J. Chem. Lett.4 (2023) 30-37

Research Article

Journal of Chemistry Letters
journal homepage: www.jchemlett.com
ISSN (online) 2717-1892 (print) 2821-0123

Determination of sulphite in wine samples with the aid of three different analytical techniques

Kingsley Chinonso Sunday[1], Patrick Enuneku Omuku[1], Collins Chibuzor Odidika[1], Ebuka Chizitere Emenike[1], Steve O. Eshiemogie[2], Kingsley O. Iwuozor[1,]*

[1] Department of Pure and Industrial Chemistry, Nnamdi Azikiwe University, P. M. B. 5025, Awka, Nigeria.
[2] Department of Chemical Engineering, University of Benin, P. M. B. 1154, Benin, Nigeria.

ARTICLE INFO

Article history:
Received 14 July 2022
Received in revised form 1 August 2022
Accepted 2 August 2022
Available online 2 April 2023

Keywords:
Chromatography
Food analysis
Spectrophotometry
Sulphite
Titrimetric

ABSTRACT

This study comparatively analyzed the free and total sulphite in thirty wine samples divided into alcoholic wines, non-alcoholic wines, and fruit juice wines using three analytical techniques, namely, the titrimetric method, the spectrophotometric technique, and the chromatographic technique (HPLC). All the wine samples using the three different analytical techniques were below the permissible limit of sulphite in wine samples as stated by regulatory bodies. Analyzing the free sulphite concentration in the wine samples gotten from the spectrophotometric analysis, it can be seen that alcoholic wine had the least free sulphite concentration (11.11–14.65 mg/10 ml). The total sulphite concentration was also found to be lowest in the alcoholic wine samples (24.49–33.62 mg/10 ml for the Spectrophotometric method; 31.21–39.26 mg/10 ml for the HPLC method; and 31–38.71 mg/10 ml for the titrimetric method). It was observed that fruit juice wines contain the highest concentration of sulphite (both free and total sulphite), followed by non-alcoholic wines, and then alcoholic wines. Statistical data analysis of the experimental results obtained for the study showed that the titrimetric technique had the highest accuracy for the determination of total sulphite in the three groups of samples, the spectrophotometric technique had the highest accuracy for the determination of free sulphite in alcoholic and non-alcoholic wines, and HPLC had the highest accuracy for the determination of free sulphite in fruit juice wines.

1. Introduction

Food is one of the most important basic needs for human sustenance [1, 2]. Its adulteration in all its forms has been an existent practice for as long as the manufacturing and processing of food has been [3]. Food adulteration is an act/process whereby food is intentionally and/or unintentionally debased through the removal of vital nutrients (wholly or partly) from the food and the substitution or addition of extraneous substances (adulterants) to the food, rendering it unsafe for consumption [4-7]. Wine is an alcoholic drink made from fermented grapes. Wines not made from grapes include rice wine and other fruit wines such as plum, cherry, pomegranate, currant and elderberry [8]. Sulphites or sulphating agents are terms used to describe sulphur-based compounds that are commonly used in a number of foods and beverages for several purposes [9]. Sulphites are the most regularly employed preservative in winemaking and are widely utilized as food additives. This is because they inhibit the growth of both enzymatic and non-enzymatic browning in a variety of processed and stored foods [10, 11]. Sulphites also improve the aroma and flavour profile of wine by binding with by-products of fermentation that are responsible for off-flavours, such as acetaldehyde [12-14].

However, dietary sulphite has been linked to a number of health problems, including difficulty in breathing and wheezing, particularly in asthmatics and sulphite-sensitive people [15]. Sulphites can also cause gastrointestinal problems [9, 16]. Hence, sulphites are potentially toxic to human health and need to be properly controlled and regulated in foods and beverages [17]. According to the European Union (EU) regulation, the maximum allowable total sulphite concentrations in wine are as follows: 250-400 mgL^{-1} for wines containing more than 5 gL^{-1} sugar, 200 mgL^{-1} for white and rose wines, and 150 mgL^{-1} for red wine [12, 18]. The acceptable daily

* Corresponding author.; Tel. 08167515860; e-mail: kingsleyiwuozor5@gmail.com
https://doi.org/10.22034/jchemlett.2022.351711.1077

intake of sulphite (expressed as SO_2 equivalent) according to the World Health Organization (WHO) is 0.7 $mgkg^{-1}$ body weight [19, 20]. The health impacts of sulphites have prompted beverage producers in the EU and America to include the warning "contains sulphites" on beverage labels whenever a concentration of 10 mgL^{-1} is exceeded [21-23].

Sulphite is present in wine in two forms: free and bound sulphites, existing in a reversible equilibrium between each other [22]. Free sulphites are those that are not bound to any wine component. They include sulphur dioxide (SO_2), bisulphite (HSO_3^-), and sulphite (SO_3^{2-}), occurring according to pH. Since the pH of wine is often in the range of 3.2-4.0, the most frequently free sulphite found in wine is the bisulphite [12]. Bisulphite is known to bind exothermally and reversibly to several compounds in wine [24, 25]. This is one of the reasons sulphites are added to wine to bind with strong-flavored compounds, like aldehydes, disabling their fragrance [25]. On the other hand, the bound form of sulphite are hydroxysulfonate adducts that bind with compounds of wine such as ketones, aldehydes, sugars, and tannins, among others [26, 27]. The summation of free and bound sulphites is called total sulphite.

Sulphites are usually difficult to detect in a sample because of the uneven distribution of food samples and the instability of sulphite in aqueous media, being easily oxidized to sulphate [28]. Nevertheless, the Optimized Monier-Williams method, which involves hydrolysis of the various sulphite complexes and sometimes distillation, has been widely used in the detection of sulphites, and is recommended by the Association of Official Analytical Chemists (AOAC) [13, 29]. This method, however, is time-consuming and tedious, requires several pre-treatment and reagent preparation processes, and cannot be used for fast and high-throughput analysis [23]. Several other analytical techniques have since been used in the determination of sulphating species in food and beverage samples, including spectrophotometric methods [11, 30], electrochemical methods [29, 31], molecular emission spectrometry [32], high performance liquid chromatography [3, 33, 34], ion-exchange chromatography [35-38], chemiluminescene [39], and flow injection analysis technique [9, 40]. However, almost all the methods have shown some disadvantages, including high cost, long processing time, poor environmental stability, and unreliability at levels below 10 $mgkg^{-1}$ [41]. Therefore, it is important to have a more convenient and reliable method for the determination of sulphites in foodstuffs.

Spectrophotometric methods have been reported as the most common and effective method nowadays [28]. High performance liquid chromatography (HPLC) is also another common method for sulphite detection. Using the HPLC method, Ni, Tang, Liu, Shen and Mo [42] investigated the sulphite in food samples and found that the results were very similar to those of the optimized Monier-Williams method, a typical example of a titrimetric method. The aim of the present study was to determine the presence of sulphites in thirty different wine samples (alcoholic wines, non-alcoholic wines, and fruit juice wines) using three different analytical techniques: high-performance liquid chromatography, spectrophotometry, and titrimetric methods. Statistical data analysis was also used to evaluate their performance in order to ascertain the best method applicable for analysing sulphites in wine samples.

2. Results and Discussion

2.1. Materials

Spectrophotometric measurements were performed with a double-beam ultraviolet-visible spectrophotometer (Uniscope, SM7504). Chromatographic analyses were carried out with high performance liquid chromatography (Shimadzu, SPD-20AV). Titrimetric analyses were performed using the Optimized Monier-Williams method. The following reagents were used in the study: sodium sulphite BP (98.48% w/w), isopropyl alcohol, sodium acetate, acetic acid, hydrochloric acid, 2,2-dithiobis(5-nitropyridine), sodium hydroxide, hydrogen peroxide, phosphoric acid, methyl red, methylene blue, D-mannitol, methanesulfonic acid, and sodium phosphate. All the reagents were of analytical grade and were used as received without any further purification. Distilled water was used throughout the study.

2.2. Sample collection

Ten (10) alcoholic wines, 10 non-alcoholic wines, and 10 fruit wines of various brands (both red and white wines) were used, purchased from a local store in Awka, the capital city of Anambra state, in south-east Nigeria. The wines were labelled A, B, and C for alcoholic, non-alcoholic, and fruit wines, respectively.

2.3. Sample preparation and analysis

2.3.1. Spectrophotometric analysis

Preparation of sample was needed for the determination of both total and free sulphite. For this purpose, 1.82 g of D-mannitol and 1.92 g of methanesulfonic acid were dissolved in a 1 L volumetric flask and made up to volume with distilled water. 10 ml of the sample was then measured into a 100-ml flask, 50 ml of wine sample was added, and the flask was properly shaken. The absorbance was taken at 525 nm using the UV spectrophotometer.

2.3.2. HPLC analysis

8ml of distilled water, 0.5 ml of 0.02 M of acetic acid-sodium acetate buffer, 1.0 ml of the prepared sample solution, and 0.5 ml of 5 M of DTNP solution (dissolved in acetonitrile) were added into 20 ml test tube and mixed thoroughly for 30 seconds. The mixture was filtered through a 0.22 µm membrane for High Performance Liquid Chromatography (HPLC) analysis.

2.3.3. Titrimetric analysis (modified Monier-Williams method)

For free sulphite determination, 3 drops of methylene blue indicator were added to 10 ml of 1% v/v hydrogen peroxide and connected to the collection end of the distillation apparatus. In a round-bottom flask, 10 ml of 25% v/v phosphoric acid was measured into 10 ml of wine sample. The vacuum pressure pump was put on to aspirate the sample for 15 min. The collection flask was then removed and titrated against a 0.1 M NaOH solution till the colour returned to green.

For bound sulphite determination, 10 ml of 1% v/v hydrogen peroxide and 3 drops of methylene blue indicator were added to the same sample in a round bottom flask. The flask was placed on a heating mantle, connected to the collection end of the distillation apparatus, and heated for 15 min. The collection flask was then removed and the solution titrated against a 0.1 M NaOH solution till the colour returned to green. In all cases, summation of free sulphite and bound sulphite gave the total sulphite.

2.4. Statistical analysis

The results obtained were analyzed using the GENSAT analytical package (2012 version). Data collected was subjected to analysis of variance (ANOVA) for both free sulphites and total sulphites. In addition, the mean and standard deviation of the three categories of wine samples for each analytical technique was determined and compared.

3. Results and Discussion

Thirty samples of different brands of wine were analyzed for their sulphite content using the three different analytical methods (titrimetric, spectrophotometric, and chromatographic methods). The result obtained is presented in Table 1 and Figs. 1-3. From table 1, it was observed that all the wine samples were below the maximum allowable total sulphite concentrations in wine samples as stated by the European Union and by Codex Alimentarius [8, 43].

Analyzing the free sulphite concentration in the wine samples gotten from the spectrophotometric analysis, it can be seen that alcoholic wine had the least free sulphite concentration (11.11–14.65 mg/10 ml). This was closely followed by non-alcoholic wine with sulphite concentration in the range of 16.16–19.7 mg/ml. Fruit juice wine was found to have the highest level of free sulphite concentration (16.92–23.74 mg/10 ml). A critical look at results gotten from the HPLC method of analysis showed a similar free sulphite concentration trend, with alcoholic wine having the least sulphite concentration in the range of 13.95 – 18 mg/10 ml, followed by non-alcoholic wine (21 – 45.71 mg/10ml), and fruit juice (20.92 – 29.11 mg/10ml). The titrimetric method of analysis also gave a similar trend as the spectrophotometric and HPLC methods. During fermentation and storage, free sulphite is vulnerable to oxidation by radical oxygenated species, and their existence delays the oxidative degradation of other wine constituents [43]. The result obtained in this study is lower than the result obtained by Arce, Báez, Muena, Aguirre and Romero [44] for free sulphite in red wine (20.4 ± 3.1 mg/l). In addition, Lowinsohn and Bertotti [10] engaged a coulometric titration technique to obtain free sulphite concentration in three different wine samples ranging from 24.96 mg/L to 55.04 mg/L. Comparing the concentration of free sulphite for the wine samples, one-way ANOVA analysis was carried out on the data, and the result is presented in Table 2. From Table 2, it can be observed that the ANOVA analysis gave a P-value < 0.05, indicating that there is a significant difference in free sulphite concentration for the three methods of analysis engaged.

The total sulphite concentration was also found to be lowest in the alcoholic wine samples (24.49–33.62 mg/10ml for the Spectrophotometric method, 31.21–39.26 mg/10 ml for the HPLC method, and 31–38.71 mg/10ml for the titrimetric method). This was followed by the non-alcoholic wine (35.96–39.39 mg/10ml for the spectrometric method, 43.92–46 mg/10ml for the HPLC method, and 43.6–45.72 mg/10ml for the titrimetric method). Fruit juice had the highest sulphite concentration (10.21–46.81 for the spectrophotometric method; 47.2–50.42 mg/10ml for the HPLC method; and 47.2–49.7 mg/10ml for the titrimetric method). All three methods of analysis produced similar trends in results. Lowinsohn and Bertotti [10], using the Monier-Williams titrmetric method analyzed three different wine samples for their total sulphite concentration.The result obtained were within the range of 80.64 mg/l to 206.72 mg/l [10]. A lower result for total sulphite of red wine (60.3 mg/l) was obtained by Arce, Báez, Muena, Aguirre and Romero [44]. However, these results were higher than that obtained in this study. An ANOVA analysis was carried out on the total sulphite concentration results of the three methods of analysis and the results are presented in **Table 3**. From the analysis, a $P < 0.05$ was obtained, signifying that there is an equally significant difference among the three methods of analysis.

To estimate the extent of accuracy for all three methods, the standard deviations (STDv) for all methods and sample results were determined.

The results of the standard deviation for the free and total sulphite concentration in the alcoholic samples are presented in Table 4.

Table 1. Result of sulphite determination in the thirty wine samples

		Spectrophotometric Method		**HPLC Method**		**Titrimetric Method**	
S/N	**Wine Samples**	**Free sulphite (mg/10ml)**	**Total sulphite (mg/10ml)**	**Free sulphite (mg/10ml)**	**Total sulphite (mg/10ml)**	**Free sulphite (mg/10ml)**	**Total sulphite (mg/10ml)**
1.	A1	14.65	33.62	16.13	38.29	15.97	38.11
2.	A2	12.63	24.47	14.19	32.20	13.87	33.00
3.	A3	13.64	28.30	15.31	35.19	15.76	35.7
4.	A4	12.88	29.79	14.28	33.14	13.91	34.00
5.	A5	13.38	25.32	17.25	39.17	16.69	38.71
6.	A6	12.37	24.26	14.57	34.72	15.01	35.71
7.	A7	13.64	26.11	18.00	39.26	17.84	38.71
8	A8	12.63	25.53	15.00	35.10	14.97	34.57
9	A9	11.11	25.32	13.95	31.21	14.00	31.00
10	A10	11.87	24.89	14.00	30.97	13.71	31.00
11	B1	17.68	37.02	21.70	44.03	22.10	43.72
12	B2	18.69	38.72	22.90	45.10	23.09	44.91
13	B3	17.17	36.17	20.97	43.92	21.00	44.09
14	B4	17.02	39.39	21.24	45.71	20.79	46.00
15	B5	16.16	35.96	20.00	43.77	20.07	43.56
16	B6	19.70	38.72	24.12	44.99	24.00	45.12
17	B7	16.16	37.02	25.17	45.22	24.90	45.00
18	B8	19.19	39.15	22.10	46.00	22.18	45.72
19	B9	17.68	39.36	21.00	44.01	20.71	43.60
20	B10	17.17	37.45	20.92	43.98	21.00	44.29
21	C1	20.45	41.92	26.79	48.23	27.19	49.00
22	C2	23.74	46.81	29.11	50.42	29.00	49.70
23	C3	20.71	42.34	25.92	47.99	26.00	48.00
24	C4	19.19	40.21	25.78	47.77	25.92	48.20
25	C5	20.20	42.12	25.69	48.11	25.71	47.70
26	C6	17.93	41.70	25.29	47.20	24.69	47.00
27	C7	17.43	41.28	26.10	48.21	24.72	48.00
28	C8	20.46	42.34	26.11	49.97	25.64	50.23
29	C9	16.92	40.85	24.98	47.92	25.19	48.29
30	C10	17.68	41.92	26.20	48.19	26.00	47.62

From the result, the spectrophotometric method of analysis for free sulphite concentration returned the least deviation from the mean, while the titrimetric method returned the least deviation for total sulphite concentration. It can therefore be inferred that to measure free sulphite concentration in alcoholic wines, the spectrophotometric method of analysis is likely to give the most accurate results, while to measure the total sulphite concentration of alcoholic wines, the titrimetric method gives the most accurate results.

For non-alcoholic wines (Table 5), the spectrophotometric method equally gave the least dispersed result for free sulphite concentration, and hence can be said to be the most accurate method of the three. The HPLC method seems to be the most accurate of the three for total sulphite concentration in non-alcoholic wines. From the results, the titrimetric method also shows great accuracy for measuring total sulphite concentration in non-alcoholic wines.

For fruit juice wine (Table 6), the HPLC method is the most accurate method for measuring free sulphite concentration, while the titrimetric method shows greater accuracy in measuring total sulphite concentration. The HPLC method also shows great accuracy in determining the total sulphite concentration in fruit juice.

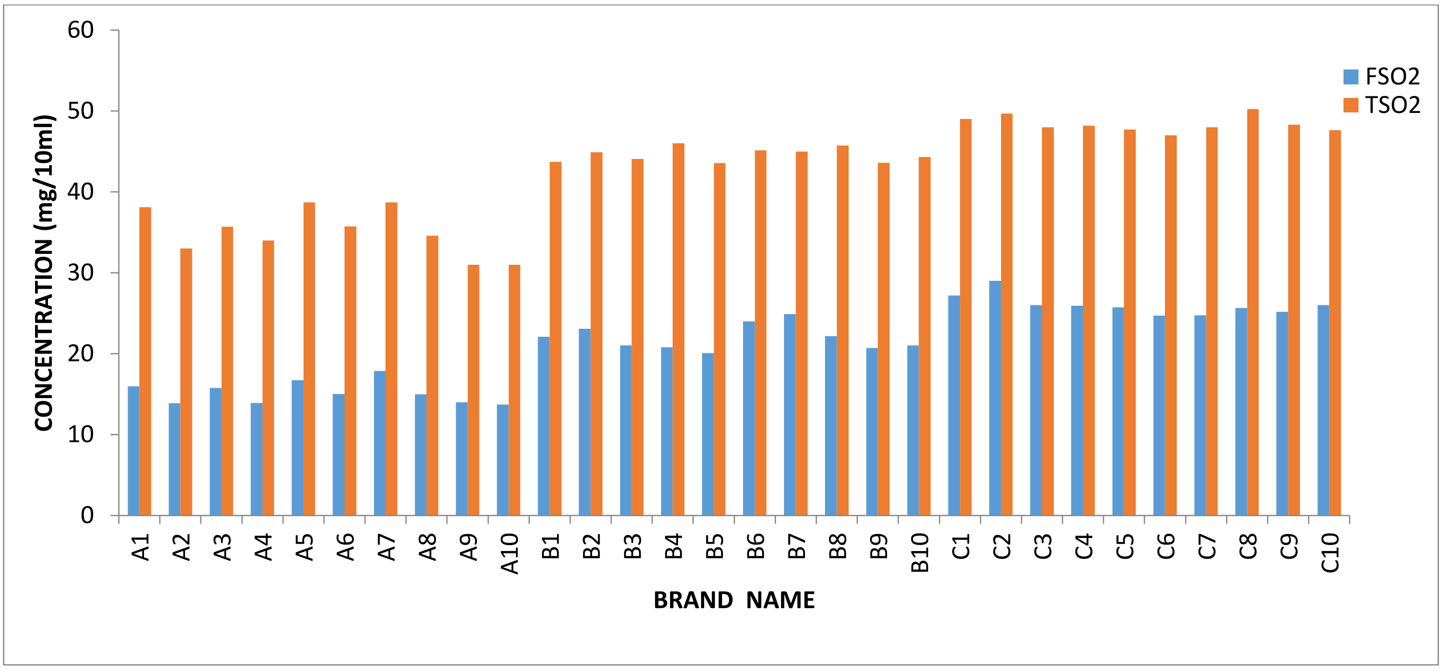

Figure 1: A plot showing the concentration of sulphite using Titrimetric Method.

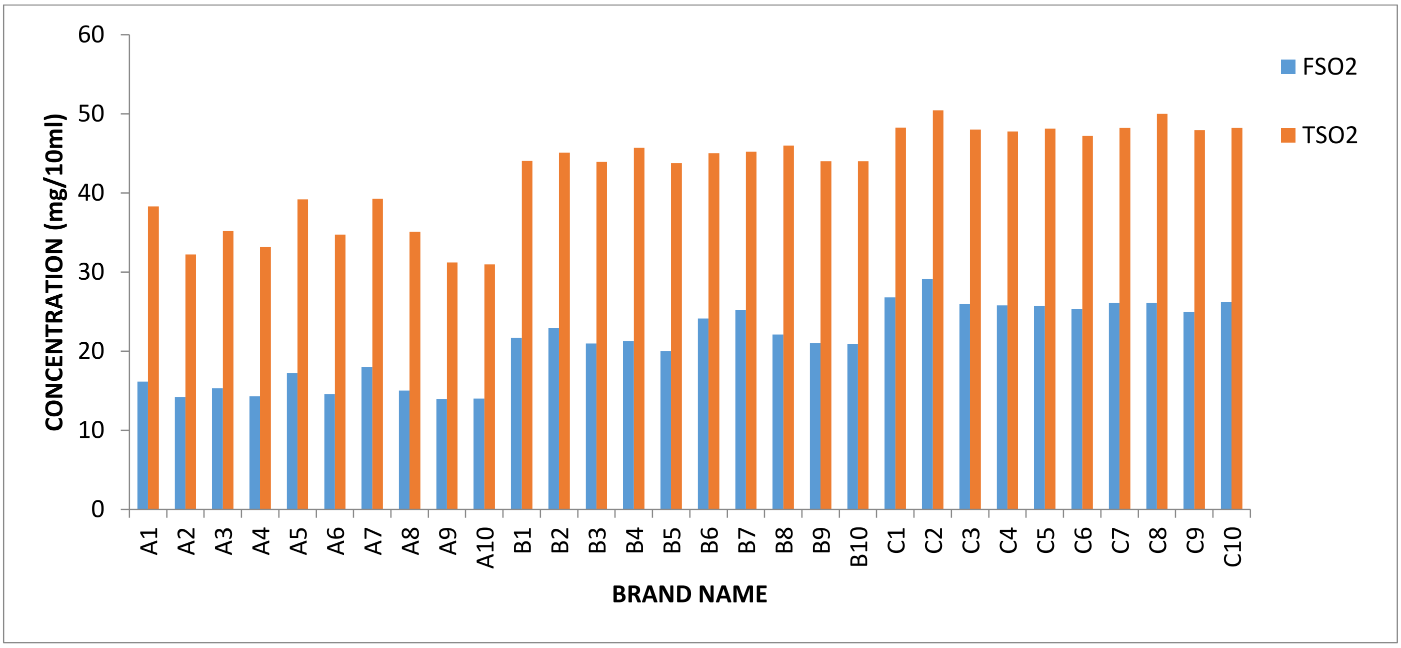

Figure 2: A plot showing the concentration of sulphite using HPLC Method.

Table 2. ANOVA analysis on free sulphite concentration

Source of Variation	SS	df	MS	F	P-value	F crit.
Between Groups	393.6671	2	196.8336	10.65022	0.000072	3.101296
Within Groups	1607.903	87	18.48164			
Total	2001.57	89				

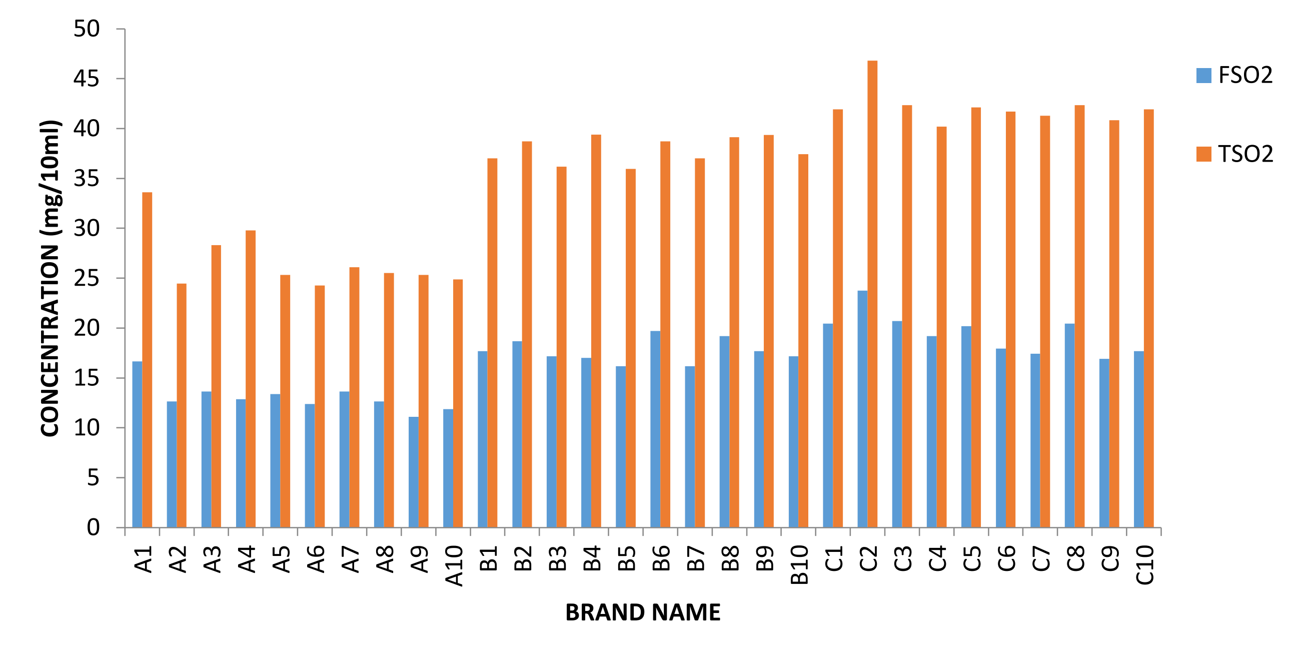

Figure 3: A plot showing the concentration of sulphite using the Spectrophotometric Method.

Table 1. ANOVA analysis of the total sulphite concentration

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	913.8271	2	456.9135	10.7972	0.000065	3.101296
Within Groups	3681.648	87	42.31779			
Total	4595.475	89				

Table 2. Mean and standard deviation for data on alcoholic wine samples

	Spectrophotometric method		HPLC Method		Titrimetric method	
	Free sulphite	Total sulphite	Free sulphite	Total sulphite	Free sulphite	Total sulphite
MEAN	12.88	26.761	15.268	34.925	15.173	35.051
STDv	1.004224	2.975561	1.421234	3.132863	1.38528	2.893542

Table 3. Mean and standard deviation for data on non-alcoholic wine samples

	Spectrophotometric method		HPLC Method		Titrimetric method	
	Free sulphite	Total sulphite	Free sulphite	Total sulphite	Free sulphite	Total sulphite
MEAN	17.662	37.896	22.012	44.673	21.984	44.601
STDv	1.19972	1.323574	1.607509	0.825564	1.580824	0.878957

Table 6. Mean and standard deviation for data on fruit juice wine samples.

	Spectrophotometric method		HPLC Method		Titrimetric method	
	Free sulphite	Total sulphite	Free sulphite	Total sulphite	Free sulphite	Total sulphite
MEAN	19.471	42.149	26.197	48.401	26.006	48.374
STDv	2.069946	1.773493	1.138499	0.998548	1.275689	0.990098

4. Conclusion

The analysis of free and total sulphite in thirty wine samples using three analytical techniques, namely, titrimetric method, spectrophotometric technique, and chromatographic technique (HPLC), were performed in this study. Three different types of wines were used for this study: alcoholic wines, non-alcoholic wines, and fruit juice wines. All the wine samples using the three different analytical techniques were below the permissible limit of sulphite in wine samples as stated by regulatory bodies. It was observed that fruit juice wines contain a higher concentration of sulphite (both free and total sulphite), followed by non-alcoholic wines, and then alcoholic wines. Statistical data analysis of the experimental results obtained for the study showed that the titrimetric technique had the highest accuracy for the determination of total sulphite in the three groups of samples, the spectrophotometric technique had the highest accuracy for the determination of free sulphite in alcoholic and non-alcoholic wines, and HPLC had the highest accuracy for the determination of free sulphite in fruit juice wines.

References

[1] K.O. Iwuozor, P.S. Mbamalu, B.O. Olaniyi, V.U. Anyanwu, E.C. Emenike and A.G. Adeniyi, Fortification of Sugar: A Call for Action. *Sugar Tech*, (2022) 1-11.

[2] S.O. Odeyemi, K.O. Iwuozor, E.C. Emenike, O.T. Odeyemi and A.G. Adeniyi, Valorization of waste cassava peel into biochar: An alternative to electrically-powered process. *Total Environment Research Themes*, 6 (2023) 100029.

[3] S. Pirsa, E. Banafshechin, S. Amiri, A. Rahimirad and J. Ghafarzadeh, Detection of fraud of palm, sunflower, and corn oil in butter using HPLC profile of tocopherols and tocotrienols by response surface method. *Journal of the Iranian Chemical Society*, 18 (2021) 1167-1177.

[4] K.O. Iwuozor, V.U. Anyanwu, B.O. Olaniyi, P.S. Mbamalu and A.G. Adeniyi, Adulteration of Sugar: A Growing Global Menace. *Sugar Tech*, (2022) 1-6.

[5] F. Ghasemi, M. Alizadeh, S. Pirsa and F. Mohtarami, Study of the Physicochemical Properties/Gas Chromatography Profile of Adulterated Pomegranate Juice by Nano-Composite-Fiber. *Journal of Agricultural Science and Technology*, 21 (2019) 1447-1458.

[6] S. Pirsa, Design of a portable gas chromatography with a conducting polymer nanocomposite detector device and a method to analyze a gas mixture. *Journal of separation science*, 40 (2017) 1724-1730.

[7] M. Alizadeh, S. Pirsa and N. Faraji, Determination of lemon juice adulteration by analysis of gas chromatography profile of volatile organic compounds extracted with nano-sized polyester-polyaniline fiber. *Food analytical methods*, 10 (2017) 2092-2101.

[8] K.O. Iwuozor, Qualitative and Quantitative Determination of Anti-Nutritional Factors of Five Wine Samples. *Advanced Journal of Chemistry-Section A*, 2 (2019) 136-146.

[9] C. Ruiz-Capillas and F. Jiménez-Colmenero, Application of flow injection analysis for determining sulphites in food and beverages: A review. *Food Chemistry*, 112 (2009) 487-493.

[10] D. Lowinsohn and M. Bertotti, Determination of sulphite in wine by coulometric titration. *Food Additives & Contaminants*, 18 (2001) 773-777.

[11] P. Musagala, H. Ssekaalo, J. Mbabazi and M. Ntale, A spectrophotometric method for quantification of sulphite ions in environmental samples. *Journal of Toxicology and Environmental Health Sciences*, 5 (2013) 66-72.

[12] A. Aberl and M. Coelhan, Determination of sulfur dioxide in wine using headspace gas chromatography and electron capture detection. *Food Additives & Contaminants: Part A*, 30 (2013) 226-233.

[13] J. Lin, Y. Zhu, W. Cheng, J. Wang, B. Wu and J. Wang, Determination of free and total sulfite in red globe grape by ion chromatography. *Food Science and Technology Research*, 20 (2014) 1079-1085.

[14] J.C.C. Santos and M. Korn, Exploiting sulphide generation and gas diffusion separation in a flow system for indirect sulphite determination in wines and fruit juices. *Microchimica Acta*, 153 (2006) 87-94.

[15] B.A.A. Al-Hadi, Spectrophotometric determination of sulphite and thiamin hydrochloride using proton transfer reaction-application to water sample and pharmaceutical formation. *Tikrit Journal of Pure Science*, 24 (2019) 74-81.

[16] E.C. Emenike, K.O. Iwuozor and S.U. Anidiobi, Heavy Metal Pollution in Aquaculture: Sources, Impacts and Mitigation Techniques. *Biological Trace Element Research*, 200 (2022) 4476-4492.

[17] K. Yoshikawa, Y. Uekusa and A. Sakuragawa, Determination of sulphite in wines using suppressed ion chromatography. *Food Chemistry*, 174 (2015) 387-391.

[18] L.M. Gonçalves, J. Grosso Pacheco, P. Jorge Magalhães, J. António Rodrigues and A. Araújo Barros, Determination of free and total sulfites in wine using an automatic flow injection analysis system with voltammetric detection. *Food Additives and Contaminants*, 27 (2010) 175-180.

[19] P. Kraikaew, T. Pluangklang, N. Ratanawimarnwong, K. Uraisin, P. Wilairat, T. Mantim and D. Nacapricha, Simultaneous determination of ethanol and total sulfite in white wine using on-line cone reservoirs membraneless gas-liquid separation flow system. *Microchemical Journal*, 149 (2019) 104007.

[20] B. Lajin and W. Goessler, Exploring the sulfur species in wine by HPLC-ICPMS/MS. *Analytica Chimica Acta*, 1092 (2019) 1-8.

[21] R.M. Ramos, L.M. Gonçalves, V. Vyskočil and J.A. Rodrigues, Free sulphite determination in wine using screen-printed carbon electrodes with prior gas-diffusion microextraction. *Electrochemistry Communications*, 63 (2016) 52-55.

[22] J.J. García-Guzmán, M.P. Hernández-Artiga, L.P.-P. de León and D. Bellido-Milla, Selective methods for polyphenols and sulphur dioxide determination in wines. *Food chemistry*, 182 (2015) 47-54.

[23] Z. Zhong, G. Li, B. Zhu, Z. Luo, L. Huang and X. Wu, A rapid distillation method coupled with ion chromatography for the determination of total sulphur dioxide in foods. *Food chemistry*, 131 (2012) 1044-1050.

[24] E.J. Bartowsky and P.A. Henschke, The 'buttery'attribute of wine—diacetyl—desirability, spoilage and beyond. *International journal of food microbiology*, 96 (2004) 235-252.

[25] R.M. Ramos, J.G. Pacheco, L.M. Gonçalves, I.M. Valente, J.A. Rodrigues and A.A. Barros, Determination of free and total diacetyl in wine by HPLC–UV using gas-diffusion microextraction and pre-column derivatization. *Food Control*, 24 (2012) 220-224.

[26] M. Koch, R. Köppen, D. Siegel, A. Witt and I. Nehls, Determination of total sulfite in wine by ion chromatography after in-sample oxidation. *Journal of agricultural and food chemistry*, 58 (2010) 9463-9467.

[27] R.M.D. Machado, M.C.F. Toledo and E. Vicente, Sulfite content in some Brazilian wines: analytical determination and estimate of dietary exposure. *European Food Research and Technology*, 229 (2009) 383-389.

[28] E. Gómez-Otero, M. Costas, I. Lavilla and C. Bendicho, Ultrasensitive, simple and solvent-free micro-assay for determining sulphite preservatives (E220–228) in foods by HS-SDME and UV–vis micro-spectrophotometry. *Analytical and bioanalytical chemistry*, 406 (2014) 2133-2140.

[29] N. Thanh, L. Decnop-Weever and W.T. Kok, Determination of sulphite in wine by flow injection analysis with indirect electrochemical detection. *Fresenius' journal of analytical chemistry*, 349 (1994) 469-472.

[30] C.R. Silva, T.F. Gomes, V.A. Barros and E.A. Zagatto, A multi-purpose flow manifold for the spectrophotometric determination of sulphide, sulphite and ethanol involving gas diffusion: Application to wine and molasses analysis. *Talanta*, 113 (2013) 118-122.

[31] R. Leubolt and H. Klein, Determination of sulphite and ascorbic acid by high-performance liquid chromatography with electrochemical detection. *Journal of Chromatography A*, 640 (1993) 271-277.

[32] T.A. Arowolo and M.S. Cresser, Automated determination of sulphite and sulphur dioxide by cool flame molecular emission spectrometry after reduction to hydrogen sulphide with sodium tetrahydroborate III. *Talanta*, 39 (1992) 1471-1478.

[33] L. Pizzoferrato, G. Di Lullo and E. Quattrucci, Determination of free, bound and total sulphites in foods by indirect photometry-HPLC. *Food Chemistry*, 63 (1998) 275-279.

[34] C.R. Warner, D.H. Daniels, D.E. Pratt, F.L. Joe jr, T. Fazio and G.W. Diachenko, Sulphite stabilization and high-performance liquid chromatographic determination: A reference method for free and reversibly bound sulphite in food. *Food Additives & Contaminants*, 4 (1987) 437-445.

[35] M. Iammarino, A. Di Taranto and A.R. Ientile, Monitoring of sulphites levels in shrimps samples collected in Puglia (Italy) by ion-exchange chromatography with conductivity detection. *Food Additives & Contaminants: Part B*, 7 (2014) 84-89.

[36] S. Pirsa, M. Alizadeh and N. Ghahremannejad, Application of nano-sized poly N-phenyl pyrrole coated polyester fiber to headspace microextraction of some volatile organic compounds and analysis by gas chromatography. *Current Analytical Chemistry*, 12 (2016) 457-464.

[37] A. Saidfar, M. Alizadeh and S. Pirsa, Application of Nano-sized Poly (N-methyl pyrrole-pyrrole) Fiber to the Headspace Solid-Phase Microextraction of Volatile Organic Compounds from Yogurt. *Journal of Chemistry Letters*, 1 (2020) 39-46.

[38] F. Ghasemi, S. Pirsa, M. Alizadeh and F. Mohtarami, Extraction and determination of volatile organic acid concentration in pomegranate, sour cherry, and red grape juices by PPy-Ag nanocomposite fiber for authentication. *Separation Science and Technology*, 53 (2018) 117-125.

[39] S. Satienperakul, P. Phongdong and S. Liawruangrath, Pervaporation flow injection analysis for the determination of sulphite in food samples utilising potassium permanganate–rhodamine B chemiluminescence detection. *Food chemistry*, 121 (2010) 893-898.

[40] C.T. Araújo, J.L. De Carvalho, D.R. Mota, C. De Araújo and N. Coelho, Determination of sulphite and acetic acid in foods by gas permeation flow injection analysis. *Food chemistry*, 92 (2005) 765-770.

[41] L. Wang and L. Xu, Cyclic voltammetric determination of free and total sulfite in muscle foods using an acetylferrocene–carbon black–poly (vinyl butyral) modified glassy carbon electrode. *Journal of agricultural and food chemistry*, 62 (2014) 10248-10253.

[42] Z. Ni, F. Tang, Y. Liu, D. Shen and R. Mo, Rapid determination of low-level sulfite in dry vegetables and fruits by LC-ICP-MS. *Food Science and Technology Research*, 21 (2015) 1-6.

[43] A.M. Pisoschi, A. Pop, I. Gajaila, F. Iordache, R. Dobre, I. Cazimir and A.I. Serban, Analytical methods applied to the assay of sulfur-containing preserving agents. *Microchemical Journal*, 155 (2020) 104681.

[44] R. Arce, C. Báez, J. Muena, M.J. Aguirre and J. Romero, Electrochemical method for sulfite determination in wines by electrochemical response using a membrane absorber system. *Journal of the Chilean Chemical Society*, 61 (2016) 3206-3210.