Analysis of Acidity-Saccharinity by NMR

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The quality of fruits can be determined by the external factors such as weights, colors and surface conditions, and the internal factors such as tastes, textural properties and nutritive values which may depend on their acidity and sweetness. In order to determine the internal factors without the destruction of the sample, several non-destructive analytical methods have been used. One of those, NMR spectroscopy, is introduced in this study.

Navel orange, minneola tangelo and Cheju blue orange were used for the experiments, which were carried out on a Bruker DPX400 (9.4 T) with 5 mm dual probe. The samples were peeled off, and their kernels were ground and filtered. 0.1 ml of the juice was collected and transferred into an NMR tube. 0.4 ml of D_2O was added and mixed. ^{1}H-NMR, ^{13}C-NMR, COSY and HMQC experiments were carried out. In cases of ^{1}H-NMR experiments, water peak was suppressed by presaturation. The 90° pulse of ^{1}H-NMR was 9.7 usec and that of ^{13}C-NMR, 9.8 usec. The pulse sequence for COSY was presaturated N-type magnitude COSY, time domain was 2K(t2)×256(t1), and spectrum window, 10.6 ppm. The pulse sequence for HMQC was TPPI BIRD program. Spectrum window was 10.6 ppm(t2)×100 ppm(t1), and time domain, 2K(t2)× 256(t1).

For the analysis of the ^{1}H-NMR spectrum of the sample, ^{1}H-NMR experiments of pure citric acid, fructose, glucose and sucrose were carried out. The peaks at 2.7 ppm shown in the ^{1}H-NMR spectrum of the sample were assigned to be two methylenes of citric acid. The peaks between 3 and 5.5 ppm were caused by three kinds of saccharides. The peaks of 4.55 ppm and 5.15 ppm were assigned as anomeric proton of glucose, and the peak 5.34 ppm, the anomeric proton of sucrose. Of 40 peaks in COSY shown in Fig. 1, 16 were assigned, which confirmed the existence of α-glucose, β-glucose, α-fructose, β-fructose and sucrose. 28 peaks in the ^{13}C-NMR spectrum and their correlated ^{1}H-peaks were assigned based on HMQC shown in Fig. 2 and listed in Table 1. While the anomeric proton of sucrose is 1, the methylene peaks of citric acid at 2.7 ppm are 4. The second and the third column of Table 2 show the integrated area of the anomeric proton of sucrose and those of the methylene peaks of citric acid divided by 4 for 8 samples, respectively. The first column of Table 2 shows their pH values. The correlated equation of the ratio of the methylene peaks of

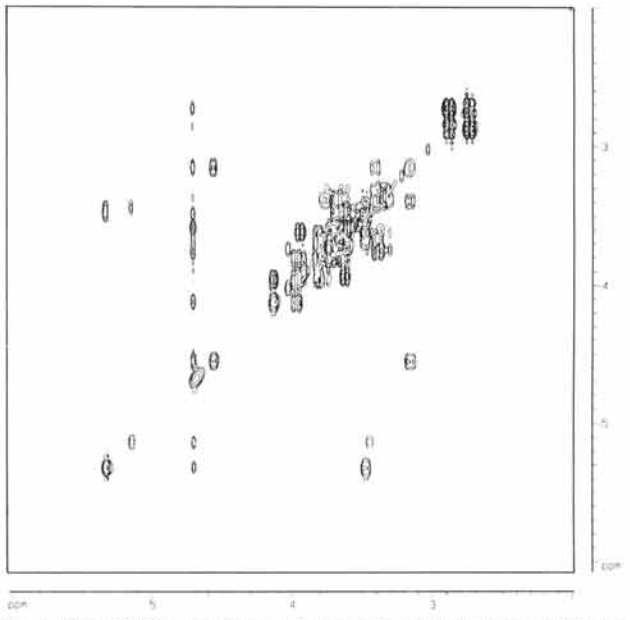

Fig. 1. The COSY spectrum of the standard sample of orange.

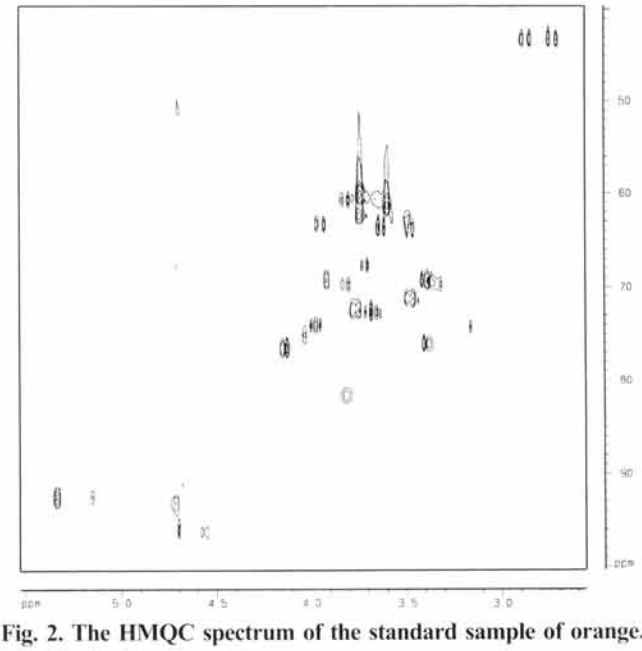

Fig. 2. The HMQC spectrum of the standard sample of orange.

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Abbreviations: COSY, correlated spectroscopy; HMQC, heteronuclear multiple quantum coherence; NMR, nuclear magnetic resonance; TPPI, time proportion phase increments.

Table 1. Assignments of the ^{1}H and ^{13}C spectral data of sucrose, glucose and fructose.

No. of C	δc	δ_H obtained from HMQC	assignments
1	43.921	2.88, 2.85, 2.75, 2.71	citric acid
2	60.494	3.74	S6
3	60.980	3.74, 3.72	αG6
4	61.139	3.61	βG6
5	61.725	3.59	S1'
6	62.736	3.74, 3.73	S6'
7	63.737	3.61, 3.64	-
8	64.280	3.50	-
9	67.954	3.71	-
10	69.595	3.41, 3.38, 3.36	S4
11	69.991	3.83	βG4
12	70.071	3.81	αG4
13	71.448	3.49, 3.48, 3.47, 3.46	S2
14	71.816	3.50	αG2, αG5
15	72.767	3.78, 3.77, 3.76	S5
16	72.952	3.69, 3.67, 3.65	S3
17	73.157	3.66	αG3
18	74.372	3.98, 3.96, 3.94	S4'
19	74.536	3.17, 3.15, 3.13	βG2
20	76.154	3.38	βG5
21	76.328	3.41	βG3
22	76.790	4.14, 4.12	S3'
23	81.773	3.83, 3.82, 3.80, 3.79	S5', αF3, αF5, βF5
24	92.477	5.15, 5.14	αG1
25	92.536	5.33, 5.32	S1
26	96.293	4.57, 4.55	βG1
27	98.451	-	βF2
28	104.041	-	αF2, S2'

Table 2. The ratio of the methylene peaks of citric acid and the anomeric proton of sucrose with pH.

pH	anomeric protons of sucrose	methylene peaks of citric acid / 4
3.23	1	0.525
3.26	1	0.480
3.79	1	0.359
3.91	1	0.295
4.01	1	0.293
4.02	1	0.289
4.17	1	0.277
4.26	1	0.220

citric acid and the anomeric proton of sucrose with pH by linear regression was as follows :

Table 3. The experimental pH values and the calculated values.

	anomeric protons of sucrose	methylene peaks of citric acid / 4	calculated pH	experimental pH	Relative error
MT1	1	0.567	3.00	3.02	0.67%
MT2	1	0.490	3.28	3.29	0.30%
CJ	1	0.452	3.42	3.45	0.88%
CN1	1	0.325	3.89	3.89	0.00%
CN2	1	0.292	3.02	4.05	1.00%

MT1; Minneola tangeloe orange 1; MT2; Minneola tangeloe orange 2; CJ; Cheju orange; CN1; California navel orange 1; CN2; California navel orange 2.

$$pH = -0.27 \times (\text{the methylene peaks of citric acid} / \text{the anomeric proton of sucrose}) + 1.38$$

where $r^2 = 0.97$.

In order to verify the equation, the other 5 samples were chosen from three kinds of oranges arbitrarily. Their pH values were measured, and their ^{1}H-NMR experiments were carried out. The experimental pH values and the calculated values are listed in Table 3. The relative errors were ranged within 1%. In the future, a comparison of author's results with those by another non-destructive analytical method will be carried out. Before that, however, the current results are reported here.

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