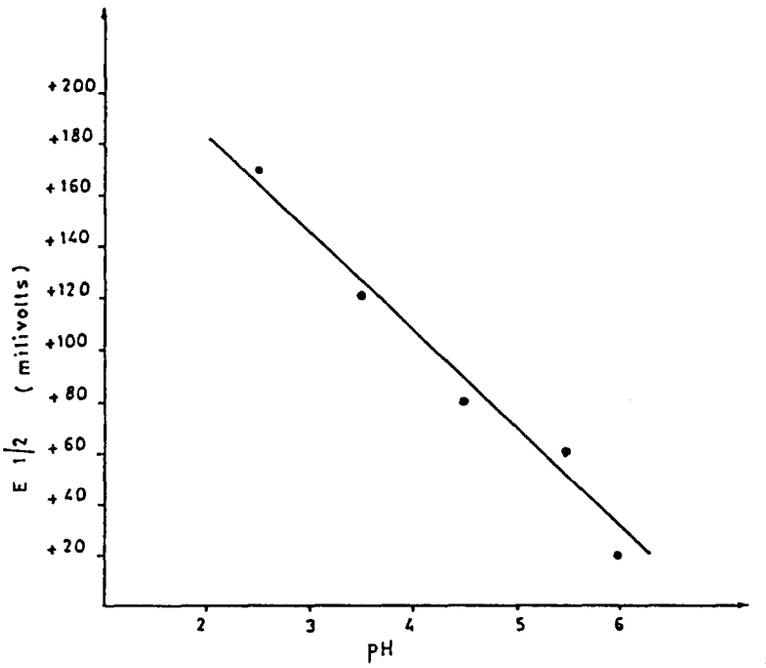

Fig. 2. The half-wave potential of ascorbic acid as a function of pH.

effects of pH on sensitivity ($p < 0.05$). Also, the sensitivity was found to be maximum at pH 4·5.

Dependence of half-wave potential on pH

The half-wave potential of the anodic current of ascorbic acid was measured between the pH values of 2·5–6·0 in citrate buffer and $E_{1/2}$ was found to shift to more positive values as the pH decreased (Fig. 2). Although the half-wave potential values differed, depending on the supporting electrolytes used, the same tendency in the shift was also observed by other authors (Coulson *et al.*, 1950; Linquist, 1975; Ruiz *et al.*, 1977; Sontag & Kainz, 1978).

pH of the supporting electrolyte

The citrate buffer of pH 4·5 was selected as the supporting electrolyte, considering the following points:

- — the maximum sensitivity was reached at pH 4·5,
- — at pH values higher than 4·5, ascorbic acid became unstable (Khan & Martell; 1967; Ogata *et al.*, 1968),
- — at lower pH values, the plateau of the wave became too steep to measure the anodic current,
- — as the pH decreased, the anodic wave was shifted towards more positive potentials and, as a result, an interference with the oxidation wave of mercury occurred.

Accuracy and precision

In order to determine the accuracy and precision of the method, the ascorbic acid solutions (app. 40 mg/100 ml), prepared by using distilled water or the citrate buffer, were analysed by the proposed technique. Samples that were prepared by using citrate buffer were considered to be the synthetic substitutes of fruit and vegetables. For each sample prepared, three replicate measurements were made by the standard addition method. The recovery values were 101 ± 1·3% when the sample was prepared with distilled water and 102 ± 0·9% when citrate buffer was used. Comparison of these recovery values by the analysis of variance showed that the mode of sample preparation did not affect the analysis ($p < 0.05$). It was also found that the amount of ascorbic acid could be determined with the relative error of −1·8 to +4·5(%), and the coefficient of variation (CV) lay between 2·1 and 4·5(%). Standard deviation of the

Table 2. Polarographic determination of ascorbic acid in synthetic samples

Mode of sample preparation	Ascorbic acid present (mg/100 ml)	Ascorbic acid found (mg/100 ml) $\bar{X} \pm \frac{t^a s}{\sqrt{N}}$
Distilled water	40·0	41.0 ± 2·3
Distilled water	40·0	39·3 ± 2·6
Distilled water	39·7	39·7 ± 1·4
Citrate buffer	41·3	41·8 ± 2·4
Citrate buffer	40·4	41·0 ± 1·6
Citrate buffer	40·2	42·0 ± 3·2
Citrate buffer	40·2	39·7 ± 2·5

[a] 90% Confidence interval.
Relative error: −1·8 to +4·5%.
Coefficient of variation: 2·1–4·5%.
Standard deviation of the method: 1·4.

Table 3. Polarographic determination of ascorbic acid in tomato and orange extracts

Product	Sample number	Ascorbic acid found (mg/100 ml) $\bar{X} \pm \frac{t^a s}{\sqrt{N}}$
Tomato extract	1	17·8 ± 1·5
Tomato extract	2	13·5 ± 0·8
Tomato extract	3	23·0 ± 2·5
Tomato extract	4	17·4 ± 2·2
Tomato extract	5	21·0 ± 1·3
Orange extract	1	39·1 ± 3·8
Orange extract	2	69·0 ± 4·6
Orange extract	3	48·6 ± 6·0
Orange extract	4	52·6 ± 6·6
Orange extract	5	53·5 ± 4·5

[a]90% Confidence interval.
Coefficient of variation: 3·4–8·2% for tomato extracts; 4·0–7·5% for orange extracts.
Standard deviation of the method: 1·04 for tomato extracts; 3·09 for orange extracts.

method, based on pooled precision data, was determined to be 1·4 (Table 2).

Determination of ascorbic acid in fruit and vegetables

Quantitative analysis was carried out in tomato and orange samples and five tomatoes or oranges were used for analysis, according to the procedure given in the experimental section. The precision of the measurements was determined by the three replicate measurements performed for the same sample (Table 3). The CV (percentage of standard deviation divided by the arithmetic mean) was found out to be 3·4–8·2% for tomatoes and 4·0–7·5% for oranges. When compared to the CV values obtained for pure solutions, the reproducibility of the results of the repeated determinations on orange or tomato extracts was found to be lower. This may be due to the fact that the analysis for natural materials included more steps.

A second polarographic wave was observed between −0·2 and +0·1 V, generally in tomato and sometimes in orange samples (Fig. 1). This interference was due to the presence of glutathione, as described by Coulson *et al.* (1950). The character of the ascorbic acid wave was influenced by the presence of glutathione (e.g. the half-wave potential shifted to more positive values and the anodic wave was steeper). When working with orange samples, a well defined anodic wave was obtained and the content of glutathione was not as high as to affect the ascorbic acid wave seriously. Tomato samples were found to yield satisfactory anodic waves, although an influence of glutathione was observed (Fig. 1). When the anodic wave of ascorbic acid did not coincide with the residual current, a correction was applied by measuring the difference between the waves of ascorbic acid and glutathione and thus the true diffusion current was obtained.

Table 4. Determination of ascorbic acid by normal polarography and 2,6-DCIP titration method

Product	Polarographic (mg/100 g) $\bar{X} \pm \frac{t^a s}{\sqrt{N}}$	2,6-DCIP (mg/100 g) $\bar{X} \pm \frac{t^a s}{\sqrt{N}}$
Tomato	13·4 ± 1·3	14·6 ± 1·0
Orange	41·2 ± 3·5	44·2 ± 2·9

[a]90% Confidence interval.

The proposed method and the standard 2,6-dichlorophenolindophenol titration were applied to tomato and orange samples. The results (mean value of three measurements) shown in Table 4 illustrate that the measurements obtained by polarography are in fairly good agreement with those obtained by titrimetric analysis.

Even though only tomato and orange samples were analysed in this laboratory, the method proposed is applicable to other fruit and vegetables when the measured ascorbic acid range is from 25 to 250 μg/ml.

CONCLUSIONS

A method is presented in which ascorbic acid can be determined easily by the polarographic technique. The principal advantages with this method are its specificity, comparative rapidity and applicability to highly coloured solutions. It is also straightforward with easy sample preparation steps, low reagent consumption and time saving. In addition it does not require expensive instrumentation. Thus these features make it suitable for analysis of vitamin C in fruit and vegetables.

REFERENCES

Adachi, Y., Ukai, N. & Kosuge, S. (1971). Browning mechanism of tomato products II. Separation and determination of reductones in tomato products. *Nippon Shokuhin Kogya Gakkai-Shi*, **18**(10), 455–60. *Chem. Abstr.*, **79**, 16986 t.

Amin, D. (1983). Application of differential pulse polarography to the assay of ascorbic acid. *Microchem. J.*, **28**(2), 174–9.

AOAC (1984). *Official Methods of Analysis of the Association of Official Analytical Chemists.* AOAC, Virginia, USA.

Birch, G. G. & Parker, K. J. (1974). *Vitamin C.* Applied Science Publishers Ltd, London, UK.

Branca, P. (1980). The polarographic determination of ascorbic acid. *Boll. Chim. Unione Ital. Lab. Prov.*, **6**(2), 143–52.

Coulson, D. M., Crowell, W. R. & Friess, S. L. (1950). Polarography of reduced glutathione and glutathione–ascorbic acid mixtures. *Anal. Chem.*, **22**(4),525–9.

Davidson, R. S. & Grieger-Block, R. A. (1977). The effect of mass transfer on apparent ascorbic acid autoxidation kinetics. *Am. Inst. of Chem. Eng. J.*, **23**(4), 529–38.

Diemair, W., Koch, J. & Hess, D. (1961). Die polarographische Bestimmung von L-Ascorbinsaeure im Wein. *Z. Anal. Chem.*, **178**, 330–5.

Falat, L. & Cheng, H. Y. (1982). Voltammetric differentiation of ascorbic acid and dopamine at an electromechanically treated graphite/epoxy electrode. *Anal. Chem.*, **54**, 2108–11.

Gerhardt, U. & Windmüller, R. (1981). Die Polarographie zur Bestimmung der Ascorbinsaeure in Gewürzen. *Fleischwirtschaft*, **61**(9), 1389–92.

Gilliam, W. S. (1945). Polarographic determination of vitamin C in fruit and vegetables. *Ind. Eng. Chem., Anal. Ed.*, **17**(4), 217–21.

Heyrovsky, J. & Zuman, P. (1968). *Practical Polarography.* Academic Press, London.

Hoffmann, H. (1984). Electrochemical analytical methods in drug quality control. *Pharm. Ind.*, **46**(8), 845–50.

Khan, M. M. T. & Martell, A. E. (1967). Metal ion and metal chelate catalysed oxidation of ascorbic acid by molecular oxygen. I Cupric and ferric catalysed oxidation. *J. Am. Chem. Soc.*, **89**, 4176–85.

Kozar, S., Bujak, A., Eder-Trijunovic, J. & Kniewald, G. (1988). Determination of L-ascorbic acid in fresh and processed fruit and vegetables by differential pulse polarography. *Fresenius' Z. Anal. Chem.*, **329**(7), 760–3.

Lau, O. W., Shiu, K. K. & Chang, S. T. (1985). Determination of ascorbic acid in vegetables and fruits by differential pulse polarography. *J. Sci. Food Agric.*, **36**(8), 733–9.

Lechien, A., Valenta, P., Nuernberg, H. W. & Patriarche, G. J. (1982). Determination of ascorbic acid by differential pulse voltammetry. *Fresenius' Z. Anal. Chem.*, **311**(2), 105–8.

Lento, H. G., Daugherty, C. E. & Denton, A. E. (1963). Polarographic determination of total ascorbic acid in foods. *Agr. Food Chem.*, **11**(1), 22–6.

Linquist, J. (1975). Voltammetric determination of ascorbic acid by use of a carbon paste electrode. *Analyst*, **100**, 339–48.

Linquist, J. & Farroha, S. M. (1975). Application of differential pulse polarography to the assay of vitamins. *Analyst*, **100**, 377–85.

Mason, W. D., Gardner, T. D. & Stewart, J. T. (1972). Determination of ascorbic acid in pharmaceutical dosage forms based on oxidation at the tubular carbon electrode. *J. Pharm. Sci.*, **61**(8), 1301–3.

Ogata, Y., Kosugi, Y. & Morimoto, T. (1968). Kinetics of the cupric salt-catalysed autoxidation of L-ascorbic acid in aqueous solutions. *Tetrahedron*, **24**, 4057–66.

Pachla, L. A. & Kissinger, P. T. (1976). Determination of ascorbic acid in foodstuffs, pharmaceuticals, and body fluids by liquid chromatography with electrochemical detection. *Anal. Chem.*, **48**(2), 364–7.

Ratzkowski, C. & Korol, J. (1977). The quantitative differential determination of ascorbic acid and erythorbic acid in foods by polarography. *J. Inst. Can. Sci. Technol. Aliment.*, **10**(3), 215–18.

Roehle, J. & Voight, F. (1984). Einfgache Methode zur Bestimmung von L-Ascorbinsaeure in Zitruskonzentraten und Saeften. *Nahrung*, **28**(5), 579–80.

Ruiz, J. J., Aldaz, A. & Dominguez, M. (1977). Mechanism of L-ascorbic acid oxidation and dehydro-L-ascorbic acid reduction on a mercury electrode. I. Acid Medium. *Can. J. Chem.*, **55**, 2799–806.

Sebrell, W. H. & Harris, R. S. (1967). *The Vitamins*, vol. 1. Academic Press, New York, USA.

Shtamm, E. V. & Skurlatov, Y. I. (1974). Catalysis of the oxidation of ascorbic acid by copper (II) ions. I. Kinetics of the oxidation of ascorbic acid in the copper (II)–ascorbic acid–molecular oxygen system. *Russ. J. Phys. Chem.*, **48**, 852–5.

Sontag, G. & Kainz, G. (1978). Bestimmung von Ascorbinsaure in Fruchtsaften und Limonaden mit Differentialpulspolarographie. *Mikrochimica Acta [Wien]*, **1**(1–2), 175–84.

Strohecker, R. & Henning, H. M. (1966). *Vitamin Assay: Tested Methods.* Verlag Chemie, Weinheim, Germany.