Comparative studies on the salt content of white bread and wholemeal bread

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Abstract. Bread contributes to the high salt daily intake of human which can cause increased blood pressure and cardiovascular health problems. Salt in bread has some important functions that affect the bread quality. However, some government organization regulated to reduce salt in bread production. Hence, this study aims to explain the salt content in commercial white bread and wholemeal bread by Mohr titration and Atomic Absorption Spectroscopy (AAS). Based on statistical analysis by using F test and t-test can be concluded there is no significant difference of salt content in both types of bread (p>0.05), however the result by Mohr titration was significantly higher than by AAS (p<0.05).

Keyword: Salt, White Bread, Wholemeal Bread, Mohr titration, Atomic Absorption Spectroscopy

1. Introduction
Bread is one of the most commonly consumed food; it is also widely considered to be a food product that supplies the most dietary salt to the diet that contributes about one-sixth of daily salt intake [3], [10]. Salt is usually added in low percentage into bread. Nevertheless, it has essential functions that affect the quality of bread. Belz et al [1] reported that salt gives sensory characteristic of bread, it also controls the yeast growth and fermentation rate, improves the bread texture and extends the shelf life by reducing spoilage.

Sodium chloride is an essential salt in the human diet. However, the intake of sodium relating to increases in blood pressure, which can cause cardiovascular health problems (Maroto et al., 2013). A recommendation of World Health Organization for maximum salt intake is 2000 mg per day for adults [6]. Hence, several countries commit to decrease the salt intake by reducing salt in food products [2]. Thus, foods salt contents are significant concerns for food processing and health care. Mohr’s titration with silver nitrate solution is commonly used to analyze salt content in foods. However, it has disadvantages due to the possibility of side reaction of silver ion with another anion such as carbonate and phosphate and the need of de-coloration for deep-colour food. Atomic absorption spectrophotometry is a method for direct detection of sodium ion concentration which might provide possible alternatives to overcome these drawbacks [4].
This study aims to evaluate and compare the salt content of white bread and wholemeal bread by using Mohr’s titration and atomic absorption spectroscopy methods. The hypothesis assumes that wholemeal bread has higher salt content than white bread.

2. Materials and Methods
The Morrisons white bread and wholemeal bread were used in this study. White bread was produced using fortified wheat flour, water, salt, wheat bran, wheat germ, calcium carbonate, yeast, wheat fiber, soy flour, calcium propionate, emulsifier, ascorbic acid, and folic acid [7]. Wholemeal bread was produced using wholemeal wheat flour, water, salt, yeast, soy flour, calcium propionate, emulsifier, wheat flour, ascorbic acid, folic acid [8]. The Mohr method can be seen in Figure 1. Which can be used to establish chloride and bromide levels in a neutral atmosphere with standard AgNO₃ solutions and the addition of K2C2O4 as an indicator. Titration in this way should be carried out in a neutral or slightly alkaline atmosphere, pH 6.5-9.0.

![Figure 1. Mohr Titration (College of Science University of Canterbury)](image1)

In Mohr’s titration there may be an excess of titrant which causes the indicator to precipitate before the equivalence point is reached which results in the end point of the titration so that it is not sharp. To anticipate, researchers can do stirring quickly.

![Figure 2. Atomic Absorption Spectrometry (Bernhard and Sperling, 2008)](image2)

In Figure 2 an Atomic absorption spectrometry (AAS) technique is an analysis used to look at the size and concentration of elements or compounds. AAS is also used in clinical analysis of health, pharmacy and also for environment and industry. This method is based on the absorption of light by atoms. Atoms absorb the light at certain wavelengths, depending on the nature of the elements. With the absorption of energy, it means obtaining more energy, an atom at the ground state is increased by its energy level of excitation level. The success of this analysis depends on the excitation process and obtains a proper resonance line.
2.1 Bread Analysis

2.1.1 Salt in Bread by Mohr Titration Method. 2g of bread was hydrolyzed in 2cm$^3$ of concentrated nitric acid and added with 10cm$^3$ of distilled water. The sample was prepared by swirling and warming the hydrolyzed bread at 80°C in Bunsen burner. Prepared sample was added to Sodium Bicarbonate until effervescence ceases. Silver nitrate solution (0.1M) was standardized with pure sodium chloride. 2cm$^3$ of Potassium Chromate was used as an indicator of silver nitrate titration, and the appearance of red brick precipitates was taken as the endpoint. The salinity of the food sample was determined based on the amount of titrated chloride ion (Chen et al., 2005).

2.1.2 Salt in Bread by Atomic Absorption Spectroscopy (AAS) Method. 1g of bread was ashed at 550°C in a Phoenix Microwave Furnace. The sample was prepared by hydrolyzing the resulting ash in 1cm$^3$ of concentrated Hydrochloric acid and diluted with distilled water into 100cm$^3$ solution. Prepared sample was diluted 20 times with distilled water, and the absorbance was measured by Atomic Absorption Spectroscopy (AAS) Pye Unicam SP9 AAS. Statistical analysis was done on all results for comparing salt content in both white bread and wholemeal bread by the same analysis method and for comparing the result of different analysis methods. The ratio of the variant can be used F test and usually to measure 2 samples from the group. This test is also similar to Bartlett while Levene, § trim, BF, PO.AD and PO.SQ use from equal-mean such as t-test or known as one-way ANOVA. Suppose we have the datasets $X_1, ..., X_n$ dan $Y_1, ..., Y_m$ with independent assumptions and identical distributions of two sample populations that are normally distributed. $H0 = \sigma_A^2 = \sigma_B^2$ versus alternative hypothesis $Ha = \sigma_A^2 \neq \sigma_B^2$ with $n - 1$ and $m - 1$ degrees of freedom

$$F = \frac{1}{n-1} \sum_{i=1}^{n} (x_i - \bar{x})^2 \left(1 - \frac{1}{n} \right)$$

Also, continued by unpaired two-sample t-test. Data are presented as the mean±standard deviation. Where $\bar{x}_1$ and $\bar{x}_2$ are the sample means, divided by the pooled sample variance, $n_1$ and $n_2$ are the sample sizes and t is a Student t quantile with $n_1 + n_2 - 2$ degrees of freedom.

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{\sum_{i=1}^{n_1} (x_i - x_1)^2 + \sum_{i=1}^{n_2} (x_i - x_2)^2}{n_1 + n_2 - 2} \times (\frac{1}{n_1} + \frac{1}{n_2})}}$$

3. Discussion

The obtained result of the salt content of white bread was compared with wholemeal bread. Information concerning salt content in white bread and wholemeal bread is presented in Table 1 and the resulting statistical analysis is presented in Table 2.

| Table 1. Salt Content in Commercial Bread |
|------------------------------------------|
| Type of Bread | Salt Level (g/100g of Bread) |
| -------------- | ------------------------------- |
|                | Mohr Titration | Atomic Absorption Spectroscopy | Bread Label$^1$ | Food Composition$^2$ |
| White Bread    | 1.47±0.15$^{ab}$ | 0.93±0.19$^{aA}$ | 1.00 | 1.2 |
| Wholemeal Bread| 1.41±0.11$^{ab}$ | 0.98±0.20$^{aA}$ | 1.00 | 1.2 |

Different subscript fonts after number shows the significant different of the data
Source: ¹Morrisons (2016); ²McCance and Widdowson (2015)
Table 2. The Statistical Analysis of Salt Content

| Variable | Probability of $F$-test | Type of $t$-test | Probability of $t$-test |
|----------|-------------------------|-----------------|------------------------|
| A        | 0.052                   | Two-Sample Assuming Equal Variances | 0.100 |
| B        | 0.308                   | Two-Sample Assuming Equal Variances | 0.363 |
| C        | 0.139                   | Two-Sample Assuming Equal Variances | 0.000 |
| D        | 0.000                   | Two-Sample Assuming Unequal Variances | 0.000 |

A: Salt content of White Bread versus Wholemeal Bread by Mohr Titration  
B: Salt content of White Bread versus Wholemeal Bread by Atomic Absorption Spectroscopy (AAS)  
C: Salt content of White Bread by Mohr Titration versus by AAS  
D: Salt content of Wholemeal Bread by Mohr Titration versus by AAS

Moreover, the amount of the sample size used in the Mohr titration using white bread as 24 samples and 1.995 gram – 2.091 gram bread contained grams of salt in range 26.00mg – 39.84 mg. After titration of the total of 24 sample that react NaCl on AgNO3 in range 5.21mg/cm$^3$ - 7.81mg / cm$^3$. In this research, we are using 32 samples used in the Mohr titration using whole wheat bread. The bread weight is 1,998gram-2.025 gram. Titration use as much as 4.55 ml-5.7ml NaCl reacts on AgNO3 as 5.39mg/cm$^3$ - 6.13 mg/cm$^3$. NaCl levels contained in the sample as much as the 24.89mg-30.97mg and NaCl concentration of 1.25 gram 1.54 gram. Atomic absorption spectroscopy (AAS) is an analytical method for the determination of metal and metalloid elements based on the absorption of radiation by the free atoms of the element. This method is very appropriate for the analysis of substances at low concentrations. This technique has several advantages compared to conventional emission spectroscopy methods.

Figure 3. Standard Curve of Na by AAS analysis

Standard curve of Na by AAS analysis can be seen in figure 3. The result using different measurement methods, Mohr titration and Atomic Absorption Spectroscopy (AAS) were compared with the composition on Bread Label and McCance and Widdowson’s Composition of Foods integrated dataset (CoFID) which are shown in Table 1. It is evident that different between values are very low. The mean values of the salt content of white bread by Mohr titration and AAS were 1.47g/100g of bread and 0.93g/100g of bread respectively. Similarly, the average values of the salt content of wholemeal bread by Mohr titration was 1.41g/100g of bread and by AAS was 0.98g/100g of bread. Also, Table 3 illustrate the calculation of the Na standard curve by AAS.
Table 3. The calculation of the Na standard Curve by AAS.

| Na Concentration µg/mL | Set 1 Absorbance | Set 2 Absorbance | Set 3 Absorbance | Set 4 Absorbance | Set 5 Absorbance | Average  |
|------------------------|------------------|------------------|------------------|------------------|------------------|---------|
| 1                      | 0.038            | 0.078            | 0.047            | 0.065            | 0.078            | 0.0612  |
| 2                      | 0.122            | 0.159            | 0.17             | 0.161            | 0.162            | 0.1548  |
| 3                      | 0.21             | 0.247            | 0.216            | 0.246            | 0.252            | 0.2342  |
| 4                      | 0.321            | 0.335            | 0.302            | 0.338            | 0.328            | 0.3248  |
| 5                      | 0.449            | 0.424            | 0.444            | 0.395            | 0.418            | 0.426   |

The resulting statistical analysis demonstrates. That there is no significant difference between the values of the salt content of white bread and wholemeal bread at a confidence level of 95% for both Mohr Titration method and AAS method; Mohr Titration p(0.100)>α(0.05); AAS p(0.363)>α(0.05). However, the result of the salt content of each bread by both methods shows significant difference statistically at α of 5%; white bread p(0.000)<α(0.05); wholemeal bread p(0.000)<α(0.05). The salt content determined by Mohr titration was significantly higher than by AAS method for the same type of bread. This is because there is different principle between Mohr titration and AAS. Chen et al. (2005) reported that AAS determination of sodium gives a fast, less tedious and more accurate alternative for the determination of salinity in processed foods.

Compared to the salt content which is stated in bread label and McCance and Widdowson’s Composition of Foods integrated dataset (CoFID), there was a significantly higher salt content of both pieces of bread by Mohr titration (1.43-1.47g/100g of bread versus 1-1.2g/100g of bread). Otherwise, the result of salt content by AAS was lower than both bread label and CoFID (0.93-0.98g/100g of bread versus 1-1.2g/100g of bread), however, it was not a significant difference. Another study by Mhurcu et.al (2011) reported that the salt content of bread in the UK was 1.01g/100g of bread. This result was similar to the result of salt content by AAS method.

4. Conclusions
In conclusion, there was no significant difference of the salt content of white bread and wholemeal bread. Regarding the statistical analysis by using alpha (α=5%) AAS method was more accurate than Mohr titration method, there was significant difference between the salt result of the same bread by these two different methods.

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