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Determination of propionates and propionic acid in bakery products using gas chromatography

[1]Khaldun M. Al Azzam., [1,]*Bahruddin, S., [1]Noor Hasani Hashim., [1]Afidah Abdul Rahim. and [2]Khairuddin Mohd Talib

[1]*School of Chemical Sciences, Universiti Sains Malaysia, 11800, Penang, Malaysia*
[2]*Food Quality Control Laboratory, Ministry of Health Malaysia, Kangar, Perlis, Malaysia*

Abstract: A simple analytical method for the determination of propionic acid and propionates in bakery products using a simple sample preparation procedure is described. The method involves the conversion of propionates to the non-ionized molecular form by adding glacial acetic acid, which is at the same time efficiently extracted into dichloromethane. After vortexing for 1 min, the extract was directly injected into a capillary gas chromatographic column with flame ionization detector. The method was applied for the determination of propionates in 112 commercial bakery samples. The levels of propionic acid plus propionates in bread, cake/rolls, burger/hot dog buns and pita breads ranged from 197-1273, 98-1846, 546-1932 and 479-1680 μg mL^{-1}, respectively. No propionate was detected in any of the 36 biscuit samples analyzed.

Keywords: propionic acid, capillary gas chromatographic column, cake/rolls, burger/hot dog buns, pita breads, biscuit

Introduction

Propionic acid and propionates are added to bakery products due to their anti-microbial effects. They are also used as mold inhibitors in animal feeds at doses over 200 μg mL^{-1} (Carlos, 2003). Propionic acid and its salt are used as raw materials in perfume, plastic industries, and so on. The salts of propionic acid have been listed as preservatives which are of the category known as generally recognized as safe (GRAS) food additives (Kırbaşlar *et al.*, 2006).

Reversed phase HPLC with UV detection for the determination of propionic acid has been reported (Destandau *et al*., 2005). The method however lacks sensitivity as the propionic acid exhibits low UV absorbing properties. Anion exchange with conductivity detection is also widely used (Ding *et al*., 1997). CE based on indirect UV detection has also been reported (Ackermans *et al.,* 1992). GC methods with (Van Huyssteen, 1970; Moreau *et al*., 2003) and without (Yang and Choong, 2001; Moreau *et al*., 2004; Guohua *et al*., 2007) derivatization, depending on the sensitivity required have also been proposed.

Another important issue in the determination of propionic acid concerns the complexity of the sample. Microextraction technique represents an interesting approach as the large potentially interfering components are effectively excluded by the pores of the fibers. An alternative way to overcome matrix interference is by trapping the analyte in a headspace region and adsorbing to solid phase microextraction fibers (Carlos, 2003). Other common approach involves liquid-liquid extraction using solvents such as dichloromethane, diethyl ether, etc. To ensure optimum extraction, the pH of the sample is adjusted to be sufficiently acidic to ensure that propionates and propionic acid are in the non-ionized molecular form (pKa ~ 4.9), (Guynot *et al*., 2005). In this paper, such extraction strategy was adopted as it is important to analyze not only propionic acid but also any possible soluble salts of propionates that could be added in bakeries. As sensitivity is not a major issue in the analysis (maximum permissible level of 2-propionic acid/propionates is 2000 μg mL^{-1}), the extracts were injected directly into a capillary GC column and was detected by a flame ionization detector (FID). The method was applied for the determination of total propionic plus propionates in bakery products that are consumed by the population of the northern states of Peninsula Malaysia. The important aim of the study was to develop simple analytical method that can be easily implemented by regulatory agencies for the monitoring of the preservative in food. The study was also important in light of the recent media reports of the excessive amounts of this preservative in bread samples.

*Corresponding author.
Email: bahrud@usm.my
Tel: +604-6533888 ext 4027, Fax: +604-6574854 35

Materials and Methods

Reagents

Standard propionic acid was obtained from Sigma-Aldrich. Dichloromethane and glacial acetic acid were obtained from R & M and (Sigma-Aldrich, United States), (meets USP testing specifications), respectively. Stock solutions of the propionic acid were dissolved in the extractant solution (dichloromethane: glacial acetic acid (1000:1, v/v). Samples were vortexed using a Vortex Mixer (Wiggea Hauser VM). All chemicals are of analytical grade reagents.

Apparatus

Samples were separated using a SRI Series 8610C gas chromatograph GC (SRI 18 instruments, Torrance, California) equipped with FID. 1 µL injection volume was used. Econo-Cap-Carbowax capillary column (30 m × 0.32 mm, i.d) and 0.25 µm film thickness was used (Alltech). Hydrogen, compressed air, and nitrogen were used at 25, 5, and 20 mL min^{-1}, respectively. The split ratio used was 29:1. The temperatures of the column, injector, and detector were 170, 220, and 270 °C, respectively.

Sample preparation

Bakery samples were bought from supermarkets and sundry shops in the states of Perlis, Kedah and Penang, Malaysia. 4 g of each sample (cut in pieces) was placed in a 50 mL plastic tube. A 20 mL of dichloromethane, glacial acetic acid extractant with the ratio of (1000: 1 v/v) was added to the sample. The tube was capped and vortexed for 1 min, then it was left for about 5 min to obtain a clear solution. The extracts were sampled using a gas-tight syringe and injected into the GC column. Internal standard was not used as only a single analyte was determined.

Results and Discussion

In the work (Isshiki *et al.*, 1981), the extractant solution composed of dichloromethane and formic acid while in the work (Lamkin *et al.,* 1987), the bread sample was ground in a meat grinder with a 3 mm hole plate 12 and was finely divided by rubbing through a No. 8 sieve. The extraction was aided by adding 0.050 M formic acid in a blender at low speed for 5 min, and an aliquot of the filtrate was analyzed directly by gas chromatography. In the work (Beljaars *et al.*, 1996), propionic acid and sorbic acid were determined in rye bread by first homogenizing test portions which were extracted with a diethyl ether-phosphoric acid mixture. In the present work, glacial acetic acid was used, but the extraction procedure has been simplified. The organic fraction was sampled with a syringe and directly injected into the GC column (no filtration). A plot of peak area (y) versus concentration of propionic acid (x, µg mL^{-1}) was linear over 50 – 2000 µg mL^{-1}. The calibration graph can be described by the equation y = 0.0051x (r^2 = 0.9991).

Table 1. Relative standard deviation for peak area and retention time for propionic acid standard (300 µg mL^{-1}) over 3 days.

Trial	Retention time (min)			Peak area		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
1	2.850	2.816	2.866	1.638	1.544	1.522
2	2.850	2..816	2.866	1.569	1.568	1.489
3	2.850	2.850	2.850	1.528	1.566	1.565
4	2.850	2.866	2.850	1.520	1.579	1.600
5	2.850	2.866	2.833	1.489	1.533	1.550
6	2.850	2.866	2.850	1.573	1.513	1.501
Mean	2.850	2.847	2.853	1.553	1.551	1.538
SD	0.000	0.025	0.012	0.05	0.025	0.042
% RSD	0.000	0.862	0.433	3.37	1.61	2.72
Mean (18 injections)	2.850			1.547		
% RSD (18 injections)	0.53			2.55		

Table 2. Levels of propionic acid found in different bakery products (n = 2) using the proposed GC method

Sample no.	Type	Concentration µg mL^{-1}
	Bread	
1	Bread 1	196.5
2	Bread 2	514.0
3	Bread 3	358.1
4	Bread 4	876.0
5	Bread 5	772.2
6	Bread 6	900.0
7	Bread 7	324.0
8	Bread 8	1273.0
9	Bread 9	1209.0
	Cake/Rolls	
10	Cake/Rolls	589.2
11	Cake/Rolls	648.2
12	Cake/Rolls	461.3
13	Cake/Rolls	496.9
14	Cake/Rolls	1555.5
15	Cake/Rolls	1147.1
16	Cake/Rolls	1190.4
17	Cake/Rolls	1177.7
18	Cake/Rolls	944.8
19	Cake/Rolls	1021.7
20	Cake/Rolls	839.8
21	Cake/Rolls	1078.0
22	Cake/Rolls	727.2
23	Cake/Rolls	757.0
24	Cake/Rolls	635.8
25	Cake/Rolls	878.2
26	Cake/Rolls	763.9
27	Cake/Rolls	97.7
28	Cake/Rolls	588.1
29	Cake/Rolls	543.6
30	Cake/Rolls	684.5
31	Cake/Rolls	676.5
32	Cake/Rolls	1455.5
33	Cake/Rolls	1192.1
34	Cake/Rolls 25	1846.0
35	Cake/Rolls 26	1571.8
36	Cake/Rolls 27	396.0
37	Cake/Rolls 28	675.0
38	Cake/Rolls 29	1063.0
	Burger/Hot Dog Bun	
39	Burger/Hot Dog Bun 1	651.4
40	Burger/Hot Dog Bun 2	546.2
41	Burger/Hot Dog Bun 3	1932.0
42	Burger/Hot Dog Bun 4	1119.0
43	Burger/Hot Dog Bun 5	1266.0
44	Burger/Hot Dog Bun 6	612.0
	Pita	
45	Pita 1	478.9
46	Pita 2	1680.0
47	Pita 3	1280.0

Table 3. Summary of results for the determination of propionic acid in bakery products

Type and Number Tested	Number Tested Positive	Percent Positive	Range, µg mL^{-1}
Bread (22)	9	40.9	197 -1273
Biscuit (36)	0	0	-
Cake/Roll (44)	29	65.9	98 – 1846
Burger/Hot Dog Bun (7)	6	85.7	546 – 1932
Pita (3)	3	100	479 -1680

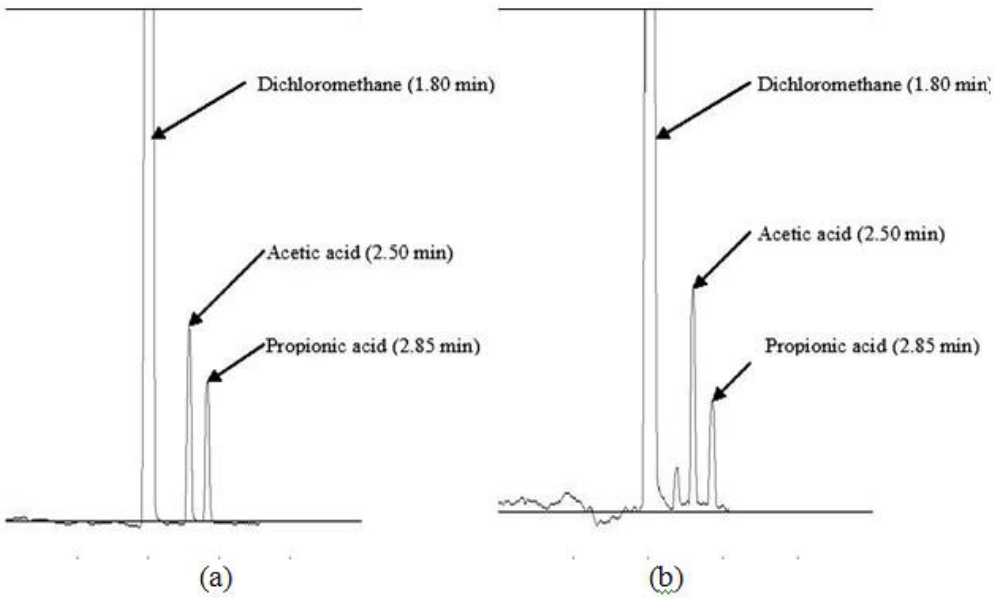

Figure 1. Typical chromatograms of (a) propionic acid standard (300 µg mL^{-1}), (b) extract of a bread sample (sample # 1). Please refer to text for chromatographic conditions.

The limit of detection (LOD) (signal-to-noise 3) and the limit of quantitation (LOQ) of the method is 9.1 and 26.3 μg mL^{-1}, respectively. The recoveries were studied by adding known amounts of propionic acid standard to the sample solution to obtain the final concentrations (75, 250 and 450 μg mL^{-1}). Mean recoveries of 99.7% were obtained, indicating the potential of this method in the determination of the analyte in bakery products. The reproducibility of the GC method was studied by repeated injections of 300 μg mL^{-1} propionic acid. Relative standard deviations of 0.53 and 2.55% for the retention time and peak area, respectively, over three days were obtained (Table 1). The sample preparation procedure involves the addition of glacial acetic acid to convert any propionates to propionic acid, and thus measures the levels of the original propionate and propionic acid that is originally present in the sample. The non-ionized molecular form was efficiently extracted into dichloromethane.

The method was applied for the determination of propionic acid and propionates in 112 bakery products that are popularly consumed in the northern states of Peninsula Malaysia. The bread samples tested represent those that are produced by the major bread producers, including established companies and hypermarkets. Typical chromatograms are shown in Figure 1. The method is not only rapid (less than 3 min) but the propionic acid peak is well separated from the solvent and acetic acid peaks. Detailed results are shown in Table 2 and are further summarized in Table 3. For the bread samples, 40.9% were found to contain propionic acid plus propionates (range, 197 – 1273 μg mL^{-1}). Of the 35 biscuits samples analyzed, none were found to contain propionic acid plus propionates. 65.9 and 85.7% cake/roll and burger/hot dog bun, respectively were found to contain propionates. All the pita bread samples tested were found to be positive (range: 479 – 1680 μg mL^{-1}). Even though the levels of propionic acid and propionates are below the Malaysia legal limits of 2000 μg mL^{-1}, a few are close to the maximum allowable level (e.g., sample # 87 and 106). It is interesting to note that these products are produced by small scale industries.

Conclusion

A GC method involving a simple sample preparation step is described. The proposed method enables the determination of the analyte in bakery products, without any overlap of matrix components with the propionic peak. No propionate was found in any of the biscuit samples analyzed. The percent of incidence in bread, cake/roll, burger/hot dog bun and pita were 40.9, 65.9, 85.7 and 100%, respectively. However, all the 112 bakery samples tested comply with the maximum legal limit of 2000 μg mL^{-1}.

References

Ackermans, M. T., Ackermans-Loonen, J. C. J. M. and Beckers, J. L.1992. Determination of propionate in bread using capillary zone electrophoresis. Journal of Chromatography 627: 273-279.

Beljaars, P. R., Van Dijk, R., Verheijen, P. J. J. and Anderegg, M. J. P. T. 1996. Gas chromatographic determination of propionic acid and sorbic acid contents of rye bread: interlaboratory study. Journal of the Association of Official Analytical Chemists 79*:* 889-894.

Carlos, I. 2003. Analysis of total propionic acid in feed using headspace solid-phase microextraction and gas chromatography. Journal of Chromatography A 1017: 161-166.

Destandau, E., Vial, J., Jardy, A., Hennion, M.-C., Bonnet, D. and Lancelin, P. 2005. Development and validation of a reversed-phase liquid chromatography method for the quantitative determination of carboxylic acids in industrial reaction mixtures. Journal of Chromatography A 1088: 49-56.

Ding, M.-Y., Chen, P.-R. and Luo, G.-A. 1997. Simultaneous determination of organic acids and inorganic anions in tea by ion chromatography. Journal of Chromatography A 764: 341-345.

Guynot, M. E., Ramos, A. J., Sanchis, V. and Marĭn, S. 2005. Study of benzoate, propionate, and sorbate salts as mould spoilage inhibitors on intermediate moisture bakery products of low pH (4.5–5.5). International Journal of Food Microbiology 101: 161-168.

Guohua. Z., Jing-Fu. L., Margareta. N. and Jan, A.J. 2007. Determination of short-chain fatty acids in serum by hollow fiber supported liquid membrane extraction coupled with gas chromatography. Journal of Chromatography B 846: 202-208.

Isshiki, K., Tsumura, S. and Watanabe, T. 1981. Gas chromatographic determination of propionic acid in bread and cake. Journal of the Association of Official Analytical Chemists 64: 280-1.

Kırbaşlar, Ş.İ., Selin. Ş. and Mehmet B. 2006. (Liquid + liquid) equilibria of (water + propionic acid + alcohol) ternary systems. The Journal of ChemicalThermodynamics 38: 1503-1509.

Lamkin, W. M., Unruh, N. C. and Pomeranz Y. 1987. Gas chromatographic determination of calcium

propionate added as preservative to bread. Journal of the Association of Official Analytical Chemists 70: 763-7.

Moreau, N. M., Goupry, S. M., Antiganc, J. P., Monteau, F. J., Bizec, B. J. L., Champ, M. M., Martin, L. J. and Dumon, H. J. 2003. Simultaneous measurement of plasma concentrations and ^{13}C-enrichment of short-chain fatty acids, lactic acid and ketone bodies by gas chromatography coupled to mass spectrometry. Journal of Chromatography B 784: 395-403.

Moreau, N. M., Delépée, R., Maume, D., Bizec, B. L., Nguyen, P. G., Champ, M. M., Martin, L. J. and Dumon, H. J. 2004. Rapid measurement of ^{13}C-enrichment of acetic, propionic and butyric acids in plasma with solid phase microextraction coupled to gas chromatography–mass spectrometry Analytica Chimica Acta 512: 305-310.

Van Huyssteen, J.J. 1970. The determination of short-chain fatty acids in aqueous solution by gas-liquid chromatography. Water Research 4: 645-659.

Yang, M.-H. and Choong, Y.-M. 2001. A rapid gas chromatographic method for direct determination of short-chain (C_2–C_{12}) volatile organic acids in foods. Food Chemistry 75:101-108.