Analysis of Vitamin K1 levels in several types of vegetables consumed by warfarin-used patients

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Abstract

Vitamin K consumption needs to be monitored in people taking warfarin since it can impact the anticoagulation response. Vegetables are the primary nutritional source of Vitamin K1, in the form of Vitamin K1. The aim of this study was to measure the level of Vitamin K1 in various vegetables that were commercially sold in three major Bandung markets and were ingested by patients using warfarin at Hasan Sadikin Central General Hospital Bandung. High-performance liquid chromatography with an ultraviolet detector set at 245 nm was the analytical technique. One hundred percent methanol was used as the mobile phase, and it was isocratically eluted at a flow rate of 0.6 mL/min with a T3 column maintained at 25°C. The results indicated that the following Vitamin K1 levels were found in lettuce: 38.4391 ± 15.2650–64.4419 ± 19.0315 μg/100 g, in napa cabbage: 56.7445 ± 0.1569–273.2828 ± 8.3061 μg/100 g, in cabbage: 27.9531 ± 1.7487–217.0457 ± 7.2201 μg/100 g, and in spinach: 305.2868 ± 3.3058–970.7098 ± 14.1167 μg/100 g. The highest Vitamin K1 level was in spinach and the lowest was in lettuce.

Key words: High-performance liquid chromatography-ultraviolet, vegetables, Vitamin K1, warfarin

INTRODUCTION

Warfarin is one of the long-term oral anticoagulant therapies given to patients with cardiovascular disease.[1] Warfarin is a less expensive and effective anticoagulant, so its use remains prevalent in health facilities even though alternative anticoagulants are available. On the other hand, warfarin has a narrow therapeutic index and response, which can be affected by meal intake.[2,3]

Consuming meals high in Vitamin K can be one of the variables influencing individual differences in warfarin response and dosage.[4] Vitamin K is a fat-soluble vitamin that is primarily derived from food. It is naturally present as Vitamin K1 (phylloquinone) and Vitamin K2 (menaquinone-n, n = 1–14). Because warfarin and Vitamin K compete to inhibit the Vitamin K epoxide reductase enzyme, which in turn reduces blood clotting, high levels of Vitamin K in the body can interfere with warfarin's effectiveness.[4-6] Low Vitamin K levels can cause instability of the anticoagulation response which may increase the risk of thrombosis or bleeding. Consequently, individuals using warfarin need to regulate their Vitamin K intake.[7]

The primary dietary source of Vitamin K comes from vegetables. The form of Vitamin K contained in vegetables is Vitamin K1.[8,9] A study on Vitamin K-containing foods was previously carried out by Salsabila at Hasan

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Sadikin Central General Hospital Bandung, involving 85 warfarin-used patients. According to the study's findings, some of the vegetables that the patients consumed were cabbage, potatoes, cucumber, broccoli, oyong, lettuce, red beans, spinach, carrots, tomatoes, napa cabbage, and water spinach.[10] Based on the studies by Shearer *et al*. and Ferland and Sadowski, as well as the Physicians Association for Nutrition (2020), the vegetables with the highest Vitamin K1 concentration are spinach, lettuce, cabbage, and napa cabbage.[11-13]

In estimating Vitamin K intake for a specific population, it is essential to consider geographical location differences to reduce inaccuracies. This is because Vitamin K levels in vegetables vary by geographical location.[8] Several previous studies have analyzed the levels of Vitamin K1 in vegetables commercially available in several countries, for example, Australia and Brazil.[8,14] However, data on vegetables' Vitamin K1 levels in Indonesia, especially in the Bandung area, are still limited. Meanwhile, it is revealed that 71 patients (83.53%) of the 85 warfarin-using patients at Hasan Sadikin Central General Hospital Bandung are locals.[10]

The most popular, readily available, affordable, and easily operated type of high-performance liquid chromatography (HPLC) is HPLC ultraviolet (UV)-Vis.[15] Vitamin K1 has chromophore groups that can absorb UV-Vis radiation so that it can be detected using a UV detector.[16] In this study, HPLC UV-Vis was used to analyze the Vitamin K1 levels of several types of vegetables, including spinach, lettuce, cabbage, and napa cabbage. The vegetables are commercially available at three main markets in the Bandung area: Gedebage Main Market, Caringin Main Market, and Ciroyom Main Market.

MATERIALS AND METHODS

Materials

Materials used in this research include Vitamin K1 standard (Merck), methanol with HPLC grade (Merck), purified water (IKA), n-hexane pro analysis (Fulltime), sodium carbonate (Merck), and also vegetable samples which were cabbage, napa cabbage, lettuce, and spinach obtained from Gedebage Main Market, Caringin Main Market, and Ciroyom Main Market.

Preparation of Vitamin K1 standard solution

One hundred milligrams of the Vitamin K1 standard was dissolved in 20 mL of methanol to obtain a stock solution of Vitamin K1 with a concentration of 5000 ppm. The working solution was prepared by taking 200 µL of 5000 ppm stock solution and then diluting it to 5 mL with methanol to make a working solution with a concentration of 200 ppm. Stock and working solutions were stored in the containers protected from light at − 20°C.

Optimization of high-performance liquid chromatography condition

Optimization of HPLC condition was carried out based on Careri *et al*.'s method.[17] The stationary phase was an Atlantis™ T3 column (4.6 × 150 mm, 3 µm) and was operated at 25°C. The mobile phase used was methanol (100%) with isocratic elution, and to prepare the mobile phase, a 0.45 µm filter membrane was used for filtering, and sonication for 15 min was used to degas the mixture. The flow rate varied between 0.6 and 1 mL/min. A UV detector with a wavelength of 245 nm was used. A system suitability test was carried out under these conditions by injecting a standard solution of 5 ppm. The resolution (R), number of theoretical plates (N), symmetry factor (Tf), and capacity factor (k') were then calculated as the system suitability test parameters.

Preparation of Vitamin K1 calibration curve

Variations of standard solutions were prepared by diluting the working solution using methanol to obtain various concentrations, namely 0.1, 0.5, 1, 5, 10, 20, and 30 ppm. Each standard solution was injected into HPLC. The peak area (area under the curve [AUC]) was plotted as the y-value and the standard solution concentration as the x-value to obtain the calibration curve equation.

Sample preparation

Sample preparation for Vitamin K1 extraction was carried out by avoiding light to minimize visible light exposure to the sample. First, each vegetable was homogenized using a blender and then frozen. The frozen homogeneous vegetables were thawed just before sample preparation. Each vegetable was weighed as much as 1 g, was added 10 mL of methanol, and then was sonicated for 15 min. The methanol extract was centrifuged at 4000 rpm for 5 min. Subsequently, 4 mL of sodium carbonate solution and a 2 mL aliquot of methanol extract were combined, and the combination was heated to 80°C for an hour. Four milliliters of n-hexane was added after the heating process, and the mixture was vortexed for 2 min before being centrifuged for 10 min at 5700 rpm. Next, the n-hexane top layer was separated. This extraction process with n-hexane was carried out three times until the n-hexane phase was approximately 12 mL. Ultimately, at 35°C, the n-hexane phase evaporated to dryness under nitrogen flow. For the samples of cabbage, napa cabbage, and lettuce, each residue was dissolved in 70 µL of methanol, whereas for the samples of spinach, it was dissolved in 150 µL.

Analysis of Vitamin K1 levels

Each prepared vegetable sample was vortexed for 1 min and injected into HPLC. Vitamin K1 levels in each vegetable sample were calculated by substituting the peak area (AUC) value on the sample chromatogram into the calibration curve equation as the y-value so that the x-value as the concentration (ppm) was obtained. The level in ppm unit was converted to µg/100 g sample unit. A difference test was also carried out on Vitamin K1 levels in samples from 3 main markets. First, the Shapiro–Wilk test was used to perform the normality test. One-way ANOVA was then used to perform the difference test if the data were normally distributed. In the meantime, Kruskal–Wallis was used to perform the difference test when the data were not normally distributed. $P<0.05$ was considered statistically significant.

RESULTS

Optimization of high-performance liquid chromatography condition

The results of the analytical method using 100% methanol with 0.6 mL/min as the flow rate obtained the retention time of 23.123 min, the number of theoretical plates of 3712.5235, the capacity factor of 4.9858, the resolution of 15.6132, and the symmetry factor of 1.2179. Meanwhile, when using 100% methanol with 1 mL/min as the flow rate, the retention time was at 12.945 min, the number of theoretical plates was 1216.004, the capacity factor was 4.8232, the resolution was 8.9844, and the symmetry factor was 0.8153. Furthermore, a fronting peak was observed at a flow rate of 1 mL/min [Figure 1].

Analysis of Vitamin K1 levels

The highest Vitamin K1 levels can be found in spinach with the range of 305.2868 ± 3.3058–970.7098 ± 14.1167 µg/100 g, followed by napa cabbage with the range of 56.7445 ± 0.1569–273.2828 ± 8.3061 µg/100 g, cabbage with the range of 27.9531 ± 1.7487–105.1026 ± 0.6855 µg/100 g, and lettuce with the range of 38.4391 ± 15.2650–64.4419 ± 19.0315 µg/100 g.

Table 1: Results of Vitamin K_1 levels in samples from 3 main markets in Bandung

Vegetable type	Vitamin K_1 (µg/100 g)	Source area
Spinach		
Gedebage Main Market	970.7098±14.1167	Tanjungsari
Caringin Main Market	305.2868±3.3058	Ciwidey
Ciroyom Main Market	450.6329±18.2195	Cimahi
Lettuce		
Gedebage Main Market	58.9659±2.7672	Majalaya
Caringin Main Market	64.4419±19.0315	Ciwidey
Ciroyom Main Market	38.4391±15.2650	Cihideung
Cabbage		
Gedebage Main Market	27.9531±1.7487	Cipacet
Caringin Main Market	217.0457±7.2201	Ciwidey
Ciroyom Main Market	105.1026±0.6855	Pangalengan
Napa cabbage		
Gedebage Main Market	56.7445±0.1569	Cipacet
Caringin Main Market	80.5650±0.7635	Ciwidey
Ciroyom Main Market	273.2828±8.3061	Pangalengan

Mean±SD. SD: Standard deviation

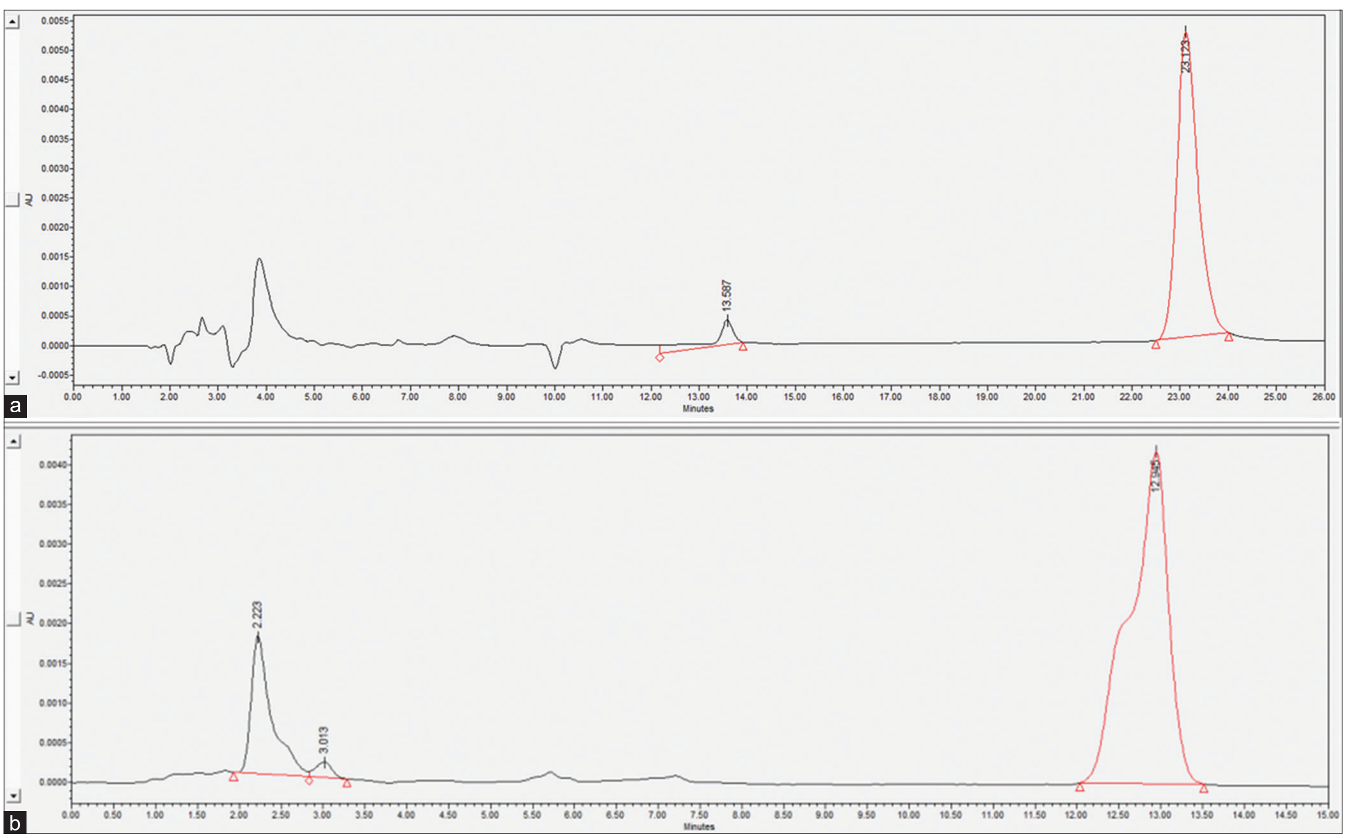

Figure 1: Chromatograms for Vitamin K1 standard in the following conditions: (a) 100% methanol at 0.6 mL/min and (b) 100% methanol at 1 mL/min

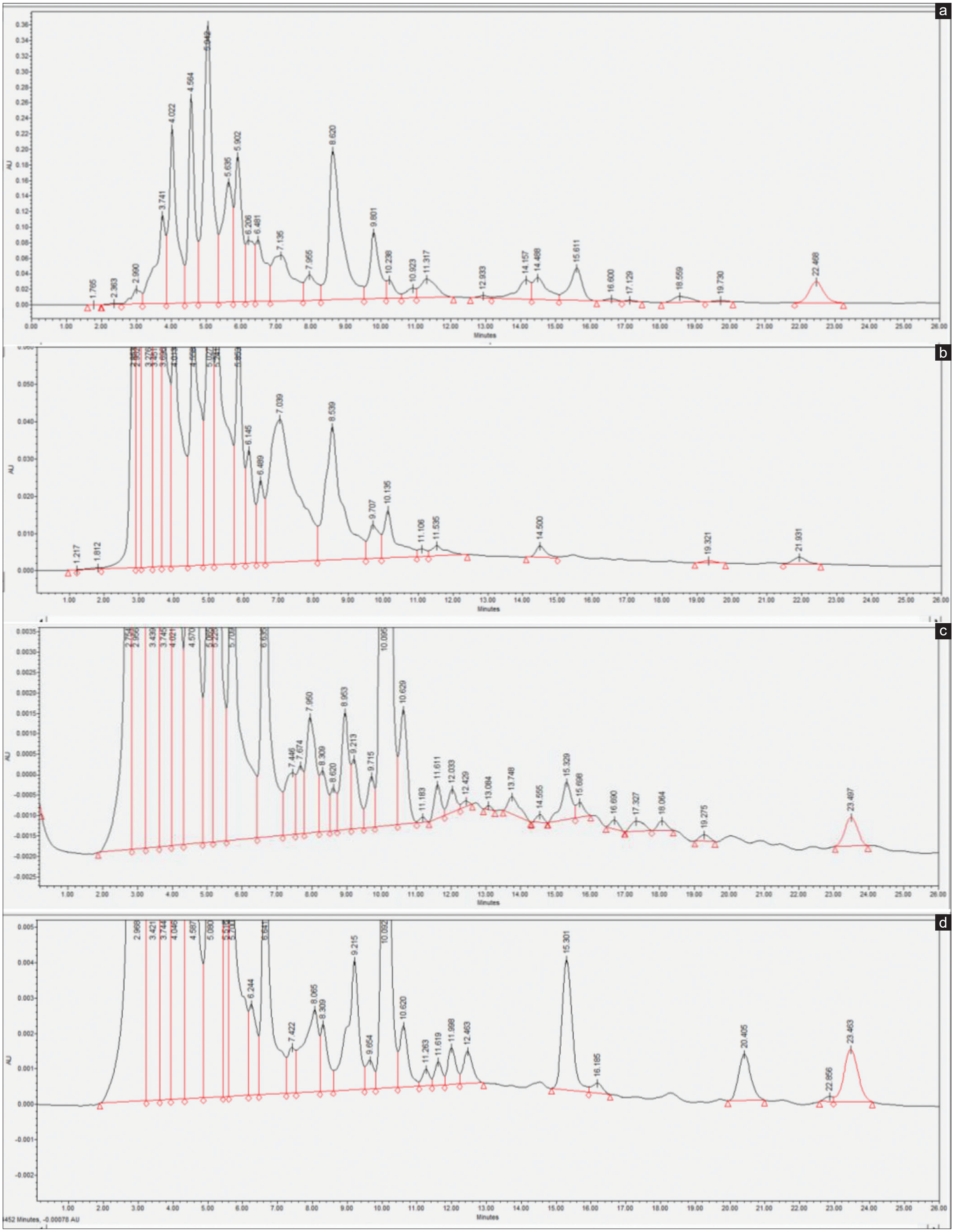

Figure 2: Sample chromatograms of (a) spinach, (b) lettuce, (c) cabbage, (d) napa cabbage

Table 1 lists the levels and the source areas of the vegetables, which were from the three major markets in the Bandung area: Gedebage Main Market, Caringin Main Market, and Ciroyom Main Market. Meanwhile, Figure 2 displays the sample chromatograms.

DISCUSSION

The analytical method using 100% methanol with 0.6 mL/min as the flow rate fulfilled all the requirements for system suitability test parameters, including number of theoretical plates (3712.5235 > 2000), capacity factor (4.9858; requirements: 2–10), resolution (15.6132 > 2), and symmetry factor (1.2179; requirements: 1–1.5). Meanwhile, when using 100% methanol with 1 mL/min as the flow rate, the analytical method fulfilled the capacity factor (4.8232; requirements: 2–10) and resolution (8.9844 > 2) requirements. However, it did not meet the requirements for the number of theoretical plates (1216.004 < 2000) and symmetry factor (0.8153; requirements: 1–1.5), and there was also a fronting peak. Hence, the condition of 100% methanol with 0.6 mL/min as the flow rate was chosen for analysis because this condition fulfilled all system suitability test parameters. Furthermore, this analytical method was simpler and quite sensitive to calculate Vitamin K1 levels, compared to existing methods that use LC-MS/MS.[18,19]

Table 1 shows that spinach, lettuce, cabbage, and napa cabbage from three main markets have varying Vitamin K1 levels. The source area of these vegetables was different to ensure that the samples represent the commercially available vegetables. Three main markets in Bandung were chosen as sampling locations (Gedebage Main Market, Caringin Main Market, and Ciroyom Main Market); this is because main markets serve as the primary distribution centers for retailers in various locations close to consumers.

Vitamin K1 levels vary for each type of vegetable; even for the same type of vegetable, there are variations in Vitamin K1 levels. Vitamin K1 levels for each type of vegetable from three main markets were statistically tested with a different test to ascertain whether there was a significant difference. The Shapiro–Wilk test showed that the Vitamin K1 levels of lettuce and cabbage were normally distributed ($P > 0.05$). In contrast, the Vitamin K1 levels of spinach and napa cabbage were not normally distributed ($P < 0.05$). Furthermore, according to the results of one-way ANOVA, cabbage from different main markets had significantly different levels of Vitamin K1 ($P < 0.05$). At the same time, lettuce did not differ significantly ($P > 0.05$). Meanwhile, according to the result of the Kruskal–Wallis test, the levels of Vitamin K1 in spinach and napa cabbage from different main markets did not differ significantly ($P > 0.05$). Although some test results were not significantly different, there were still variations. The variation of Vitamin K1 levels in vegetables from three main markets follows the theory that growing conditions, including differences in geographic location, cause varying Vitamin K1 levels in vegetables.[8,20] Based on the secondary data obtained, which was information about the source area of each vegetable purchased from traders, each type from three different main markets also has a different source area. In addition to geographical location differences, because of their sensitive nature to daylight and fluorescent light, other factors that can cause variations in Vitamin K1 levels in vegetables include the amount of sunlight and also climates such as temperature, soil, and irrigation.[12,14,21]

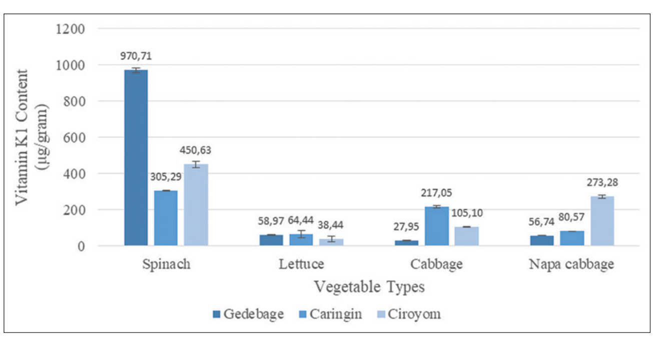

Figure 3: Vitamin K1 levels in analyzed vegetables

The ranges of Vitamin K1 levels from various literature collected are: lettuce, 10.3–519 µg/100 g; cabbage, 28.6–618 µg/100 g; napa cabbage, 32.6–250 µg/100 g; and spinach, 244.7–1001 µg/100 g.[8,11-13,22] Based on Figure 3, we can deduce that out of the four vegetables determined in the Bandung area, spinach had the greatest levels of Vitamin K1, ranging from 305.2868 ± 3.3058 µg/100 g to 970.7098 ± 14.1167 µg/100 g. These results follow the research from Beulens *et al*. and Booth who stated that dark green vegetables contain a higher Vitamin K1 level. In addition, the results align with the determination of lettuce, which has a paler green color than spinach and lower Vitamin K1 level ranging from 38.4391 ± 15.2650 µg/100 g to 64.4419 ± 19.0315 µg/100 g.[23,24] However, in this study, we found that napa cabbage contained Vitamin K1 which ranged between 56.7445 ± 0.1569 µg/100 g and 273.2828 ± 8.3061 µg/100 g, while cabbage ranged between 27.9531 ± 1.7487 µg/100 g and 217.0457 ± 7.2201 µg/100 g, where the Vitamin K1 level was higher than lettuce. This condition was also shown in the study by Faria *et al*. It indicates that cabbage and napa cabbage, which are not green, also contain a pretty high Vitamin K1 and are higher than lettuce.[14] Therefore, Hasan Sadikin Central General Hospital Bandung patients using warfarin must consider their consumption of cabbage and napa cabbage when controlling their diet.

In contrast to other research conducted in other nations, such as Australia[8] and Brazil,[14] Vitamin K1 levels in several vegetables varied. According to this study, spinach had a greater Vitamin K1 level than it did in Australia, which ranged between 244.7 and 286.2 µg/100 g, and higher than in Brazil, which ranged between 316 and 423 µg/100 g. Meanwhile, Vitamin K1 levels in lettuce were higher than in Australia, which ranged between 10.3 and 43.2 μg/100 g, and lower than in Brazil, which ranged between 86 and 147 μg/100 g. Next, Vitamin K1 levels in cabbage in this study were higher than in Australia, which ranged between 28.6 and 94.4 μg/100 g, and lower than in Brazil, which ranged between 319 and 353 μg/100 g. There was no data on Vitamin K1 level for napa cabbage in Australia and Brazil. However, in a study by Arianto (2014), the Vitamin K level of napa cabbage from a market in Surabaya was 326 μg/100 g, whereas the results in this study were higher.[22]

A study by Kim *et al*. indicates that there was a relationship between Vitamin K intake from food and the stability of anticoagulation response in patients undergoing long-term warfarin therapy. The study found that patients with higher Vitamin K intake with a certain amount (>195 μg/day) had a more stable anticoagulation response, as indicated by a smaller coefficient of variation of INR.[25] Similarly, research by Kim *et al*. showed that warfarin patients with lower Vitamin K levels in their bodies had a more unstable anticoagulation response, as indicated by more frequent PT-INR checks.[7] Regularly taking a certain amount of Vitamin K may help maintain the stability of warfarin's anticoagulation response.

The data on Vitamin K1 level in several types of vegetables obtained from this study are expected to provide valuable information for healthcare professionals, such as dietitian, to estimate Vitamin K intake and manage stable dietary patterns in order to help maintain the stability of warfarin's anticoagulant effect for warfarin-used patients.

CONCLUSION

All four types of vegetables – spinach, lettuce, cabbage, and napa cabbage – from the three main markets in Bandung were found to have Vitamin K1. In our investigation, we discovered that lettuce had the lowest Vitamin K1 levels while spinach had the greatest. These findings may help warfarin-used patients to choose vegetables consumed so that the warfarin's anticoagulant effect is not influenced.

Authors' contributions

All the authors have contributed equally.

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Conflicts of interest

There are no conflicts of interest.

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