Journal of Applied Food Technology

Home page: https://ejournal2.undip.ac.id/index.php/jaft

Comparison of NaCl Levels in Seasoning Powder Formulation of Nagara Bean Flour (*Vigna unguiculata Ssp. cylindrica*) and Oyster Mushroom Using the Mohr Method by Direct Titration and Ash Mineral Titration

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Abstract

The use of excessive salt in the food product poses a potential health problem. For this reason, efforts should be made to reduce salt consumption. One of food products containing a significant amount of salt is flavor enhancer. The method of measuring salt content is crucial in seasoning powder to provide a good description of the salt content. Salt content measurement in foodstuffs was analyzed by the Mohr method that can be carried out by direct titration or by kiln to obtain mineral ash. The direct titration method will give faster results than the mineral ash titration. This research aims to obtain a comparison of salt content through direct titration and mineral ash titration, the deviation, and correlation between two methods. The results of the NaCl measurement content with the direct and the ash mineral titration method were significantly different, the correlation regression of the direct titration followed the equation Y = 15.368 + 1.021X. The direct titration showed higher measurement results than the ash mineral titration method. Mohr's method by ash titration is more precise and reliable for measuring NaCl in seasoning powder but requires a longer time.

Article information:
Received: 21 October 2021
Accepted: 13 September 2022
Available online: 25 September 2022

Keywords:
NaCl
Mohr's method
direct titration
ash mineral titration
seasoning powdert

doi: 10.17728/jaft.12547

Introduction

Dietary sodium intake is associated with an increased risk of hypertension and cardiovascular disease (Webster *et al.*, 2010; Capuano *et al.*, 2013; Cook *et al.*, 2016; Frieden, 2016). Currently, adult' sodium intake is three times the recommended daily intake. The recommendation of reducing sodium intake to 1.4 g (6 g salt) per day. Food companies must carry out strategies to reduce the sodium or salt content of foods and among others by adding flavor enhancers to increase the saltiness of foods so that lower salt concentrations can be used to achieve the same effect (Baines & Brown, 2015).

Flavor enhancers are chemical compounds that strengthen and intensify the taste effects of other flavor compounds in the mouth when food is consumed. Flavor enhancers are added to food to enhance taste and are considered food additives and not flavoring agents. Flavors can play an important nutritional role, especially in less flavorful foods, by providing the required appeal (Löliger, 2000). Foods and ingredients that are high in free amino acids or made from protein hydrolysates have been used to improve the sensory quality of various foods (Bellisle, 1999). The umami flavor helps to enhance the food flavor by giving it a savory taste. Glutamate is often used as a flavor enhancer in food, increasing the savory taste provided by glutamic acid, which occurs naturally in protein foods such as meat, seafood, stews, soups, sauces (Rangan & Barceloux, 2009).

The seasoning powder formula consists of a salt formulation with the addition of nagara bean tempeh flour and white oyster mushroom flour that identified containing glutamate which is expected to provide a strong enough umami taste to reduce the use of salt in seasoning. The salt content was analyzed by the Mohr method where the testing process was carried out directly titrated with a silver nitrate solution and through a kiln process before obtaining purer mineral ash. Johnson and Olson, (1985); Kindstedt and Kosikowski, (1984) tested the salt content of cheese with the Volhard method not different from measurements using

electrodes. Tests using the selective electrode method are more reliable, have higher accuracy and are more reproducible on the NaCl content of cheese (Rajkovic *et al.*, 2010).

The use of the direct titration method is often done because it is easy and faster than the furnaced method so it is useful in quality control activities. The direct method or ash titration method is equally often used for analyzing the salt content of solids, so it is necessary to validate the difference of the salt content measurement among the methods. The method of determining salt content by argentometry is carried out by titration, the endpoint of the titration is indicated by the formation of the brownish-red precipitate (Rochmawati and Purnadianti, 2019; Korkmaz, 2011; Young, 1893). Under acidic conditions, a dichromic acid complex with silver is formed which is easily soluble so that the brownish-red titration endpoint is not formed. The use of ammonia solution to adjust the pH of the chloride solution can give overestimated measurement results. On the other hand, the presence of ammonium salts in the material is not a nuisance if the pH is maintained at 6.5-7.2. otherwise it can cause the endpoint to be late (Block & Waters, 1967).

This study was aimed to validate the measurement results of salt content in seasoning powder from the formulation of salt, nagara bean tempeh flour, and oyster mushroom flour using the Mohr method through direct titration and mineral ash titration and obtain the correlation of the values.

Materials and Methods

Materials

Nagara beans from Hulu Sungai Selatan, South Kalimantan Indonesia, tempeh starter (Raprima), white oyster mushroom, commercial salt with brand Kapal (filtered of 100 mesh), white sugar (filtered of 100 mesh), chemicals for analysis are $AgNO_3$ (Merck), K_2CrO_4 (Merck), NaOH (Merck), Phenophtalein indicator (Merck), ethanol 96% (Merck), oven (Memmert), furnace (Memmert), filter paper and glassware for analysis.

Methods

Preparation of Tempeh

Nagara beans were soaked in water in a ratio of 1: 4 for 5 hours then the skin is peeled and cleaned. Nagara beans were steamed for 10 minutes then drained and cooled, added tempeh starter of 0.2% and wrapped in plastic, then fermented for 42 hours. The tempeh produced was sliced and dried at 60°C for 48 hours, then grounded and filtered at 100 mesh.

Preparation of Oyster Mushroom Flour

The mushrooms were washed and blanched for 10 minutes then dried at 60°C for 48 hours. Crushed dried mushrooms were filtered at 100 mesh

Formulation of Seasoning Powder

Seasoning powder was formulated using salt, nagara bean tempeh flour, white oyster mushroom flour, and white sugar. The proportions of salt: nagara bean tempeh flour: white oyster mushroom flour is shown in Table 1.

Table 1. The proportions of salt: nagara bean tempeh flour: white oyster mushroom flour

Salt: nagara bean tempeh flour: white oyster mushroom flour	White Sugar (%)
50: 25 : 25;	0
40: 30 : 30;	25
30: 35 : 35	30
20: 40: 30.	

Parameter of Analysis

Analysis of NaCl by Mohr Method (direct titration)

The sample is weighed 0.5g, put into a 250 mL Erlenmeyer, add 100 mL of distilled water, 3 mL of 0.1N NaOH, and 1 mL of 5% potassium chromate indicator, shaken evenly, then titrated with 0.1 M silver nitrate solution.

Analysis of Salt content (NaCl) by Mohr's Method (ash mineral titration)

The sample was weighed 1 g and put in the furnace as in the method of determining the ash content. The ash was washed with as little aquadest as possible and transferred to a 250 ml Erlenmeyer. Add 1 ml of 5% potassium chromate solution and titrate with 0.1 M silver nitrate solution. The endpoint of the titration is when the first cloudy red color appears. The NaCl content is calculated by the formula:

$$\% \, NaCl = \frac{T \, x \, M \, x \, 5.84}{W} \quad \text{.....(1)}$$

With:

T = titer

M = molarity of silver nitrate

W = sample weight

Preparation of 0.1 N NaOH solution (Sudarmadji et al. 1997)

Weighed 4 grams of NaOH pellet, dissolved with distilled water in a volumetric flask to a volume of 1000 mL

Standardization of 0.1 N . NaOH Solution

Carefully weigh approximately 0.1 g of oxalic acid ($C_2H_2O_4.2H_2O$) in a 250 mL erlenmeyer, add 25 mL of distilled water. After dissolving, 2-3 drops of phenolphthalein indicator are added and titrated with NaOH solution which will be standardized to a pink color.

$$N \, NaOH = \frac{g \, oxalic \, acid \, x \, 2}{0.126 \, x \, mL \, NaOH} \quad \text{.....(2)}$$

Preparation of 0.1 N $AgNO_3$ Solution (Sudarmadji et al. 1997)

Weighed 16,989 g $AgNO_3$ which had been dried at 120°C for 1 hour, dissolved in distilled water to exactly 1000 mL.

Standardization of 0.1 N $AgNO_3$ Solution

Weighed 200 mg of KCl in erlenmeyer, then dissolve with 25 mL of distilled water, add 2-3 drops of K_2CrO_4 5% and titrate with $AgNO_3$ solution which will be standardized until orange red color (color of Ag_2CN_2O)

$$N \, AgNO_3 = \frac{g \, KCl}{0.07455 \, x \, mL \, AgNO_3} \quad \text{.....(3)}$$

Analysis of Data

Analysis of salt content (NaCl) data through direct titration method and ash titration method, which includes standard deviation, regression correlation test and 2-tailed difference test using SPSS version IBM 24

Result and Discussion

NaCl Content

The seasoning powder formulation used a decreasing percentage of salt to the proportion of nagara bean tempeh flour and oyster mushroom flour. Similarly, the addition of sugar was carried out to obtain its effect on strengthening the umami effect of the nagara bean tempeh flour and white oyster mushroom flour. This seasoning powder is expected to have a strong umami taste so that it can be used as a flavor enhancer to reduce the use of salt. Therefore, the measurement of salt content in this formulation was carried out to determine the level of NaCl in it.

It is recommended that the maximum recommended salt intake is 6 g/day which is equivalent to sodium 2400 mg/day, this can reduce the risk of hypertension (Krauss *et al.*, 2000). Consumers should choose low-salt foods and limit the amount of salt added to food. Therefore, measuring the salt content in seasoning powder is important to determine the salt content in the ingredients consumed. The precise measurement method will show the actual amount of NaCl content in the seasoning powder. It is good because the formulation of this seasoning powder is used to reduce the salt content in the ingredients through components that can provide an umami taste so can reduce the salt consumption.

The choice of method to determine sodium chloride (NaCl) in materials is important to provide quality assurance. There are various methods available to determine salt, with their respective advantages and limitations, one of which is Mohr's method of argentometry which is carried out by titration. Measurement of salt content in solids based on the Mohr method is often carried out by two methods, namely by direct titration or the material furnaced to obtain mineral ash. In principle, the determination of the chloride concentration is carried out by titration using silver nitrate, the addition of silver nitrate will form a silver chloride precipitate.(Sezey and Adun, 2019). The end point of the titration occurs when chloride ions are precipitated by showing a red-brown precipitate change due to the reaction of silver ions and chromate ions as indicators.

$\mathrm{NaCl} + AgNO_3 \rightarrow AgCl + NaNO_3$.....(4)

$Ag^+ + Cl^- \leftrightarrow AgCl$.....(5)

$2Ag^+ + {CrO_4}^{2-} \leftrightarrow Ag_2CrO_4$.....(6)

Table 2 showed the total NaCl content of seasoning powder measured by direct method tends to be higher than the ash titration method with a standard deviation ranging from 0.10-6.90, while the measurement of NaCl content using the ash titration method resulted in a smaller standard deviation in the range of approx. 0.12 – 1.94. The variation of NaCl content value in the direct titration method tends to be greater than the ash titration method (Figure 1). The NaCl measured by the direct method is higher than the actual salt proportion in the formulation, while the ash titration method is lower. It is presumably due to direct methods which can provide late endpoints. At alkaline pH (pH > 8), some Ag^+ will be precipitated into silver carbonate or silver hydroxide, so that more $AgNO_3$ solution as titrant is needed. In the measurement of NaCl by ash titration method, the titrated component is purer because it consists of mineral components that are left behind when furnaced, including sodium chloride.

Table 2. Salt content (NaCl) measured by Mohr's method (direct titration and ash mineral titration)

Salt : nagara bean tempeh flour : mushroom flour	Sugar (%)	Mean Value [1]	Standard deviation [1]	Mean Value [2]	Standard deviation [2]	Standard deviation*
50:25:25	0	62.89	6.90	44.00	1.94	13.36
40:30:30	0	51.21	2.54	36.53	0.91	10.38
30:35:35	0	48.53	5.50	26.44	0.27	15.62
20:40:40	0	33.30	0.69	18.14	1.91	10.72
50:25:25	25	49.68	0.10	34.00	1.47	11.09
40:30:30	25	39.30	1.24	28.52	0.84	7.63
30:35:35	25	38.98	0.59	21.44	0.54	12.40
20:40:40	25	31.19	4.21	14.55	0.14	11.77
50:25:25	30	49.36	1.71	33.19	0.37	11.43
40:30:30	30	40.70	0.21	28.32	2.25	8.76
30:35:35	30	35.92	2.95	20.63	0.23	10.81
20:40:40	30	28.50	2.25	13.11	0.12	10.88

1) direct titration

2) being furnace-ash mineral titration

*) standard deviation of value salt content from 2 methods (1 and 2)

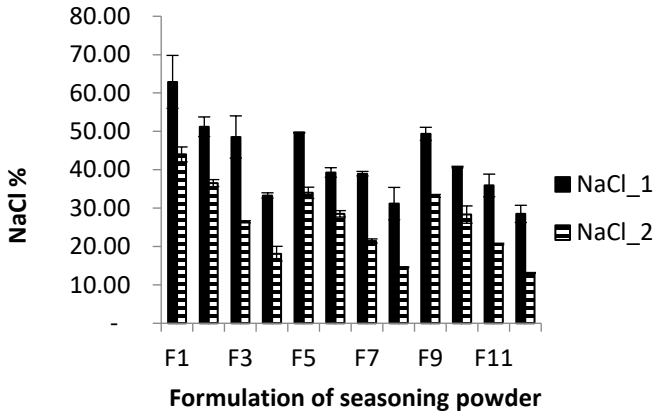

Figure 1. NaCl content measured by Mohr's method direct titration (NaCl_1) and ash mineral titration (NaCl_2)

$AgCrO_4$ is red color precipitates at neutral pH conditions and forming after the formation of AgCl. Chromate has a higher solubility than AgCl, so AgCl is formed first after C^{l-} is bound, Ag will react with chromate indicator to give a reddish color. Measurements by the direct titration method must pay attention to the condition of the material being tested. The success of the measurement using the direct method if the condition of the solution must be neutral – slightly alkaline, in the range of 6.5 – 9.0. In the formulation of seasoning powder which has a composition with the addition of tempeh flour, peanuts, and mushroom flour, it has slightly acidic properties so if a direct titration is carried out, it must be conditioned to be neutral. In the direct method, 100 mL of distilled water and 3 mL of NaOH solution were added to obtain neutral conditions, while in the mineral ash titration method, a little water was added before the K_2CrO_4 indicator.

Under acidic conditions, the chromate ion changes to chromic acid ($HCrO_4$-) and then to bichromate. Chromic and bichromatic acids combine with Ag+ to become more soluble salts and not form a red-brown precipitate. The solubility of silver chromate increases due to the protonation of the chromate anion, therefore the pH of the solution must be maintained at 7. There may be errors in this method because the chromate solution used has an intense color so it may require additional Ag+ to form Ag_2CrO_4, this affects the late equivalence point.

The difference between the measurement results of NaCl in the direct method and the ash method is slightly high, ranging from 7.63 to 15.62 and an average difference of 11.24. The difference between these two methods is slightly high, therefore the direct titration method on solids with a slightly acidic pH, it will be more precise to use the ashing method first.

Correlation and Deviation

The results of the normality test showed the NaCl measurement data in seasoning powder with the direct method or the mineral ash titration method fulfill the normality requirements ($p>0.05$) both with Kolmogorov Smirnov, Shapiro Wilk, and Skewness (Table 3). It is supported by the distribution of data which close to the normality line (Figure 2).

The method of measuring NaCl in seasoning powder by formulation of salt: nagara bean tempeh flour: oyster mushroom flour: sugar either by direct Mohr titration method or mineral ash titration was correlated by correlation value of the two data. In Table 4, the correlation value of R and R^2 is quite high, the dependent variable in this case NaCl with the direct titration method is linearly related to the mineral ash titration method with a significance <0.05. The linear equation of NaCl content between the two methods is described by equation 7, where Y is the NaCl content from direct titration measurement and X is the NaCl content from ash titration

$Y = 15.38 + 1.021X$……..(7)

Based on equation 7, there is a difference of 15.38 between the measurement results in the direct method and the ash titration method. The salt content resulted from the direct measurement method tends to be higher than the proportion of salt added to each formula. Meanwhile, the measurement using the ash titration method tends to have the measured NaCl content lower than the proportion of salt in the formulation (Table 2). The results of the pairwise difference test between the two methods are significant (<0.05) in Table 5, indicates that the measurement results of both methods are significantly different, this is correlated with a large enough difference or gap so that the direct method measurement results cannot be assumed to be the same as the ash titration method. In the direct method, there must be an appropriate pH control (pH 8) so that the addition of $AgNO_3$ is not too excessive, because some Ag^+ is precipitated into silver carbonate or silver hydroxide. It causing the measured NaCl content to be more than the added salt formulation.

Table 3. The results of the normality test on the measurement of NaCl in the direct and ash method.

Metode	p value Kolmogorov-Smirnov	p value Shapiro wilk	p value Skewness
NaCl 1 (direct titration)	0.181	0.430	1.190
NaCl 2 (being furnaced)	0.200	0.484	0.508

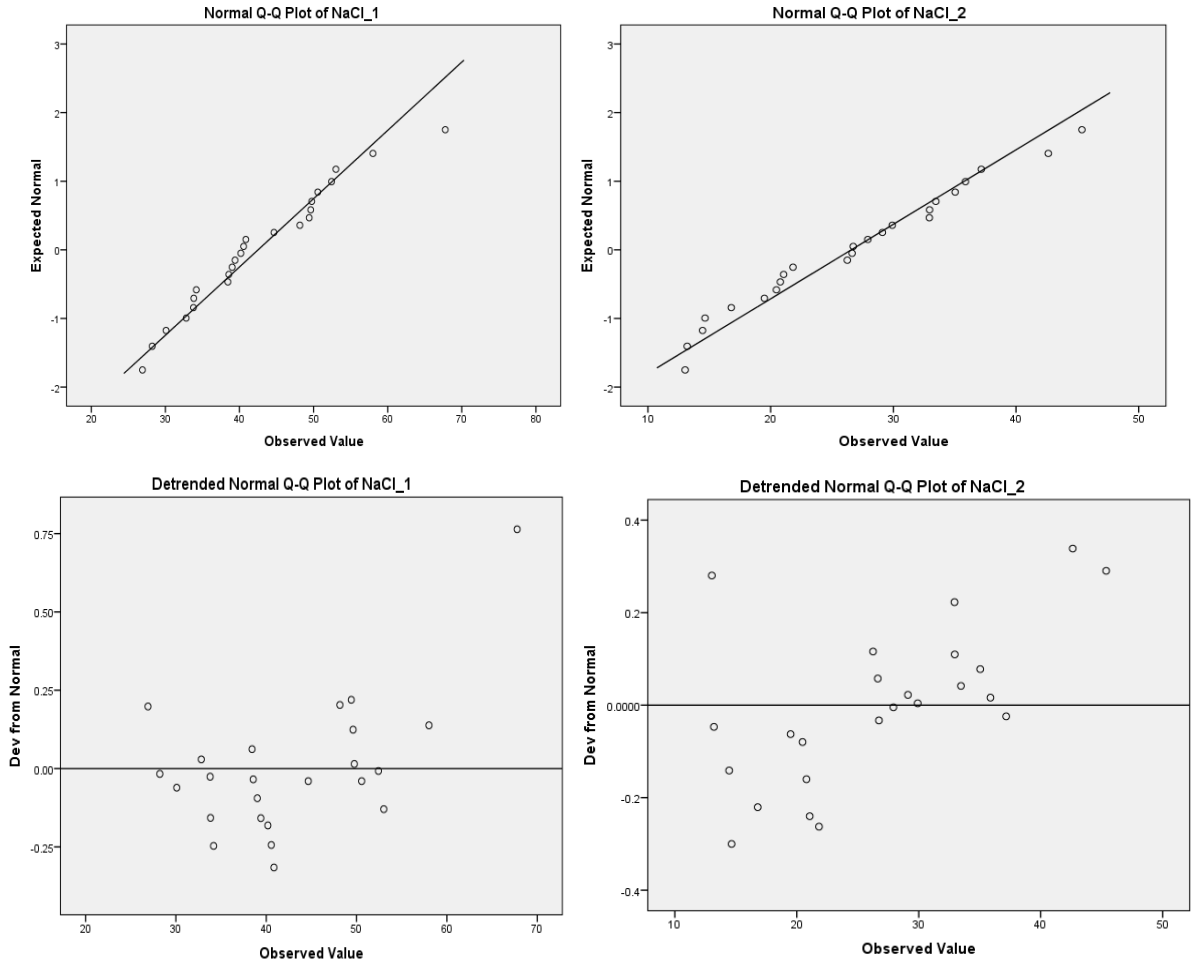

Figure 2. Normality of NaCl measurement data with direct method (NaCl_1) and ash method (NaCl_2)

Table 4. Correlation analysis of NaCl content by direct and ash mineral titration methods

R Value	R^2 Value	Significance	Correlation
0.936	0.876	0.000	Y = 15.368 + 1.021X Y = NaCl (direct methods) X = NaCl (ash mineral titration)

Table 5. Two-tailed Difference Test NaCl content from the direct and ash method

	Mean	Std. Deviation	Std Error Mean	t (df)	Significance
Pair NaCl1-NaCl2*	15.934	3.537	0.722	22.071 (23)	0.000

*) NaCl1: direct titration; NaCl2: ash mineral titration

Conclusion

Measurement of NaCl content in Mohr's method by direct titration and ash titration was significantly different with an average value of the difference of 15.93 %, both methods were positively correlated, the value of NaCl by direct titration was higher than the ash titration method. The pH of the solution in direct titration affects the use of $AgNO_3$ as a titrant, if the solution is acidic, it will not form an endpoint. Whereas, if the solution is too alkaline, it will cause the formation of silver carbonate or silver hydroxide which causes excess $AgNO_3$. Therefore, Mohr's method by ash titration is more precise and reliable for measuring NaCl in seasoning powder.

References

Baines, D., Brown, M. 2015. Flavor Enhancers: Characteristics and Uses. Encyclopedia of Food and Health. 1st ed. Elsevier Ltd. DOI:10.1016/B978-0-12-384947-2.00297-X.

Bellisle, F. 1999. Glutamate and the UMAMI taste: Sensory, metabolic, nutritional and behavioural considerations. A review of the literature published in the last 10 years. Neuroscience and Biobehavioral Reviews 23(3): 423–438. DOI:10.1016/S0149-7634(98)00043-8.

Block, J., Waters, O.B. 1967. Interference of the ammoniiun ion in Mohr's method for the determination of chloride. Talanta 14(9): 1130–

1131. DOI:10.1016/0039-9140(67)80152-8.

Capuano, E., Veer, G. van der, Verheijen, P.J.J., Heenan, S.P., Laak, L.F.J. van de, Koopmans, H.B.M., Ruth, S.M. van. 2013. Comparison of a sodium-based and a chloride-based approach for the determination of sodium chloride content of processed foods in the Netherlands. Journal of Food Composition and Analysis 31(1): 129–136. Elsevier Inc. DOI:10.1016/j.jfca.2013.04.004.

Cook, N.R., Appel, L.J., Whelton, P.K. 2016. Sodium Intake and All-Cause Mortality Over 20 Years in the Trials of Hypertension Prevention. Journal of the American College of Cardiology 68(15): 1609–1617. DOI:10.1016/j.jacc.2016.07.745.

Frieden, T.R. 2016. Sodium reduction-saving lives by putting choice into consumers' hands. JAMA - Journal of the American Medical Association 316(6): 579–580. DOI:10.1001/jama.2016.7992.

Johnson, M.E., Olson, N.F. 1985. A Comparison of Available Methods for Determining Salt Levels in Cheese. Journal of Dairy Science 68(4): 1020–1024. DOI:10.3168/jds.S0022-0302(85)80924-3.

Kindstedt, P.S., Kosikowski, F. V. 1984. Measurement of Sodium Chloride in Cheese by a Simple Sodium Ion Electrode Method. Journal of Dairy Science 67(4): 879–883. Elsevier. DOI:10.3168/jds.S0022-0302(84)81381-8.

Korkmaz, D. 2011. Determination of Chloride Ion Concentration by Titration (Mohr's Method). University of Cantearbury 1–2.

Krauss, R.M., Eckel, R.H., Howard, B., Appel, L.J., Daniels, S.R., Deckelbaum, R.J., Erdman, J., et al. 2000. AHA Dietary Guidelines Revision 2000: A statement for healthcare professionals from the Nutrition Committee of the American Heart Association. Circulation 102(18): 2284–2299. DOI:10.1161/01.CIR.102.18.2284.

Löliger, J. 2000. The Use and Utility of Glutamates as Flavoring Agents in Foods Function and Importance of Glutamate for Savory Foods 1. Journal of Nutrition supp 915–920.

Rajkovic, M., Sredovic, I., Miloradovic, Z. 2010. Comparison of different methods for determination of sodium chloride in cheese. Journal of Agricultural Sciences, Belgrade 55(1): 65–77. DOI:10.2298/jas1001065r.

Rangan, C., Barceloux, D.G. 2009. Food Additives and Sensitivities. Disease-a-Month 55(5): 292–311. Elsevier Inc. DOI:10.1016/j.disamonth.2009.01.004.

Rochmawati, D.A.N., Purnadianti, M. 2019. Determination of rate chloride (Cl-) with Argentometric Mohr on Drinking Water Refill Tidiness in the Village Purwodadi Districts Kras Regency Kediri. (Icps): 462–464. DOI:10.5220/0007545004620464.

Sezey, M., Adun, P. 2019. Validation of mohr's titration method to determine salt in olive and olive brine. Journal of the Turkish Chemical Society, Section A: Chemistry 6(3): 329–334. DOI:10.18596/jotcsa.496563.

Webster, J.L., Dunford, E.K., Neal, B.C. 2010. A systematic survey of the sodium contents of processed foods (American Journal of Clinical Nutrition (2010) 91, (413-20)). American Journal of Clinical Nutrition 92(4): 1003. DOI:10.3945/ajcn.2010.30174.

Young, W.G. 1893. On a source of error in the volumetric estimation of chlorides by Mohr's method. The Analyst 18(MAY): 125–130. DOI:10.1039/AN8931800125.