Simultaneous Determination of Nine Kinds of Fish Sauce Organic Acids by Ion Chromatography with Suppressed Conductivity Detection

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Abstract: A method for the determination of nine kinds of fish sauce organic acids was developed by ion chromatography with suppressed conductivity detection. The separation of the organic acids was achieved on hydrophilic anion exchange column with potassium hydroxide as gradient eluent with gradient pump. The detection was performed by a conductivity detection mode. The calibration graphs of peak area for all analytes were linear in the ranges about two orders of magnitude. The recoveries of addition standard are within 93.65%～104.32%. The results indicated that this method is highly accurate and efficient with good reproducibility and high recovery.

Key words: ion chromatography; organic acid; fish sauce

抑制型离子色谱同时测定分离检测鱼酱油中的九种有机酸

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摘　要：建立了用亲水性阴离子交换分离柱，KOH为淋洗液作梯度淋洗，电导检测，同时分离和检测鱼酱油中九种有机酸的离子色谱法。方法对所测有机酸的线性范围均在2个数量级以上，回收率在93.65%～104.32%之间，该方法用于鱼酱油样品的分析结果显示具有准确、高效、回收率高和重现性好的特点。

关键词：离子色谱；有机酸；鱼酱油
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Fish sauce, a fermented product, was a brown seasoned liquid commonly used as dressing in Asian countries [1]. It has been used in various prepared foods as sauces with the merits of characteristic favorable taste and nutritive values. It was basically produced from a mixture of fish and salt after fermentation. The fermented products, amino acids and peptides, had considerable attraction on the sensory characteristics of fish sauce. In southeast Asia, fish sauce was not only popular as a dressing But in some regional areas and certain social classes, it was the main source of protein in the diet and became a necessity in the household.

Organic acids were the important group of compounds in fish sauce for their influence in some respects like organoleptic properties (flavor, color, and aroma). The level and nature of the organic acids present in fish sauce might provide information concerning the origin of the raw materials, microbiological growth and even processing techniques. The total acidity of fish sauce was expressed as L-Lactic acid, the major organic acid in fish sauce. These organic acids might come directly from the processes of fish sauce fermentation.

Ion chromatography with suppressed conductivity detection allowed separation and quantification of organic acids in grape juices, wines, vinegars and fruit juices [2-3]. The advantages of ion chromatography were its specificity and sensibility in the determination of organic acids due to the use of a conductivity detector and the minimization of the sugar interferences. Furthermore, sample treatments such as extraction and/or derivation were not necessary. Sample

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preparation was a simple dilution and filtration process[6]. This kind of chromatography was a real option for the determination of samples with very low quantity of organic acids and for routine analysis. Although HPLC analysis method had been used for analysis of organic acids in fish sauce [4] in general, but the sample pretreatment procedure was specifically needed and the organic acids extraction and isolation procedures were tedious, expensive and time-consuming [5]. Inspection of the cited literature revealed that the determination of organic acids in fish sauce by ion chromatography with suppressed conductivity detection has not yet been reported.

Thus, the objective of this study was to introduce a new method to evaluate the concentrations of organic acids in fish sauce by ion chromatogram system with suppressed conductivity detection.

1 Materials and Methods

1.1 Ion chromatography instrument

The instrument was a Dionex Model 2000 ion chromatograph (Sunnyvale, CA, USA) with a GP 40 gradient pump, an ED 40 electrochemical detector, a Dionex IonPac AG11-HC (50 mm×4 mm) and IonPac AS11-HC (250 mm×4 mm) columns, a 25μl sample loop and an anion self-regenerating suppressor (SRS) operated in the auto suppression recycle mode. The suppressor current setting was 238 mA and the detector full scale setting was 2000μs. Data acquisition and instrument control were performed with the Dionex Chromeleon (6.60 SP2) program. Data collection was carriedout intriplicates.

1.2 Chemicals and reagents

Organic acids standard samples (L-Lactate, Acetate, Formate, Pyruvate, Malate, Tartrate, α-Ketoglutarate, Oxalate, Fumarate, and Citrate) were purchased from Sigma (St. Louis, MO, USA). All chemicals used were of the analyticalgrade.

Deionized distilled water with a specific resistance of > 18.2 MΩ was obtained from a Millipore (Bedford, MA, USA) Milli-Q plus PF water purification system and was used to prepare all reagents and standards.

1.3 Fish sauce samples

1.3.1 Purchased fish sauce samples

Five fish sauce samples used were purchased from Qingdao supermarket, nation-wide or producing area markets separately.

1.3.2 Preparation of fish sauce from squid by-products

Fish sauce produced from squid processing by-products was manufactured in our laboratory. Producing technology was as follows:

The squid (*Symplectoteuthis oualaniensis*) by products composed of heads, viscera,skin and fins were thawed by tap water, cut into pieces and chopped into plasm. To remove the lipid, the chopped by-products were boiled and cooled to 10 ℃. The lipid component floating up to the surface was removed with a spoon. Fish sauce mush was prepared by fermenting the mixture of defatted waste, with soy sauce koji, salt, and distilled water. The pH of fish sauce mush was adjusted to the designed pH by 1.0 mol/L hydrochloric acid solution or 1.0 mol/L sodium hydroxide solution respectively. The prepared fish sauce mush was fermented for 30 days in 48 ℃ and ripened. The ripened fish sauce mush was centrifuged at 10000r/min for 15 min. The obtained supernatant was heated at 90 ℃ for 10 min and the lipid was removed with a spoon as well. The defatted fish sauce obtained was filtered with a filter paper (No.5) and subjected to chemical analysis.

1.4 Ion chromatographic analysis

Before analysis each fish sauce sample was diluted 100-fold with deionized water. The diluted fish sauce samples were filtered with 0.22μm filters. The filtrates were injected onto the column for the chromatographic analysis [6-7]. Each sample was analyzed three times by three repeated injections. A potassium hydroxide gradient used was shown in Table 1. Eluent flow-rate was 1.2 ml/min.

Table 1 Potassium hydroxide elution gradient conditions

Time (min)	0.00	2.00	6.00	1.00	10.01	18.00	23.00	25.00	30.00	35.01
Eluentconcentration (mmol/L)	2.00	3.43	3.43	5.00	15.00	20.00	20.00	30.00	60.00	2.00

1.5 Statisticalanalysis

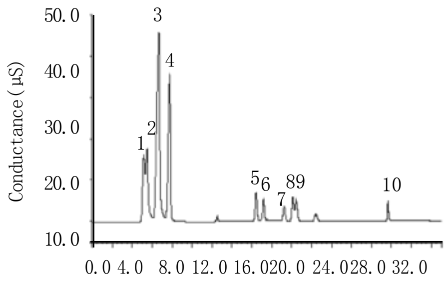
Analysis of variance and the Duncan's new multiple range test were performed with the statistical MAC statistical analysis package.

2 Results and Analysis

2.1 Chromatogram of standard organic acids

As shown in Fig.1, ten kinds of standard organic acids were separated completely by the suggested method. Retention times for L-Lactate, Acetate, Formate, Pyruvate, Malate, Tartrate, α-Ketoglutarate, Oxalate, Fumarate and, Citratewere 5.177, 5.510, 6.693, 7.760, 16.437, 17.173, 19.230, 20.120, 20.457, and 29.657 min respectively.

2.2 Calibration curves



1. L-Lactate (40×10^{-6}); 2. Acetate (10×10^{-6}); 3. Formate (10×10^{-6}); 4. Pyruvate (10×10^{-6}); 5. Malate (10×10^{-6}); 6. Tartrate (10×10^{-6}); 7. α-Ketoglutarate (10×10^{-6}); 8. Oxalate (10×10^{-6}); 9. Fumarate (10×10^{-6}); 10. Citrate (10×10^{-6}).

Fig.1 Chromatogram of organic acid standards

Linearity was investigated with the stock solution containing ten kinds of organic acids, which were diluted serially. Five concentrations (ranging for L-lactate, 5×10^{-6}～200×10^{-6}; Acetate, 0.5×10^{-6}～20×10^{-6}; Formate, 0.1×10^{-6}～20×10^{-6}; Pyruvate, 0.1×10^{-6}～20×10^{-6}; Malate, 0.1×10^{-6}～20×10^{-6}; Tartrate, 10×10^{-6}～160×10^{-6}; α-Ketoglutarate, 0.2×10^{-6}～10×10^{-6}; Oxalate, 0.2×10^{-6}～16×10^{-6}; Fumarate, 0.2×10^{-6}～10×10^{-9}; Citrate, 0.5×10^{-6}～25×10^{-6}) of the ten analytes solutions were injected in triplicate and then the calibration curves were constructed by plotting the conductivity versus the concentration (mmol/L) of each analyte. The results were shown in Table 2. Responses of the ten analytes were linear in the working range respectively ($r^2\geqslant0.9976$).

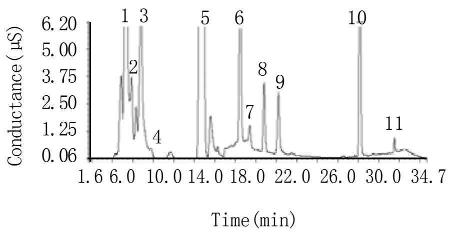
The relative standard deviation (RSD) for L-Lactate, Acetate, Formate, Pyruvate, Malate, Tartrate, α-Ketoglutarate, Oxalate, and Citrate were 2.8%, 1.2%, 1.5%, 3.3%, 2.33%, 1.8%, 1.7%, 2.2%, 4.1%, and 1.86% respectively.

Table 2 Linear regression equations and correlation coefficients

Analyte	Range of linear (mg/L)	Regressive equation	γ^2	RSD (%)
L-Lactate	5～200	Y=13.129X－1.391	0.9983	2.8
Acetate	0.5～20	Y=8.472X－3.439	0.9976	1.2
Formate	0.1～20	Y=0.639X－1.462	0.9984	1.5
Pyruvate	0.1～20	Y=0.978X－0.0415	0.9995	3.3
Malate	0.1～20	Y=13.900X－5.064	0.9986	2.3
Tartrate	10～160	Y=13.292X－13.193	0.9994	1.8
α-Ketoglutarate	0.2～10	Y=9.763X－0.0340	0.9993	1.7
Oxalate	0.2～16	Y=9.938X +0.909	0.9993	2.2
Fumarate	0.2～10	Y=18.657X	0.9986	4.1
Citrate	0.5～25	Y=16.452X－2.223	0.9994	1.9

2.3 Detection of organic acids in fish sauce samples

Fish sauce samples were diluted 100-fold and filtered by 0.22μm filter. Filtrates were analyzed directly. Chromatogram of the fish sauce sample from squid processing by-products was shown in Fig. 2. As shown in Fig.2, nine organic acids were separated by potassium hydroxide gradient conditions (Table 1). Eluent flow-rate was 1.2 ml/min.



1. L-Lactate; 2. Acetate; 3. Formate; 4. Pyruvate; 5. Chloride; 6. Malate; 7. Tartrate; 8. α-Ketoglutarate; 9. Oxalate; 10. Phosphate; 11. Citrate.

Fig.2 Chromatogram of fish sauce samples from squid by-products

2.4 Percent recovery from sample matrix

Diluted fish sauce samples were spiked with standard organic acids samples for the recovery and stability study. An aliquot of fish sauce sample was diluted 10-fold with deionized water. Another aliquot of fish sauce sample was diluted with the mixture of ten standard organic acids solution. The final concentrations for L-lactate, Acetate, Formate, Pyruvate, Malate, Tartrate, α-Ketoglutarate, Oxalate, and Citrate were 10.00, 1.00, 2.00, 0.10, 2.00, 1.00, 1.00, 1.00, and 2.00 mg/L respectively. An aliquot of fish sauce sample was diluted 100-fold with deionized water directly, serving as an unspiked "blank". Five detections were carried out for each sample. Fumarate was not tested because fumarate had not been detected originally in fish sauce samples. Two unknown waves crests in chromatogram of fish sauce samples were judged as chloride and phosphate at the base of retention time respectively by injected standard chloride and phosphate solution. Chromatogram of fish sauce samples was shown Fig. 2. The recovery data of standard samples were shown in Table 3. Percent recoveries for for L-lactate, Acetate, Formate, Pyruvate, Malate, Tartrate, α-Ketoglutarate, Oxalate and Citrate were 97.86%, 96.7%, 104.33%, 93.60%, 94.68%, 103.35%, 98.74%, 95.49%, and 98.77% respectively.

2.5 Contents of organic acids in different fish sauces

Table 4 showd the content of nine organic acids in six fish sauces from different producing areas. The variability in total content of nine organic acids is obvious among factories and among countries. The total organic acid contents are highest in fish sauce from squid processing by-products. L-Lactate is the main organic acids and the total content is

Table 4 Concentrations of organic acids in different fish sauces

Analyte	Contentrations of organic acids (g/L)					Prepared sample (g/L)
	Japan	Korea	China	Thailand	Vietnam	
L-lactate	N D	8.99±0.13	2.862±0.03	4.437±0.038	3.060±0.03	14.480±0.15
Acetate	0.0513±0.001	0.093±0.001	7.343±0.067	1.092±0.009	1.756±0.018	0.794±0.015
Formate	N D	0.025±0.001	0.221±0.002	0.279±0.003	N D	0.0352±0.001
Pyruvate	N D	N D	N D	N D	0.0266±0.001	0.0023±0.001
Malate	0.171±0.006	1.30±0.013	N D	0.0238±0.001	0.229±0.002	1.598±0.015
Tartrate	0.0207±0.004	0.81±0.09	0.678±0.006	0.0432±0.001	0.463±0.005	0.280±0.003
α-Ketoglutarate	1.465±0.007	N D	N D	N D	0.795±0.01	0.594±0.005
Oxalate	0.0213±0.001	0.48±0.05	0.313±0.003	0.256±0.002	0.0469±0.003	0.367±0.005
Citrate	0.0805±0.001	0.0479±0.001	N D	1.143±0.014	0.976±0.008	1.071±0.018
Total	1.810±0.021	11.753±0.286	11.416±0.11	7.274±0.068	7.352±0.077	19.274±0.202

Note: Data, mean ± SD; ND, undetected.

Table 3 Analytical results of organic acids in fish sauce and recovery data of standard samples

Analyte	Concentration (mg/L)	Addition (mg/L)	Values of detection (mg/L)	Percent recovery (%)
L-Lactate	144.800	10.00	154.586	97.86
Acetate	0.794	1.00	1.761	96.7
Formate	3.520	2.00	5.607	104.33
Pyruvate	0.230	0.10	0.324	93.60
Malate	15.984	2.00	17.878	94.68
Tartrate	2.796	1.00	3.830	103.35
α-Ketoglutarate	5.943	1.00	6.930	98.74
Oxalate	3.666	1.00	4.621	95.49
Citrate	10.707	2.00	12.682	98.77

19.274 ± 0.202 g/L. The content of organic acids in fish sauce from Japan is low and the total content is 1.810±0.021 g/L while α-Ketoglutarate is the main organic acids. The total organic acid contents in fish sauce from China and Korea are similar and the contents of total organic acids are 11.416±0.110 g/L and, 11.753±0.286 g/L respectively. But the variety of main organic acid is different between them. L-Lactate and Acetate is main organic acids in fish sauce from China, Whereas L-Lactate and Malate are main organic acids in fish sauce from Korea. The contents of total organic acids in fish sauce from Thailand and Vietnam are basically the same (7.274 ± 0.068 g/L and 7.352 ± 0.077 g/L respectively). L-Lactate, Acetate, Citrate are the main organic acids in both of them. Differences of contents and varieties of organic acids in fish sauce among countries come from different materials and fermentation technology. Lactate may be produced by lactic acid bacteria during fermentation or may be originated from fish muscle. Judging from the low Lactate and high Acetate results in the fish sauces of China, the acetic acid fermentation is surpassing the lactic acid fermentation as shown in these samples [8].

3 Conclusion

The results showed that fish sauce can be analyzed for nine organic acids compositions by ion chromatography with suppressed conductivity. The columns were available for fish sauce analysis of organic acids. Complex organic acids mixtures in fish sauces could be monitored simultaneously. The developed method is highly accurate and efficient. The developed method can provide the analyst with some of the information of organic acids in fish sauce and can be applied for guiding fermentation process of fish sauces.

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