Inquiry-based laboratory practice enzyme kinetics to improve students’ critical thinking ability

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Abstract. The purpose of this study was to identify the effects of practicum using inquiry-based laboratories of enzyme kinetics on local material to improve students’ critical thinking ability. The inquiry-based laboratory practice of enzyme kinetics has four stages to keep the students’ effort on track: pre-lab activities, an in-lab discussion, in-lab work and a post-lab assignment. This study used a quasi-experimental method, with single group pretest-posttest design. The subjects of this study involve 55 students enrolled in biochemistry practicum course from the sixth semester of chemistry study program. The critical thinking ability test instrument consist of 12 reasonable multiple choice question that includes two indicators of critical thinking ability i.e. basic support and inference and also supported by student worksheet. Data analysis of students’ score test using t-test. There was a statistically significant improvement of students’ critical thinking skills (symp.sig 0.000). Furthermore to see the increase in each students’ critical thinking indicator, N-gain is calculated. Based on implementation of the inquiry-based laboratory, it is reported that students’ critical thinking skills improved in medium category and also for students’ critical thinking indicator of ‘inference’. But in the students’ critical thinking indicator of ‘basic support’ they get high category.

1. Introduction
Laboratory learning is very important for students. This can be achieved through laboratory experience, including: increasing mastery of lecture material, developing scientific practice skills, developing scientific reasoning, fostering interest in science, developing teamwork skills, and understanding how scientific research is carried out [1].

However, most of the practicum activities are used traditional laboratories. There students only follow the steps or procedures in all the practical guide and student worksheets. This resulted in traditional laboratories giving less involvement of students in scientific practice and not giving students the opportunity to think critically [2].

Some skills are needed by students can only be achieved through laboratory experience. Inquiry based laboratories provide opportunities for students to be actively involved in practical activities, starting from: formulating titles, formulating problem formulations, making hypotheses, analyzing data and drawing conclusions. There activities were one way to make the student laboratory experience more meaningful [3]. Several studies have reported that inquiry-based laboratories have been widely used to improve problem-solving abilities, flexible thinking abilities, and the ability to make decisions based on available evidence [4]. In addition, inquiry-based laboratories have also been used to improve critical thinking skills [5], communication skills [6], mastery of concepts [1], increase self-confidence [7], analytical skills [8], and improve academic achievement [9].
The inquiry-based laboratory includes several stages that must be followed by students including: 1) developing knowledge, 2) formulating problems, 3) formulating hypotheses, 4) collecting data, 4) interpreting data, and 5) making conclusions. The inquiry-based laboratory design that using in this research is the design includes pre-lab activities, lab discussions, lab work and post-lab assignments. The inquiry-based laboratory practicum environment emphasizes the use of critical thinking and science process skills compared to memorizing concepts [10]. With the inquiry-based laboratory students are not guided to carry out step-by-step instructions, but students are given the opportunity to understand scientific concepts, improve science process skills and improve problem-solving skills. Thus, inquiry-based laboratory considered to have a positive impact on improving higher order thinking skills [11].

Critical thinking involves rational assessment and differentiation of the elements of reasoning. In contrast to memorizing or remembering simple information, methods to encourage critical thinking have a purpose to stimulate the analytical and evaluative processes of the mind [12]. Critical thinking indicators that will be use in this study were adapted from Ennis included, 1) elementary clarification, 2) basic support, 3) inference, 4) advanced clarification, and 5) strategy and tactics [13]. Critical thinking refers to student activities where they can develop knowledge and understanding of scientific ideas and how scientists study nature [5].

One of the experiments conducted by students in biochemical lectures is the experiment in the form of biochemical practicum is a compulsory subject in chemistry study programs. Biochemical practicum, especially on practicum enzymes, uses enzyme sources originating from the surrounding environment or commonly known as local materials [14]. Local materials that contain the enzyme polyphenol oxidase (PPO), including: apples, bananas, eggplants, potatoes, pears, rambutan, and sweet potatoes [15,16]. Previous study that has been carried out including: Amida’s research that uses PPO enzyme sources from three types of eggplants (eggplant round, eggplant purple and eggplant green). Research conducted to improve creative thinking skills and generic science skills. Students’ generic science and creative thinking skills are measured using essay test question and supported by student worksheets [17]. In addition, Wahyudi's research used PPO enzyme sources from three potato varieties. The study was conducted to see an increase students’ creative thinking skill by using two research design [18]. However, the use of PPO enzyme sources derived from bamboo shoots is still not widely done. So that researchers will use the source of PPO enzymes derived from bamboo shoots.

2. Method

2.1. Research Methods and Design
This study was used a quasi-experimental research method with single-group pretest-posttest design. This design was used because the subject may not be chosen randomly, researchers use it as a whole, the subject group has been determined, for the subject to be given a pretest first, giving treatment to one group and given a posttest [19]. This research uses only one student class.

2.2. Research Locations and Subjects
This research was conducted at the Organic Chemistry and Biochemistry Laboratory (LKOB), a state university in Bandung, West Java. The research subjects were even semester students of chemistry study programs enrolled in biochemical practicum courses consisting of 55 students.

2.3. Research Flow
The research procedure was carried out through three stages, namely 1) the stage of research preparation, 2) the stage of research implementation, and 3) the final stage. Activities at the implementation stage of the research consisted of: biochemical practicum syllabus study of enzyme kinetics, inquiry-based laboratory studies, material studies and optimization (preliminary research) PPO enzyme practicum, and indicators of critical thinking abilities to be achieved in the syllabus, making research instruments (test questions and students’ worksheet) and instrument validation. Activities at the implementation stage of the research consisted of: providing pretest, answering student worksheet (LKM) and giving posttest. While the activities at the final stage include: collecting research data, data analysis and discussion of research findings.
This research was conducted using Google Forms of a state university in Bandung, West Java. Students who take part in this program enrolled in biochemical practicum courses. The research carried out through online media because of the Covid-19 pandemic. This research follows the critical thinking indicators developed by Ennis. However, in this study only focus on two indicators namely basic support and inference

2.4. Data analysis technique
Processing of pretest and posttest data through normality test, homogeneity test and significance test. Data analysis performed with the help of SPSS 20.0 software for windows. The normality data using the Kolmogorov-Smirnov test, while the homogeneity test carried out with the Levene statistics test. Finally the significance test using the Wilcoxon test.

3. Result and Discussion
Preliminary research has been conducted to determine the optimum conditions of PPO enzyme activity. PPO enzyme sources that are used come from two types of bamboo shoots, namely yellow bamboo shoots and green bamboo shoots. The optimum conditions determined in the form of enzyme specifications, optimum temperature, optimum pH and the best inhibitors. Preliminary research results showed that PPO enzyme activity was highest in catechol substrate, temperature 37 °C, pH 7 and the least activity in lemon juice inhibitor. This is in accordance with Wahyudi's research, in which the optimum results obtained for PPO enzymes from potatoes are catechol as a substrate, temperature of 35 °C, pH 7 and EDTA as inhibitors [18]. And then, Eidhin’s research optimum conditions for PPO enzyme activity from potato sources are at the temperature of 30 °C, a range of pH 6-7.5 and ascorbic acid as the most effective inhibitor [20]. In addition, Dziki’s research where the optimum PPO enzyme conditions from broccoli has an optimum catechol substrate with a pH of 5.7 and sodium sulfate as an inhibitor [21].

Based on the results of normality and homogeneity test, it was found that the pretest and posttest data did not meet the parametric statistical test requirements. Then, the hypothesis test conducted was a nonparametric statistical test, in this case was used the Wilcoxon test. Wilcoxon test results can be seen in Table 1.

Table 1. The results of statistical tests of critical thinking ability

| Indicators            | Asymp. Sig. | Conclusion       |
|-----------------------|-------------|------------------|
| Critical thinking ability | 0.000       | significantly different |
| Basic support         | 0.000       | significantly different |
| Inference             | 0.000       | significantly different |

Based on the results of hypothesis testing using the Wilcoxon test it found that the value of sig. 0.000 then H₀ rejected. That is, there is a significant difference between the results of the pretest and posttest on critical thinking ability of students after the inquiry-based laboratory practicum. In addition, for ‘basic support’ critical thinking indicator also obtained sig values. <0.05 then H₀ was rejected [19]. That is, there is a significant difference between the results of the pretest and posttest for basic support indicators of student after the inquiry-based laboratory practicum. The results of this study were in line with research conducted by Weaver’s research, show that the inquiry-based laboratory learning in organic compound synthesis courses conducted through three stages of learning: 1) literature-based proposals, 2) experimentation and data analysis, 3) communication of research results. The results showed that the increased ability of critical thinking and problem solving students [9].

The assessment results were support by the answer of students’ worksheet. Among them students able to reveal the optimum conditions for PPO enzyme activity based on the intensity of colour changes and quantitatively from the maximum absorbance results, it was support by several other research [1,2,3,17,18].

This is also the case with the ‘inference’ critical thinking indicator, where the value of sig. <0.05. There is a significantly different between the results of the pretest and posttest for student inference
indicators after the inquiry-based laboratory practicum. This research was in accordance with previous research, among them: the inquiry laboratory design on biochemistry practicum shows that 30% was a statistically significant improvement in students' critical thinking ability. The inquiry laboratory design on biochemistry practicum, consists of pre-lab activities, lab discussion, practicum and post-lab assignments [10].

This result also supported by students’ worksheet assessment data. The inference indicators assessed on students’ worksheet are based on students’ ability to determine suitable substrates used for PPO enzymes, the right method to obtain PPO enzyme extracts, and are able to reveal that storage of enzyme extracts at low temperatures and dark reagent bottles to prevent PPO enzymes from oxidizing [5,6,9,10,11,13].

This research inquiry-based laboratory practicum conducted online due to the middle of the Covid-19 pandemic. Practicum activities consist of pre-lab activities, lab discussions, practicum and post-lab assignments. Pre-lab activities in this case the provision of pre-tests carried out twice, namely for the qualitative analysis pretest (consisting of eleven questions in 40 minutes) and quantitative analysis (consisting of one question within ten minutes). Discussion in the lab is carried out by working on an students’ worksheet in discussion consisting of 4-5 people in one group. The work of the students’ worksheet carried out for two weeks. This discussion activity includes the determination of practical goals, problem formulation, hypothesis and optimum conditions of PPO enzymes [14,17,18]. Here students are not directly done the practicum activities, but providing preliminary research data that has been done by researchers. The last stage, the post-lab assignment in this case by administering the posttest conducted in the last week. Students’ postings administered via Google form for 50 minutes. The posttest questions given were twelve questions and the questions were the same as the pretest questions.

Next, the results of the N-Gain calculation for critical thinking skills carried out to see the increase in students' critical thinking ability in Table 2.

| Data               | Question          | N-gain | Categorization |
|--------------------|-------------------|--------|----------------|
| Critical thinking ability | 1-12              | 0.62   | Medium         |
| Basic support      | 2,4,6,7,8,10      | 0.87   | High           |
| Inference          | 1,3,5,9,11,12     | 0.33   | Medium         |

Based on table 4 the overall critical thinking ability of students has increased in the medium category. This evidenced by the acquisition of an N-gain value of 0.62. Furthermore, for the inference indicator also increased with a medium category. However, basic support indicator increased with high category. This is in line with research conducted that biochemical practicum with inquiry-based laboratories improve students' critical thinking ability using four stage to keep the students' effort on track: pre-lab activities, an in-lab discussion, in-lab work and a post-lab assignment [10].

4. Conclusion

The inquiry-based laboratory of enzyme kinetics using local material can improve students' critical thinking ability based on the significance different of pretest-posttest data (asym.sig. 0.000). The students’ critical thinking improvement in medium category, after implementation of inquiry-based laboratory that consist of: pre-lab activities, an in-lab discussion, practicum and post-lab assignment. Students’ critical thinking indicator of ‘basic support’ reach a high category improvement. Students get ‘inference’ critical thinking indicator in medium category, although supported by the answers of students’ worksheets where students create report of the title of the practice, formulate the problem, formulate a hypothesis, analyze the data and draw conclusions.
5. References

[1] Murthy P P, Thompson M and Hungwe K 2014 Development of a semester-long, inquiry-based laboratory course in upper-level biochemistry and molecular biology Journal of Chemical Education 91 11 1909-1917

[2] Wheeler L B, Maeng J L and Whitworth B A 2015 Teaching assistants’ perceptions of a training to support an inquiry-based general chemistry laboratory course Chemistry Education Research and Practice 16 4 824-842

[3] Sesen B A and Tarhan L 2013 Inquiry-based laboratory activities in electrochemistry: High school students’ achievements and attitudes Research in Science Education 43 1 413-435

[4] Cook A L, Snow E T, Binns H and Cook P S 2015 Self-reported student confidence in troubleshooting ability increases after completion of an inquiry-based PCR practical Biochemistry and Molecular Biology Education 43 5 316-323

[5] Arias H, Lazo L and Cañas F 2014 Experimental activities in the laboratory of analytical chemistry under an inquiry approach Journal of the Chilean Chemical Society 59 4 2747-2752

[6] Gray C, Price C W, Lee C T, Dewald A H, Cline M A, McAnany C E and Mura C 2015 Known structure, unknown function: An inquiry-based undergraduate biochemistry laboratory course Biochemistry and Molecular Biology Education 43 4 245-262

[7] Pilcher L A, Riley D L, Mathabathe K C and Potgieter M 2015 An inquiry-based practical curriculum for organic chemistry as preparation for industry and postgraduate research South African Journal of Chemistry 68 236-244

[8] Ural E 2016 The effect of guided-inquiry laboratory experiments on science education students’ chemistry laboratory attitudes, anxiety and achievement Journal of Education and Training Studies 4 4 217-227

[9] Weaver M G, Samoshin A V, Lewis R B and Gainer M J 2016 Developing students’ critical thinking, problem solving, and analysis skills in an inquiry-based synthetic organic laboratory course Journal of Chemical Education 93 5 847-851

[10] Hall M L and Vardar-Ulu D 2014 An inquiry-based biochemistry laboratory structure emphasizing competency in the scientific process: A guided approach with an electronic notebook format Biochemistry and Molecular Biology Education 42 1 58-67

[11] Irwanto, Saputro A D, Rohaeti E and Prodjosantoso A K 2019 Using inquiry-based laboratory instruction to improve critical thinking and scientific process skills among preservice elementary teachers Eurasian Journal of Educational Research 19 80 151-170

[12] Zhang L and Kim S 2018 Critical thinking cultivation in chinese college english classes English Language Teaching 11 8 1-6.

[13] Tawil M and Liliasari 2013 Berpikir Kompleks dan Implementasinya dalam Pembelajaran IPA (Makassar: Badan Penerbit Universitas Negeri Makassar)

[14] Maulana I, Budio S P and Hidayat M T 2015 Pengaruh variasi dolimit material lokal kabupaten bangkalan sebagai substitusi agregat dalam pembuatan batako terhadap kuat tekan dan absorbsi Jurnal Mahasiswa Jurusan Teknik Sipil 1 3 1-4

[15] Liu S, Luo W and Huang H 2016 Characterization and behavior of composite hydrogel prepared from bamboo shoot cellulose and β-cyclodextrin International journal of biological macromolecules 89 527-534

[16] Luo Z, Wu X, Xie Y and Chen C 2012 Alleviation of chilling injury and browning of postharvest bamboo shoot by salicylic acid treatment Food Chemistry 131 2 456-461

[17] Amida N, Supriyanti F M T and Liliasari 2018 Ekspersen kinetika enzim menggunakan model inkuiri terbimbing untuk meningkatkan keterampilan berpikir kreatif mahasiswa Jurnal Pendidikan dan Ilmu Kimia 2 1 72-77

[18] Wahyudi A, Liliasari S and Supriyanti T 2019 Isolation and characterization of polyphenol oxidases (PPO) on potatoes (Solanum tuberosum) using age and environmental control Journal of Engineering Science and Technology 2 3 1-9

[19] Wiersma W and Jurs S G 2009 Research Methods in Education (USA: Pearson Education)
[20] Eidhin D N, Degn P and O’beire D 2010 Characterization of polyphenol oxidase from rooster potato (Solanumtuberosum cv rooster) *Journal of Food Biochemistry* 34 1 13-30

[21] Dziki U G, Szymanowka U and Baraniak B 2007 Characterization of polyphenol oxidase from broccoli (brassica oleracea var. botrytis italica) florets *Food Chemistry* 105 3 1047-1053