Potentiality of ethanol curcumin extract as biosensor for detection of sodium tetraborate

A Wulandari¹, T C Sunarti¹ and F Fahma¹

¹Study Program of Agroindustrial Technology, Department of Agroindustrial Technology, Bogor Agricultural University, Indonesia

e-mail : titi-cs@apps.ipb.ac.id

Abstract. Sodium tetraborate in foods is very dangerous and fatal if consumed continuously even if only in small quantities. Because the dangers of sodium tetraborate in food products for health, it is necessary to detect this chemical. Bioactives from local resource such as curcumin are natural ingredients that produce color with certain specificity. Curcumin is extracted from turmeric (Curcuma longa L.) in ethanol crude extract. The change color of curcumin against certain chemical that contain boron make this bioactive potential to be developed as biosensor to detect sodium tetraborate. The result showed that the curcumin had visual LOD of 50 ppm in the range 50-500 ppm of sodium tetraborate. The curcumin detection time for sodium tetraborate was 1-2 minutes. Curcumin has good selectivity against sodium tetraborate, its performance was not interfered with the presence of other chemicals (sodium benzoate, sodium nitrite, formalin, and sodium cyclamate). The selectivity and sensitivity of curcumin to sodium tetraborate are the basic information in the development of biosensor. Therefore, curcumin is highly potential to be developed as a biosensor to detect sodium tetraborate in food products.

1. Introduction
Safe, high quality and nutritious food is very important for growth, maintenance and improvement of health status and improvement of community intelligence [1]. Therefore, nutritious food, safe to consume, and have good quality become important requirements that must be fulfilled before consumed by society so that the body stays healthy. Along with the increase of science and technology, the process of food processing becomes vary. In general, the food processing always attempts to get a product that has good quality, good taste, and attractive colors to be favored by many consumers. Therefore, certain additional ingredients are often added. But for the ignorance and indifference of certain traders, they often add harmful chemicals to food products like sodium tetraborate. Based on the annual report of NADFC [2], sodium tetraborate is found on many food products such as noodles, meatballs, and tofu. Sodium tetraborate in foods is very dangerous and fatal if consumed continuously even if only in small quantities. Boric acid can decrease metabolic

* Corresponding author, E-mail address : titi-cs@apps.ipb.ac.id
concentrations such as glucose, glycogen, and lactate related with the formation of boron and hydroxy complexes. It can cause poisoning if it reaches 2g kg\(^{-1}\) in liver and brain tissue, and is lethal if it exceeds 5g kg\(^{-1}\) in adults and 3g kg\(^{-1}\) in neonates [3]. Because the dangers of sodium tetraborate in food products for health, it is necessary to detect this chemical. Common methods for detection of sodium tetraborate include spectrophotometric method [4,5], titimetric method [6], and flame test method [7]. However, these methods have limitations such as multi-stage processes, requiring specialized experts, can only be used on a laboratory scale, time-consuming, require sophisticated, complicated, and relatively expensive equipment [8,9], so it is not suitable for daily analysis. Thus, a new method for detecting sodium tetraborate in a simple, fast, cheap, and easy to use is needed.

Colorimetric biosensor, a method often used for this purpose, is easy to use, inexpensive, and can be applied on site [10]. The diversity of natural plant resources in Indonesia is a good source for dye and bioactive compounds that will offer the opportunity to become a biosensor with unique specificity and sensitivity. In this study, biosensor for sodium tetraborate detection was developed from bioactive of curcumin from turmeric (Curcuma longa L). Curcumin is a yellow-orange natural dye compound found in turmeric (Curcuma longa (L)) or other curcuma compounds [11]. Curcumin was widely used as a colorimetric biosensor for heavy metal detectors such as lead [12], iron [13], fluorides [14], and measurements of boron concentrations [15]. The ability of curcumin for measurement of boron concentration in seawater allows it to be used as a biosensor in detecting the presence of boron elements in food products such as sodium tetraborate. Thus, the purpose of this study is to investigate the potential of curcumin as a biosensor to detect sodium tetraborate in food products based on the color change.

2. Materials and methods

2.1. Materials
Turmeric (Curcuma longa), ethanol, aquadest, formalin, sodium tetraborate, sodium nitrite, sodium cyclamate, sodium benzoate, and urea. The equipment used include UV-VIS spectrophotometer (Thermo Scientific, Genesys 10S), vacuum filter (Value, 2 Stage Vacuum Pump, VE 2100 N), Whatman filter paper No. 41, filter fabric, analytical scale (Sartorius BL 2105), centrifuge (IEC Clinical Centrifuge, USA), water bath and magnetic stirrer (Lab tech Multi-Position), blender, blower (Memmert), and other glassware used for analysis.

2.2. Extraction of curcumin from turmeric.
A total of 100 grams of turmeric powder added to 70% ethanol solvent by 700 mL (material: solvent = 1: 7 (w / v)), then macerated for 2 days with stirring speed of 400 rpm at room temperature. Then the macerated solution was filtered with filter fabric. The resulting filtrate was filtered again with Whatman filter paper No. 41 using a vacuum filter. The filtrate was further heated with a water bath at 50 °C to obtain a concentrated colored curcumin filtrate. The filtrate was centrifuged at 3000 rpm for 15 minutes to separate the remaining solids. Then, the centrifugation supernatant was stored in a dark colored glass bottle in the refrigerator then analyzed further [16,17].

2.3. Sensitivity examination of curcumin against sodium tetraborate
The sensitivity test was performed by reacting curcumin with sodium tetraborate with various concentrations of sodium tetraborate of 10, 25, 50, 100, 250, 500, 1 000, 2 500, 5 000, and 10 000 ppm (curcumin: sodium tetraborate = 1:4 (v/v)). After ± 30 min, the color change was observed with the digital camera and the absorbance was measured by a UV-VIS spectrophotometer in the range 280-800 nm.

2.4. Selectivity examination curcumin against chemicals
Curcumin extract in each test tube was added with chemicals commonly found in food products (sodium benzoate, sodium nitrite, sodium cyclamate, urea residue, formalin, and sodium tetraborate) at
a concentration of 1%. The ratio of curcumin: analytical chemical was 1:4 (v/v). After ± 30 min, the color changes and spectrum characteristics were observed using the UV-VIS spectrophotometer in the range 280-800 nm.

3. Results and discussions

3.1. Sensitivity of curcumin to sodium tetraborate

The sensitivity of curcumin to sodium tetraborate (borax) was tested at various borax concentrations of 10, 25, 50, 100, 250, 500, 1000, 2500, 5000, and 10000 ppm. The sensitivity test result is shown in Figure 1. Figure 1 showed the change of curcumin color from yellow to orange to brown from sodium tetraborate concentration of 10-10000 ppm. Based on Figure 1, visually, with the naked eye method, limits of detection (LOD) of curcumin biosensor to detect the presence of sodium tetraborate was 50 ppm. It occurred because the color change of curcumin from yellow to orange starting at a concentration of sodium tetraborate of 50 ppm (Figure 1).

Color changes occurred in different times for each concentration. The color change of curcumin from yellow to orange-brown became faster along increasing concentrations of sodium tetraborate. The response time of color change curcumin reacting with sodium tetraborate was about 1-2 minutes. The spectrum of curcumin sensitivity against sodium tetraborate from various concentrations has also been observed using a UV-VIS spectrophotometer in the wavelength range of 280-620 nm. The result of absorbance of curcumin against sodium tetraborate at various concentrations can be seen in Figure 2. The results showed that the maximum absorbance of curcumin-sodium tetraborate mixture with sodium tetraborate concentration 0-100 ppm occurred at $\lambda$ 360 nm and $\lambda$ 420 nm, whereas at the sodium tetraborate concentration of 250-10000 ppm, the maximum absorbance occurred at $\lambda$ 420 nm (Figure 2).

![Figure 1. The Change of curcumin to sodium tetraborate (0-10000 ppm).](image)

The appearance of multiple shoulder peaks (other small peaks besides the main peaks) is common in many conjugate systems, and often depends heavily on the type of solvent. There was a bathochromic shift on a mixture of curcumin-sodium tetraborate from $\lambda$ 360 nm (sodium tetraborate concentration of 0-100 ppm) to $\lambda$ 420 nm (sodium tetraborate concentration of 250-100000 ppm). This wavelength shift indicates a change in curcumin structure that it causes the color change from yellow to orange-brown. This shift indicates the occurrence of electron transition to the higher energy. The existence of conjugation generally leads to a bathochromic and hypochromic shift in its absorbance.
Figure 2. The Spectra of mixture curcumin-sodium tetraborate with concentration of sodium tetraborate of 10-10000 ppm (a), 50-500 ppm (C) at λ 420 nm.

In addition to the bathochromic shift, this study also tends to experience a maximum absorbance shift (hypochromic shift) in the mix of curcumin-sodium tetraborate from concentrations of 0 to 100 ppm at λ 360 nm and λ 420 nm. This hypochromic shift indicated a decrease in curcumin color intensity. It was indicated by decreased absorbance as the sodium tetraborate concentration increased (Figure 3). Figure 3 (a) showed a tendency the decrease of absorbance peak (hypochromic shift) of curcumin-sodium tetraborate mixture along with increasing sodium tetraborate concentrations at a maximum wavelength of 360 nm and 420 nm from a concentration of sodium tetraborate of 0-100 ppm. The decrease of absorbance of curcumin-sodium tetraborate also occurred at a sodium tetraborate concentration of 250 ppm-10 000 ppm at λ 420 nm that were shown in Figure 3 (b). These decreases of absorbance indicated that the curcumin color faded from yellow to orange to brown. This was due to the presence of sodium tetraborate. Raj and Dhesingh [12] also reported that there was a decrease of absorbance of nanofiber curcumin along with an increase of lead concentration from 10-100 μM at a wavelength of 456 nm.
Figure 3. The spectra of curcumin against sodium tetraborate at various concentrations of 0-100 ppm (a) and 250-10,000 ppm (b) at λ 280-620 nm.

The wavelength of 360 nm is an area of ultraviolet light that can absorb the invisible colors, while at λ 420 nm is a light area that can absorb visible colors. With the latter wavelength, the light can be reflected as the yellow color to appear to our eyes, so the reddish brown color due to the presence of sodium tetraborate at these concentrations would not be reflected well by visible light from the spectrophotometer at that wavelength so that it resulted the decrease of absorbance.

3.2. Analytical performance of curcumin sensitivity to sodium tetraborate

The analytical performance test of curcumin sensor analysis against sodium tetraborate can be seen in Figure 4. Figure 4a shows the curcumin-sodium tetraborate spectra with a sodium tetraborate of 0-10000 ppm at λ 420 nm. There was an increase in absorbance as the sodium tetraborate concentration increased at the certain point until it was constant that it was shown in Figure 4a. The curve was linear in sodium tetraborate concentration of 50-500 ppm and it curved at > 500 ppm.
Figure 4  The spectra of curcumin against sodium tetraborate of 10-10 000 ppm (a), 50-500 ppm (b) at λ 420 nm.

The curcumin-sodium tetraborate complex absorbance curve follows Lambert Beer’s law stating that lights absorbed or transmitted by a solution are an exponential function of the concentration of matter and the thickness of the solution. The absorbance of a solution with a certain thickness and concentration will get a straight line in the area where Lambert-Beer’s law applies [18,19]. Thus, in an increase in concentration will result in the phenomenon of the Beer Lambert law where the absorbance curve warp at higher concentration area. It happened in this study. At the high concentration of sodium tetraborate (> 500 ppm), the absorbance curve started to curve to form a constant curve because the curcumin color changed to deep brown. An analytical method has good sensitivity and accuracy when
it results a linear curve with a coefficient of determination > 0.997 or close to 1 [20]. In this research, the complex curcumin-sodium tetraborate absorbance curve with good linearity was generated in the sodium tetraborate concentration range 50-500 ppm at $\lambda_{\text{max}}$ 420 (Figure 4b). This curve resulted a linear equation $y = 0.0001x + 1.0236$ with correlation coefficient 0.9815. Therefore, the curcumin biosensor for sodium tetraborate had good sensitivity and accuracy in the range concentration of sodium tetraborate of 50-500 ppm.

3.3. Selectivity of curcumin against sodium tetraborate

The selectivity of the curcumin biosensor against sodium tetraborate was also evaluated by various other chemicals that were found predominantly in food products, namely sodium cyclamate, benzoate, sodium tetraborate, and formalin with concentrations of 1%. The selectivity of curcumin against sodium tetraborate was also demonstrated by its spectral characteristics against various analytical chemicals. It was analysed with UV-VIS spectrophotometers in the wavelength range of 400-620 nm.

The results showed that the maximum absorbance of curcumin, mixed curcumin, curcumin-benzoate, curcumin-formalin, and curcumin-sodium tetraborate had maximum absorbance at $\lambda_{420}$ nm (Figure 5a).

At a wavelength of 420 nm, the light absorbed the color purple and reflected the yellow color. This corresponds to the color of curcumin, which is yellow. The absorbance results at $\lambda_{420}$ nm showed no significant color change between curcumin and chemicals such as cyclamate, nitrite, benzoate, urea, and formalin. There were decreases in curcumin color intensity at $\lambda_{420}$ nm after it reacted with cyclamate, nitrite, benzoate, and formalin. They were shown by hypochromic shifts. While the reaction of curcumin with sodium tetraborate had the highest absorbance that it was shown by hyperchromic shift at $\lambda_{420}$ nm (Figure 5a). This showed a strong interaction between curcumin and sodium tetraborate that it was indicated by the color change to brown orange. That was in accordance with research conducted by Xu et al. [21] on curcumin-based chemosensors for Cu$^{2+}$ detection.

The maximum absorption between curcumin with each chemical at 520 nm (red light reflecting wavelength) showed clearly that the mixture of curcumin-sodium tetraborate had maximum absorbance value rather than the mixture curcumin and other chemicals (Figure 5 (b)). It showed that curcumin had good selectivity against sodium tetraborate and it was not affected by the presence of other chemicals (sodium benzoate, nitrite, cyclamate, and formalin). That was in accordance with Raj and Dhesingh [12] research on lead detection with curcumin where the results showed that lead had maximum absorbance compared to other metal ions at certain maximum wavelength.
Conclusion

Curcumin has good sensitivity to sodium tetraborate with detection sensitivity in the range 50-500 ppm where the visual LOD is 50 ppm. The curcumin detection time for sodium tetraborate is 1-2 min. Curcumin has good selectivity against sodium tetraborate, its performance is not interfered with the presence of other chemicals (sodium benzoate, sodium nitrite, formalin, and sodium cyclamate). The selectivity and sensitivity of curcumin to sodium tetraborate are the basic information in the development of biosensor. Therefore, curcumin is very potential to be developed as a biosensor to detect sodium tetraborate in food products.
5. References

[1] Saparinto C and Hidayati D 2006 Bahan Tambahan Pangan (Food Additives), in Indonesian. Cetakan I (Yogyakarta: Kanisius)

[2] [NADFC] National Agency for Drug and Food Control 2016 Annual report of result of security and quality supervision food products [in Indonesian]. http://www.pom.go.id/new/admin/dat/20171127/laptah2016.pdf [2018-01-07]

[3] See A W, Salleh A B, Fatimah A B, Yusof N A, Abdulamir A S and Heng L Y 2010 Risk and health effect of boric acid Am. J. Applied Sci. 7(5) 620-627

[4] Grotheer E W 1979 Spectrophotometric determination of boric acid in boron powder with curcumin Anal. Chem. 51 (14)

[5] Horwitz W 2015 Official Methods of Analysis of AOAC Internationanal 18th edition Volume 1 Agricultural Chemical USA Chapter 47 pp.13-14

[6] Williams S 1984 Official Methods of Analysis. 14th edition. Association of Official Analytical Chemists. Arlington. pp 379-381

[7] Roth H J and Blaschke G 1988 Analisis Farmasi (Pharmacy Analysis) Sarjono Kisman, Slamet Ibrahim, Translator (in Indonesian) (Yogyakarta: Gadjah Mada University Press) pp 430–431, 482–493

[8] Nash T 1953 The colorimetric estimation of formaldehyde by means of the Hantzschreaction Biocheml. J. 55 416–421

[9] Chiou J, Arthur H H L, Hang W L and Wing-tak W 2015 Rapid testing methods for food contaminants and toxicants J.Integr.Agric. 14(11) 2243–2264

[10] Kaur N and Kumar S 2011 Colorimetric metal ion sensors Tetrahedron. 67 9233–9264.

[11] Sharma R A, Gescher A J and Steward W P 2005 Curcumin: the story so far Eur J Cancer. 41 1955–1968

[12] Raj S and Dhesingh R S 2016 Curcumin based biocompatible nanofibers for lead ion detection Sens. Actuators B. 226 318-25

[13] Saithongdee A, Narong P and Apichat I 2014 Electrospin curcumin-loaded zein membrane for iron (III) ions sensing Sens. Actuators B. 202 935–940

[14] Wu F Y, Sun M Z, Xiang Y L, Wu Y M and Tong D Q 2010 Curcumin as a colorimetric and fluorescent chemosensor for selective recognition of fluoride ion J Lumin. 130 304–308

[15] Liu Y-M and Lee K 2009 Modifications of the curcumin method enabling precise and accurate measurement of seawater boron concentration Marine Chem. 115:110–117

[16] Setyowati A and Suryani C L 2013 Peningkatan kadar kurkuminoid dan aktivitas antioksidan minuman instan temulawak dan kunyit (Increased levels of curcuminoids and antioxidant activity of ginger and turmeric instant drinks, in Indonesian) J Teknol Pert Agritech. 33 (4) 363-370

[17] Waghmare P, Dheeraj P and Pramod K 2015 Extraction, isolation, purification and identification of curcumin: a review article. Eur J Biomedic Pharm Sci. 2(3) 108-123.

[18] Pecsok R L, Shileds L D, Cairns T and Mcwilliam I G 1976 Modern Methods of Chemical Analysis 2nd ed (New York: John Wiley & Sons, Inc)

[19] Skoog D A and West D M 1971 Principles of Instrumental Analysis (New York: Holt, Rinehart and Winston, Inc)

[20] Chan C C, Lam H, Lee Y C and Zhang X 2004 Analytical Method Validationand Instrumental Perfornment Verification. (New Jerse: John Wiley and Sons. Inc. Publication) pp 1-3

[21] Xu G, Wang J, Si G, Wang M, Xue X, Wu B and Zhou S 2016 A novel highly selective chemosensor based on curcumin for detection of Cu^{2+} and its application for bioimaging. Sens. Actuators B. 230 684–689
Acknowledgements
This research specially thank to the Ministry of Research, Technology and Higher Education of the Republic of Indonesia for the funding and support to this research (Hibah PMSDU Contract no. 1105/IT3.11/LT/2017).