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Quantitation of organic acids in sugar refinery juices with capillary zone electrophoresis and indirect UV detection

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ABSTRACT

During sugar refinement, monitoring of organic acids such as formate, tartrate, succinate, malate, glycolate and acetate in the process "juices" is important for process control. Matrix effects can lead to problems in conventional chromatographic ion analysis of these solutions. Capillary zone electrophoresis, with indirect UV detection, has been shown to be a good alternative, requiring almost no sample preparation, other than dilution, and with fast analysis time (less than 7 min). A co-elution problem for the formate–tartrate pair could be solved by adding small amounts of bivalent metal ions to the electrophoresis buffer. Quantitative analyses of the organic acids in the juices from beet sugar production and from the processing of a hydrolysed chicory root extract (*Cichorium intybus*) are reported.

INTRODUCTION

Organic acids can be determined with ion-exchange HPLC and refractive index detection. However, in the case of oligosaccharide- and polysaccharide-containing solutions, quantitation becomes difficult, if not impossible, because the small organic acid signals are partly or even totally obscured by the response of the accompanying sugars. Many attempts have been made [1] to work out alternative GC procedures, but the necessary isolation and derivatization steps, although performing well with standard mixtures, invariably fail to achieve reliable quantitation in the case of real-life matrices, especially with the more hydrophilic species such as formate and acetate.

Another alternative is electrokinetic analysis. The well-documented acid–base properties have made the carboxylic acids well suited as substrates for capillary isotachophoresis (ITP) [2,3] and also for capillary zone electrophoresis (CZE). The lack of chromophores, however, requires the use of a potential gradient [4] or a conductivity [5–7] detector. As this type of detection is not available on the instrumentation that has become commercially available, much attention is now being focused on CZE analysis with indirect UV detection [8–13]. This detection technique [14] relies on the presence of a UV-active buffer component with the same charge as the analytes. In the case of anions, an electroosmotic flow modifier [4], together with reversed polarity (cathode at the injection side), is applied to ensure movement of all anions towards the detector.

Samples from two different processes were analysed. Formate was determined in sugar beet juices and related samples (Fig. 1). Formate, tartrate, malate, succinate and glycolate were determined in chicory root extract juices.

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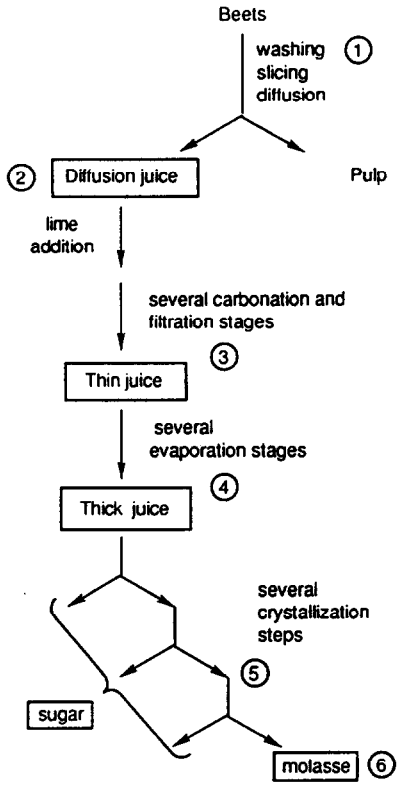

Fig. 1. Simplified scheme of beet sugar refinement. For fructose and oligofructose syrups from the chicory root juice, additional enzymatic hydrolysis steps (between first and second evaporation) are included for breakdown of the inulin to fructose. Raftilose results from complete hydrolysis, while Raftisweet is obtained by controlled partial hydrolysis.

EXPERIMENTAL

CZE was performed on a Quanta 4000 (Millipore, Bedford, MA, USA). Fused-silica capillaries were 75 μm internal diameter, 375 μm external diameter, 60 cm long, with the detection window at 53 cm.

Electropherograms were obtained with a phthalate buffer, prepared with deionized water (Milli-Q, Millipore) and adjusted to the appropriate pH with sodium hydroxide. Also, varying amounts of an electroosmotic flow modifier (OFM Anion-BT, Millipore) were added, resulting in a reversed electroosmotic flow and necessitating the use of a reversed-polarity source (high negative voltage at the column inlet). The indicated volume percentages of OFM refer to the volume of the commercial solution used for preparing the buffer (5%, v/v, is equivalent to 1 m*M* active substance [11]). The required calcium ion concentration was obtained by adding the appropriate amount of calcium chloride.

Standard samples were obtained by dissolving the acids, or their salts, in deionized water. Beet sugar juices, wash water samples and chicory root extract juices were obtained from a Belgian sugar refinement company (Tiense Suikerraffinaderij, Belgium). Highly viscous syrups, from the evaporation stage on, were stored in a refrigerator, while the less viscous samples from earlier stages were received frozen and kept in a deep freezer. The only treatment of the samples consisted of thawing where necessary and dilution with deionized water. Because of the high density of some of the samples, all organic acid concentrations are expressed as a w/w ratio (ppm).

Samples were introduced hydrodynamically (elevation, 10 cm; injection time, 20 s), analysed with an applied voltage of −20 kV (330 V/cm) and indirectly detected at 254 nm. Selection of the injection time was based on relative standard deviation data for peak area, which were found to decrease with injection time (for 10 ppm formate: 13.8%, 8.6%, 4.5% and 3.4% at, respectively, 5, 10, 15 and 20 s injection time). In between runs of standard mixtures, the column was rinsed with separation buffer for 2 min. For the actual samples, however, it was found necessary to use a more thorough rinsing procedure consisting of 0.1 *M* sodium hydroxide (1 min), deionized water (1 min) and separation buffer (2 min). The column was exclusively used for the analyses described here. Before storage, it was purged with water and then dried in an air stream. After remounting, it was flushed with buffer for at least 5 min.

RESULTS AND DISCUSSION

Separation optimization

The objective of this work was the separation and determination of formate, (DL-)tartrate, malate, succinate and glycolate. The initial selection of the background electrolyte (5 m*M* phthalate at pH 5.6) was based on literature data [11]. The osmotic flow modifier (OFM) concentration could be regulated to optimize the separation of most anions, except for the formate–tartrate pair

(Fig. 2a and b). Changing the pH in the range 5–7 did not improve resolution (Fig. 2c). It is significant that, in a recently published analysis of dental plaque [9] using these conditions, formate was identified in the electropherogram, but not tartrate (the hardened form of dental plaque is, just like wine deposits, also known as *tartar*).

The relative mobility of ions can be influenced by changing their charge state through selective complexation. This principle is commonly used in the analysis of cations with hydroxyisobutyric acid (HIBA) or citrate [15]. However, the complementary principle, cationic complexation to optimize the separation of anions, has not been much applied. Only recently, the separation of Pb^{2+}-complexed sulphate from organic anions has been reported [16,17]. The addition of alkaline earth cations to the buffer has been found effective for the separation of formate and tartrate (Fig. 3). With 0.2–0.6 m*M* Ca^{2+} added to the buffer, effective separation was obtained by selective retardation of the tartrate. Unfortunately, oxalate was also retarded, and although it was not a target compound overlap with the formate peak would result in poor quantitation of the latter (Fig. 4). Therefore, the following

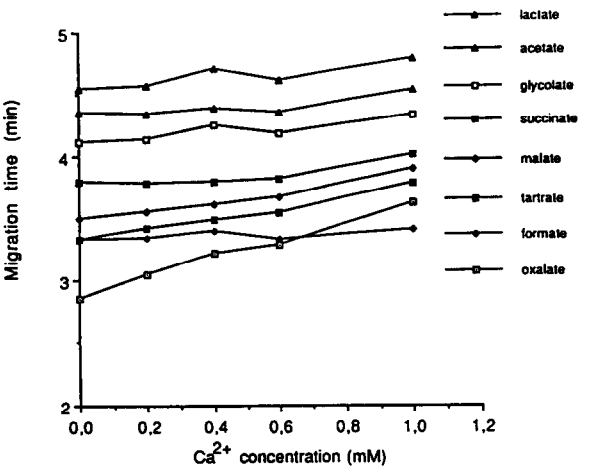

Fig. 3. Effect of Ca^{2+} concentration on the separation of organic acids. Same conditions as in Fig. 2a, except for the addition of Ca^{2+}.

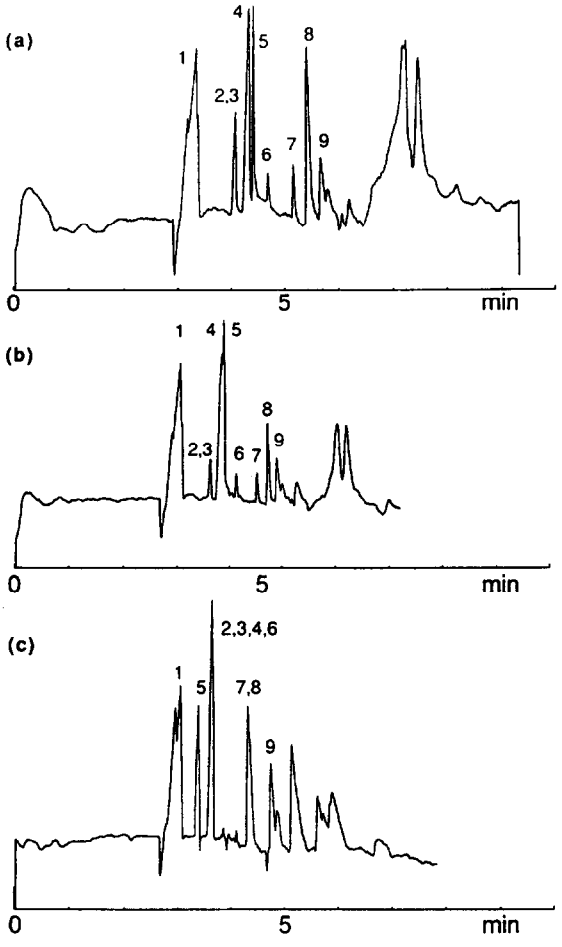

Fig. 2. Separation of organic acids in a chicory root thick juice (Raftisweet): (a) pH 5.6, 2.0% (v/v) OFM; (b) pH 5.6, 2.5% (v/v) OFM; (c) pH 7.0, 2.0% (v/v) OFM. Other conditions: see Experimental section. Peaks: 1 = inorganic anions; 2 = formate; 3 = tartrate (spiked); 4 = malate; 5 = citrate; 6 = succinate; 7 = glycolate; 8 = acetate; 9 = lactate.

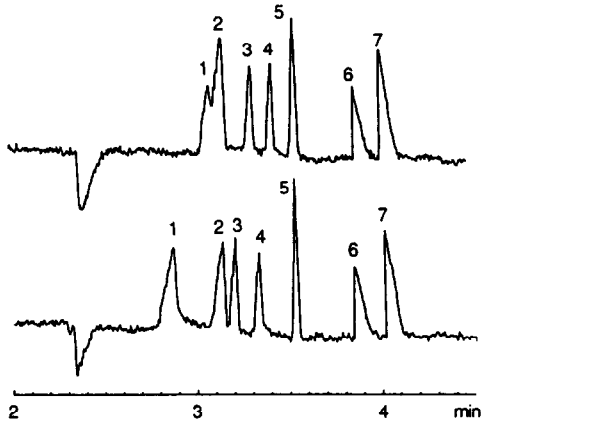

Fig. 4. Effect of Ca^{2+} concentration on the separation of organic acids (standards, 30 ppm each). Same conditions as in Fig. 2a, except for the addition of Ca^{2+}: 0.6 m*M* (top) or 0.2 m*M* (bottom). Peaks: 1 = oxalate; 2 = formate; 3 = tartrate; 4 = malate; 5 = succinate; 6 = glycolate; 7 = acetate.

approach was used: analysis was generally performed with 0.6 m*M* Ca^{2+} added to the buffer, which gave optimal separation between tartrate and formate. For diffusion juices, which still contain oxalate, a buffer containing 0.2 m*M* of Ca^{2+} was preferred. It must also be pointed out that, with the Ca^{2+} present, citrate is strongly retarded.

Good linearity was observed in the calibration graphs for the five acids of interest. In all cases the correlation coefficient was better than 0.999.

Quantitation applied to sugar refinery juices

For analysis, the native samples were diluted so that the actual concentration of the acids is in the 10–50 ppm range. Initially, extreme variability was observed. Triplicate, successive analyses gave reasonable standard deviations on the observed area (typically 1–5%), but the standard deviation for sets obtained by repetition of these triplicate analyses could be as high as 20%. Sample inhomogeneity was apparently not the cause of this effect, as this was equally well observed with new dilutions or repetitions on the same diluted sample. All efforts to correlate this with variations in analytical conditions such as temperature variation or small variations in buffer composition failed and forced us to conclude that the variability was inherent in the samples themselves. Indeed, some of the soil microorganisms, or their associated enzymatic activity, can survive the heat treatment during the diffusion process and result in further breakdown of some or build-up of other components in the mixture. The key step in achieving good performance was to give up automated analysis. Inevitably, extensive use of an autosampler requires that samples are prepared a relatively long time before analysis, and, consequently, have ample time to develop enzymatic activity.

This approach worked well, especially for the very viscous samples, which are relatively stable but start degrading after dilution. However, as will be indicated later, problems remain with some of the less viscous, inherently unstable samples. We would like to remark that addition of 0.1% sodium azide is not a good approach in this case as the large amount of azide ions interferes with the determination of the earlier-eluting organic carboxylates, especially formate.

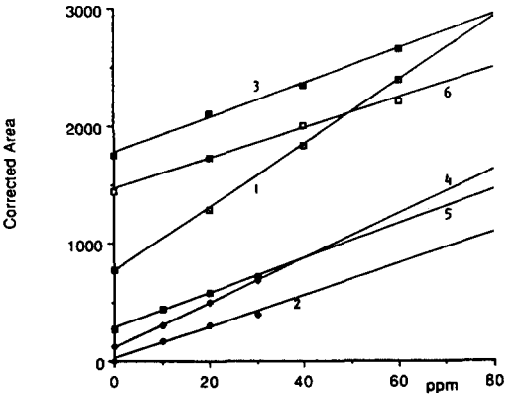

Fig. 5. Analysis of a chicory root evaporation juice by standard addition. Conditions as in Fig. 2a, except for the addition of 0.6 m*M* Ca^{2+} to the buffer. (See Discussion section for details on sample handling.) 1 = Formate; 2 = tartrate; 3 = malate; 4 = succinate; 5 = glycolate; 6 = acetate.

When care was taken to prepare the dilutions immediately before analysis, reliable results were obtained. This is further indicated by the response linearity, as verified by standard addition. The curves in Fig. 5 were obtained by preparing a dilution which was immediately stored at 0°C. For the successive points in the graph, aliquots were taken from the stored dilution, spiked with the appropriate amount of standard and analysed three times immediately.

For actual analyses, the system was calibrated

TABLE I

RUN-TO-RUN AND DAY-TO-DAY VARIABILITY FOR THE MIGRATION TIMES IN REAL SAMPLES (POOLED FROM DIFFERENT CHICORY ROOT JUICES)

Compound[a]	Run-to-run		Day-to-day	
	Mean (min)	R.S.D. (%) ($n = 10$)	Mean (min)	R.S.D. (%) ($n = 3$)[b]
Formate	3.26	1.0	3.51	13.7
Malate	3.63	1.0	3.91	14.1
Succinate	3.71	1.1	4.00	14.3
Glycolate	4.07	1.2	4.41	15.3

[a] Tartrate is not included as it occurred only rarely in these samples.

[b] Based on observations for 3 days covering a 3-month period (November 1992 to January 1993).

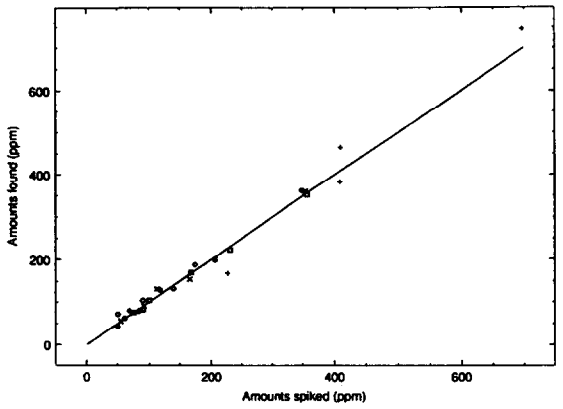

Fig. 6. Agreement between spiked amounts and observed differences between spiked and unspiked samples for the analyses from Table II (malate in diffusion juice not included, see Discussion section). The line represents the one-to-one correspondence.

daily. Although the run-to-run variability of the migration times was very good, considerable day-to-day variation occurs (Table I). As the capillary is cooled with ambient air, and the apparatus was situated in a non-thermostatted room, we believe that this variability is related to temperature variations in the surroundings.

Table II summarizes some typical results that were obtained for the analysis of five anions in chicory root juices (Raftilose). For a blind test, nine unidentified samples were obtained, consisting of three original and six spiked samples. With the exception of malate in the diffusion juices, good agreement was found between the amounts spiked and the amounts calculated after analysis of spiked and unspiked samples (Fig. 6).

The unreliability of the malate determinations in diffusion juices was related to sample instability, not to the analytical method. With capillary zone electrophoresis, it could further be demonstrated that the disappearance of malate was correlated with an increase in the acetate and lactate content (Fig. 7). Also, an unidentified compound was formed. Even when the sample was stored at low temperature (2–4°C) degradation of malate occurred. Apparently, degradation of malate in the unspiked sample led to an overestimation of the spiked amounts. Of course, in such a complex mixture, to conclude from Fig. 7 that the malate is effectively converted to lactate would require a more detailed study.

Table III summarizes typical results for the formate determinations in beet sugar-related samples (Fig. 8). Here again, the relatively large standard deviations for the diffusion juices reflect the stability problems of this type of sample.

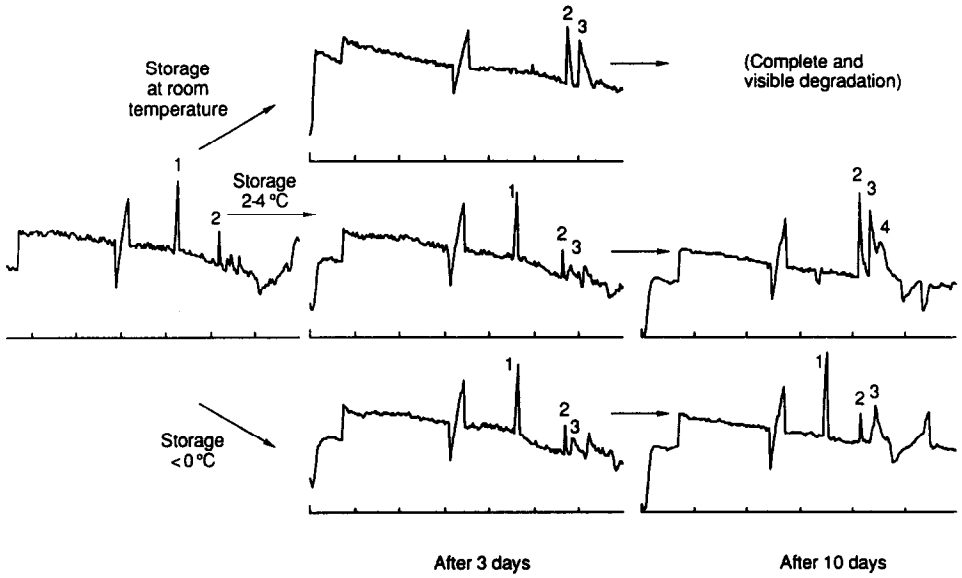

Fig. 7. The degradation of malate in a chicory root diffusion juice as monitored by CZE. The fresh sample (leftmost electropherogram) was received frozen. Conditions as in Fig. 5. Peaks: 1 = malate; 2 = acetate; 3 = lactate; 4 = unknown.

TABLE II

DETERMINATION OF ORGANIC ACIDS IN CHICORY ROOT JUICES. BLIND TEST WITH SPIKED SAMPLES

Juice analysed[a]	Formate				Tartrate				Malate				Succinate				Glycolate			
	b	*c*	*d*	*e*	*b*	*c*	*d*	*e*	*b*	*c*	*d*	*e*	*b*	*c*	*d*	*e*	*b*	*c*	*d*	*e*
R	n.d.[f]	–	0	–	n.d.	–	0	–	567	1.6	0	–	28	3.3	0	–	n.d.	–	0	–
M	76	1.7	76	76	73	3.8	51	73	1751	1.7	237	1184[g]	83	1.3	56	57	44	1.0	51	44
P	84	2.2	91	84	105	3.4	90	105	1921	3.1	444	1354[g]	120	2.6	92	92	81	0.7	89	81
K	274	2.4	0	–	n.d.	–	0	–	2631	0.8	0	–	93	1.5	0	–	150	3.1	0	–
H	379	2.5	100	105	63	9.7	62	63	2800	1.4	227	169	225	3.7	113	132	235	0.6	91	85
I	498	1.2	231	224	80	5.3	83	80	3015	0.6	409	384	294	3.3	207	201	349	3.2	206	199
F	474	3.3	0	–	n.d.	–	0	–	5557	2.2	0	–	214	1.2	0	–	252	1.3	0	–
B	644	2.9	168	170	130	6.2	118	130	6023	1.1	410	466	369	3.0	166	155	441	1.8	174	189
C	827	1.7	357	353	746	10.3	140	191	6303	0.2	697	746	575	1.6	354	261	615	4.1	348	363

[a] R, M, P = diffusion juices; K, H, I = evaporation juices (first stage); F, B, C = evaporation juices (second stage).
[b] Amounts found [ppm (w/w), average of three determinations].
[c] R.S.D. (%) on *b*.
[d] Amounts spiked [ppm (w/w)].
[e] Difference between amounts found and amounts found in the unspiked sample, to be compared with *d*.
[f] No detectable amounts.
[g] See text for a discussion of these values.

TABLE III

DETERMINATION OF FORMATE IN BEET SUGAR-RELATED SAMPLES

	Sample	ppm[a]	R.S.D. (%)	Method[b]
1[c]	Wash water (before washing)	n.d.[d]	–	A
1	Wash water (after washing)	6.9	0.9	A
2	Diffusion juice	6.2	9.6	B
2	Diffusion juice	4.2	5.9	B
2	Diffusion juice	6.3	3.4	B
3	Thin juice	58.3	0.4	A
3	Thin juice	64.5	0.9	A
5	Mother liquor II	1479	1.2	A
5	Mother liquor II	1589	1.5	A
6	Molasses	2333	2.0	A

[a] Average of three measurements (w/w).
[b] Refers to Ca^{2+} content of the separation buffer (A = 0.6 m*M*, B = 0.2 m*M*), all other conditions as in Fig. 2a.
[c] The number refers to the production stages in Fig. 1.
[d] No detectable amounts.

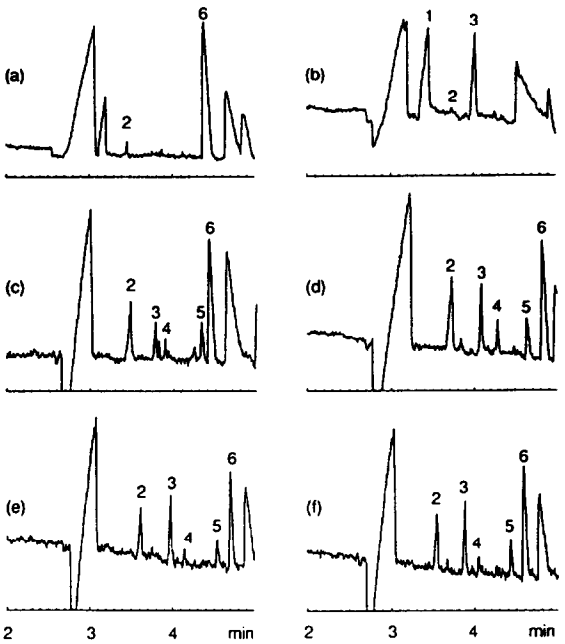

Fig. 8. Some typical beet sugar-related samples analysed in this work. Conditions as in Fig. 5, except for (b): 0.2 m*M* Ca^{2+}. (a) Wash water; (b) diffusion juice; (c) thin juice; (d) thick juice, (e) mother liquor II; (f) molasses. Peaks: 1 = oxalate; 2 = formate; 3 = malate; 4 = succinate; 5 = glycolate; 6 = acetate.

CONCLUSIONS

Determinations, similar to those mentioned in Tables II and III, have now been routinely used for several months and have provided process control parameters that were previously difficult to obtain. The minimal required sample preparation and short analysis time makes this type of analysis very attractive.

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