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Advanced Nanoemulsion Production on Ginger (*Zingiber officinale*) for Product Quality Improvement

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Abstract. Indonesia is one of the world largest exporters of ginger with approximately 30% increment per year. The main problem faced by the ginger oil industry in Indonesia is its inability to meet the requirements of the quality characteristics defined in the international standards. The content of zingiberene in the Indonesian ginger oil is relatively small due to the process of conventional distillation, which causes zingiberene to undergo thermal degradation. To overcome this problem, it is necessary to seek for alternative development of the suitable process in ginger oil production. Enzymatic extraction technique is a promising and prospective technology because it stimulates the phase equilibrium shift by degrading the cell wall structure of the plant so that the solute can be extracted properly. Lately, the application of nanoemulsion technology is growing very rapidly both at home and industrial scale. Ginger oil nanoemulsion products also are an innovation in which they show more stable properties and better storage capacity. Therefore, it is necessary to develop a scheme for the production process of ginger oil nanoemulsion through enzymatic extraction techniques using immobile isolated cow rumen enzymes. Experiments were conducted in an enzymatic extractor tools that consisted of a 5 liter/day capacity tank equipped with a heater, temperature indicator, pH indicator, magnetic stirrer and rotating speed indicator. Experimental results showed that the longer the homogenizer time, the smaller the particle size, which shows more nanoemulsions are formed.

INTRODUCTION

Indonesia has been known as one of the mega-biodiversity countries which produce approximately 57% or 49 out of 80 essential oil varieties. One of the plants which produce high-demanded essential oil in the world and grow well in Indonesia is Ginger (*Zingiber officinale*). In addition, Indonesia is included in the 5 world largest exporters of ginger and the amount is estimated to increase by an average of 32.75% per year. The data obtained from the Indonesian Chamber of Commerce in 2011 showed that the projected demand for oil export will be 905.43 tons. This amount is quite high although most of it is still in the form of fresh ginger rhizomes, pickled ginger and dried ginger. Meanwhile, the export of processed ginger in the forms of ginger oil and ginger oleoresin is still relatively small, precisely 0.4% of Indonesia's total essential oil exports [1] .

Ginger oil is known to have multiple functions, such as in cosmetic, food, therapeutic and pharmaceutical industries. Therefore, it has a high value in the world market. In the European market, the price of ginger oil from China is US $ 65 per kg and from India is US $ 85 per kg.

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The main problem faced by the ginger oil industry in Indonesia is that it has not met the requirements of the quality characteristics specified in the international standards. The quality parameters, for instance the optical rotation value, are very different from the applicable standards. The desired optical rotation is negative (-), while the three examples of Indonesian ginger oil are positive (+) [2]. This positive optical rotary value is primarily due to the distillation process that generates a relatively small zingiberene content compared to camphene and curcumene content. Meanwhile, the general commercial ginger oils that meet the international standards have negative optical rotating value because they have a greater zingiberene content than curcumene and camphene content. The optical rotating value expresses the purity of ginger oil, with reference to zingiberene content [3]. It indicates that Madagascar ginger oil has a positive rotating value, and relatively small zingiberene, camphene and curcumene content.

The composition of Indonesian zingiberene ginger oil is relatively small, because in conventional distillation, zingiberene undergoes thermal degradation. Zingiberene is a thermolabile compound [4.] The conventional distillation process takes between 10-18 hours to produce ginger oil. This process increases the risk of thermal degradation of zingiberene compounds. Along with the length of time needed for the distillation process, the energy needed for heating is also higher and less economical. Ginger oil extraction process is generally carried out using ethanol, isopropyl alcohol and petroleum ether. However, the process efficiency is relatively low because it has reached its natural limit (phase equilibrium) and the process and acquisition rate cannot be increased [5]. Although the supercritical fluid extraction method is able to increase the yield of ginger oil, yet the production costs and equipment prices are relatively expensive due to the required operating conditions and specific process tools [6].

To overcome such problems, it is necessary to seek for alternative development of the appropriate process for ginger oil production. The alternative process must be able to extract ginger oil quickly so as to minimize energy use and be able to control temperature considering that ginger oil has a zingiberene compound—which is thermolabile. Therefore, if zingiberene compounds are not degraded during the extraction process, the zingiberene content in ginger oil will be relatively high.

Enzymatic extraction technique is a promising and prospective technology because it stimulates phase equilibrium shift by degrading the cell wall structure of the plant so that the solute can be extracted properly [7, 8]. Therefore, it is necessary to alternate the process of extracting ginger oil through enzymatic extraction. The technology is suitable for taking thermolabile compounds because it has better temperature control than conventional heating processes.

Enzymatic extraction from several sources has been reviewed by several researchers. Extract pectin from mandarin orange peel using an enzyme was obtained from *Aspergillus aculeatus* [9]. The results showed that the extract pectin content was able to reach 21.3% (b/b). Pectin was extracted from pumpkin fruit using an enzyme complex obtained from *Aspergillus owamori* [10]. Their results showed that the yield reached 14% with DE by 53%. These results are much higher than the use of enzymes from Bacillus macerans and Trichoderma viride. The enzyme complexes contained in *Aspergillus awamori* are cellulose, xylanase, b-glucosidase, endopolygalacturonase and pectin esterase [10].

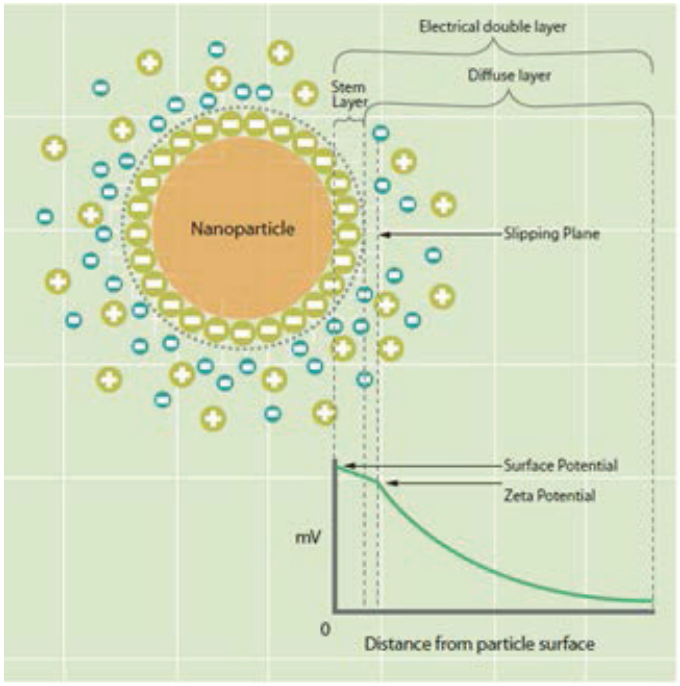

FIGURE 1. The Zeta potential measurement

Enzymatic extraction techniques are believed to have many advantages over conventional extraction, including the potential high yields, selectivity, and environmentally friendly properties [7- 12]. Although there are several patents for the enzymatic extraction process, according to [13, 14] the process efficiency is less than 80%. It is because around 15-20% of the total production costs are used for the supply of enzymes. The fundamental weakness of the enzyme extraction process scheme is that the commercially enzyme is expensive while it can be used for one time only.

Therefore, to reduce production costs, cellulose and protease enzymes sourced from rumen are used[8, 11, 15, 16]. Enzymes isolated from cattle rumen have advantages over commercial enzymes, since they are more stable at high temperatures, higher specific activities, higher optimum pH, and lower production costs [16, 17].

Cow rumen liquid comes from slaughterhouse waste. If it is not handled properly, such a waste has the potential to pollute the environment. During this time, the contents of the rumen have only been discarded and only a few have used it as compost. Currently, the number of cows slaughtered in a year is not less than 1.75 million, and around 1.5 million comes from local cattle while the rest is imported. With the amount of rumen fluid reaching 31 liters per cow, the amount of rumen fluid from cattle reaches 54.25 million liters/year [18].

Currently, the technique that can be applied so that the enzyme can be used repeatedly is an enzyme immobilization technique. This technique has several advantages since the enzymes can be used repeatedly, the process can be stopped quickly by removing the enzyme from the solution, the stability of the enzyme can be repaired, and the resulting solution is not contaminated by enzymes [12, 19, 20]. The process can be done on media, e.g., alginate matrix. The advantage of immobilization with alginate gel is that it is safe, fast, inexpensive, lightweight, simple and can be used for all types of enzymes [12, 21].

Theoretical analysis shows that the enzymatic extraction productivity with immobile isolated cow rumen enzymes are about 3.5 times higher than conventional enzymatic extraction. Therefore, recycling of enzymes becomes very important. The application of immobilized enzyme immunity of cow rumen is expected not only to be able to keep the enzyme in the extraction system during its active period, but also to produce better quality extract products compared to conventional enzyme extraction.

Lately, the application of nanoemulsion technology is growing very rapidly both at home and industrial scale. The nature of the product in the form of nanoemulsion is more easily adsorbed homogeneously to the target cell. Its application in the field of food, pharmaceuticals, and aromatherapy is easier to be absorbed by the body in which it provides acceptable sensory properties. In addition, ginger oil nanoemulsion products also have more stable properties with better storage capacity. For this reason, it is necessary to develop a scheme for the production

process of ginger oil nanoemulsion through enzymatic extraction techniques using immobile isolated cow rumen enzymes. However, the problem is how to condition the specific ability of protease and cellulase enzymes to degrade the structure of plant cell walls so that ginger oil can cross into the enzyme phase as much as possible, in addition to reducing the drag phase to the continuous phase. To that end, the focus of the research is directed at designing and fabricating enzymatic extractor devices and determining economic technical data that are very important before the commercial application.

MATERIALS AND METHOD

Research materials include: ginger rhizome, cow rumen liquid, alginate, and chemicals needed for the enzymatic extraction process. Materials for product analysis include: phosphate buffer, ethanol, distilled water, sodium hydroxide, hydrochloric acid and phenolphthalein indicator. Chemicals for the enzyme isolation process are ammonium sulfate, while the chemicals used in the enzyme immobilization process are sodium alginate, calcium chloride and manganese sulfate. The chemicals used in the enzyme activity test process are casein and TCA. The main material of ginger rhizomes are obtained from the local market in Semarang, Central Java, Indonesia. Cattle rumen liquid is also obtained from Semarang RPH, and the chemicals are purchased at CV. Jurus Maju Semarang.

The main tool used in this research is the enzymatic extractor. The series of enzymatic extractor tools consists of a 5 liter/day capacity tank equipped with a heater, temperature indicator, pH indicator, magnetic stirrer and rotating speed indicator. Meanwhile, the tools used for the enzyme isolation process and enzyme immobilization are centrifugation, magnetic stirrer, analytical balance and glass equipment.

The tools used for cell culture are liquid nitrogen tank, 1 ml micropipette (Gilson), conical tube (Nunclon), tissue culture flask (Nunclon), Laminar Air Flow Cabinet (Gelman Sciences), label sticker, centrifugator (Sarfal MC12V) and microscope phase contrast (Zeiss MC 80), and CO_2 Incubator (Heraeus). The tools used for harvesting the cells are sterile pasteur pipettes, 1 ml and 200 µl (Gilson) micropipets, sterile white tips, conical tubes (Nunclon), contrast phase microscopes (Zeiss MC 80), haemocytometer (Neubauer), counters, and digital camera (Canon Power Shoot A80.4,0 megapixels).

Research variables include: (i) fixed variables: distilled water volume (3000 ml), ginger powder weight (100 gr), extract cooling temperature (100C), and extract cooling time (30 minutes); and (ii) Variables change: enzyme-substrate ratio (0.1; 0.15; 0.2; 0.25), pH (3; 3.5; 4; 4.5), temperature (30, 35, 40, 45, 500C), and stirring speed (50, 60, 70, 80 rpm).

Enzymatic Isolation of Rumen

Rumen fluid is derived from the content of the rumen of the cow by filtration under cold conditions. The filtration liquid was centrifuged at a speed of 10,000 g for 10 minutes at 4ºC to separate the supernatant from the cell and fill the microbial cells. The supernatant was then collected as a coarse enzyme source. The supernatant was then reacted with 60% ammonium sulfate and stirred using a magnetic stirrer for 1-hour, and allowed to stand for 24 hours at 4^oC. The supernatant was centrifuged at a speed of 10,000 g for 15 minutes at 4^oC. The obtained (enzyme) deposits were then dissolved in a pH 7.0 phosphate buffer with a ratio of 10: 1 (precipitate from 100 ml of rumen supernatant dissolved in 10 ml of phosphate buffer pH 7.0).

Enzymatic Immobilisation on Alginate Matrix

Sodium alginate solution was made by adding ion-free water to a concentration of 1.5%. In the sodium alginate solution, an enzyme was added in a ratio of 3: 2 and stirred. The mixture was put into 2ml macropipette and slowly tested into $CaCl_2$ solution while stirring with a magnetic stirrer. Enzyme-containing gel was removed by filtration. Immobilization with the addition of Mn cation was carried out by adding 50 mM $MnSO_4$.

Determination of Protease Activity

Measurement of protease activity will be carried out according to the Murachi method using 1% casein as a substrate. A total of 5 grams of the immobilized enzyme was added to 5 ml of the substrate. In the reaction mixture, 1 ml 30% TCA solution was added. The mixture was heated at a temperature of 500°C for 20 minutes. Coagulated protein was separated and the filtrate was measured at a wavelength of 280 nm. The protease activity unit is expressed as the amount of enzyme that causes an increase in absorbance at a wavelength of 280 nm, which is equivalent to 1 ug of tyrosine/enzyme ml/20 minutes.

Determination of Cellulose Activity

Cellulase activity was measured using the Miller method, by adding 1 ml of enzyme extract in 1% CMC substrate at 0.2 M phosphate buffer pH 7, incubated at 500C for 60 minutes. The reaction was stopped by adding 2 ml of dinitro salicylic acid and boiled for 15 minutes, then cooled down to room temperature and measured at a wavelength of 540 nm.

Enzymatic Extraction

Extraction was carried out in an enzymatic extractor with water as a solvent. Enzymes were added to ginger powder with a certain weight ratio. Subsequently, distilled water was added and the pH of the solution was set at a certain pH using a phosphate buffer solution. The extraction process was carried out at a certain temperature. Before the feed and solvent were put into the extractor, the extractor was also conditioned at the desired temperature. The extraction time calculation (t = 0) started when the stirrer was begun to run at the desired rotation. During the extraction, a number of samples were taken every 10 minutes. Samples in the form of immobilized enzyme matrix were taken and the mixture was filtered to separate the insoluble biomass. The filtrate was analyzed in terms of the specific gravity analysis using pycnometer, refractive index analysis using a refractometer, optical rotary analysis using a polarimeter, solubility analysis in alcohol, analysis of acid numbers and ester numbers and chemical composition analysis performed using GC.

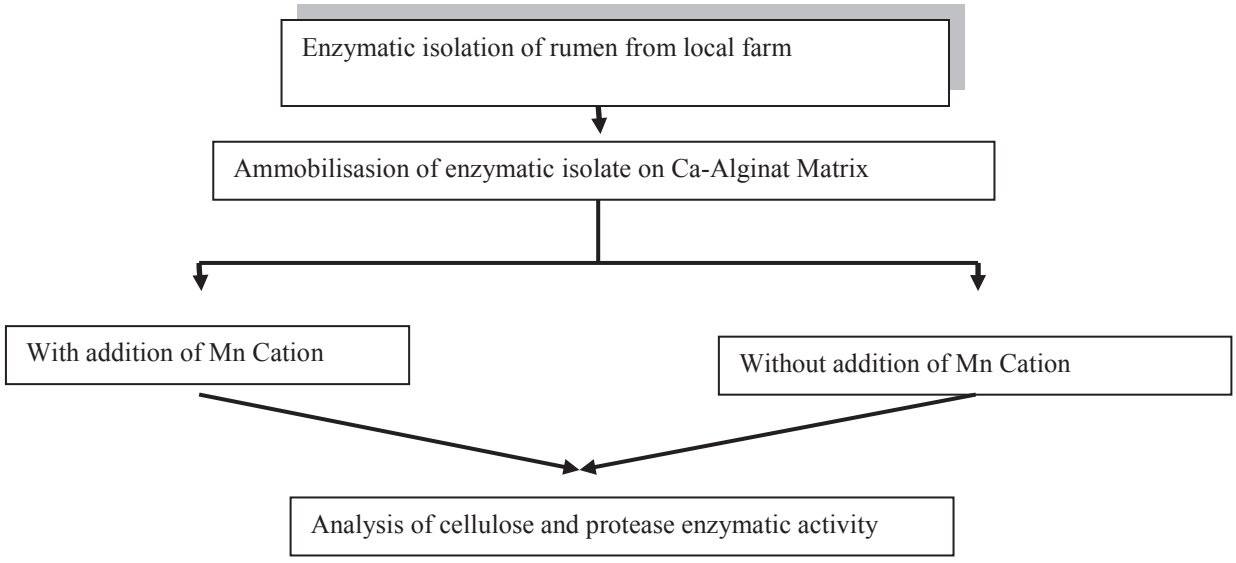

FIGURE 2. The Procedure of Productivity Study

Productivity Study of Ginger Oil

The productivity of the ginger oil bio-extraction process depends on the activity of the selected immobilized enzyme. Meanwhile, enzyme activity can be increased by the addition of certain cations or compounds. Cations that have a positive effect on the activity of rumen fluid cellulase enzyme isolates include Fe, Mg, Mn, Zn, Cu, Co, and Ca [16]. Meanwhile, cations that have a positive effect on proteases are Ca, Na and Mn [22]. Considering that the enzyme immobilization process will be trapped with Ca-alginate media, the productivity study was carried out by examining the effect of the addition of Mn cation compounds on the increase in immobilized enzyme activity.

Production of Zingiberene Ginger Oil Nanoemulsion

Production of nanoemulsion was carried out by dispersed phase factor (solvent-free ginger oil) and dispersing phase in the form of a mixture of whey protein concentrate with maltodextrin, and a mixture of maltodextrin with tween 20. The ratio of ginger-surfactant oil is varied at 10-50%. Homogenization was done between 15-25 minutes using the Ultra Turrax homogenizer at a speed of 22,000 rpm temperature and microfluidizer pressure [23].
The ginger extract obtained from the enzymatic extraction stage was prepared as an oil phase mixed with co-surfactant in the form of ethanol to obtain a concentration of 10%. Buffer solutions were made using NaOH and/or HCl to pH 7. Tween 80 as much as 10% v/v oil phase was dissolved in a buffer solution. The samples were prepared by mixing the oil phase and water phase with a ratio of 3: 7 and stirred using an Ultra Turrax homogenizer with a variation of 15-25 minutes at a speed of 22.000 rpm and a temperature of 30ºC. The process of making nanoemulsion was carried out with and without the addition of antioxidants to examine the effect of antioxidants on the stability of the size of nanoemulsion. Response measured the emulsion stability [24]. Nanoemulsion structures was done using Scanning Electron Microscopy (SEM) [23]. The dispersion analysis and size of nanoemulsion granules were carried out with Particle Size Analyzer—which can measure the size distribution with a range of 2-7.000 nm.

RESULTS AND DISCUSSION

TABLE 1. The product of Nanoemulsion on storage for one month at 4 ºC

Sample	Oil Concentration (%)	Homogenization time (minute)	ZP (mV)
1	10	15	-31.2
2	10	25	-32.5
3	30	15	-50.5
4	30	25	-44.7
5	50	15	0.2
6	50	25	-22
7	-	-	-

The physicochemical characteristics of nanomaterial have an important role in the process of absorption of nano-level material at the cellular level (cellular uptake). The physicochemical properties consist of the size, shape and surface charge of the nanomaterial. Of the three physical and chemical properties, the surface charge is a parameter that plays a major role in the process of absorption of nano materials in the cell membrane. This surface charge is associated with the zeta potential value, which is represented in mV unit.

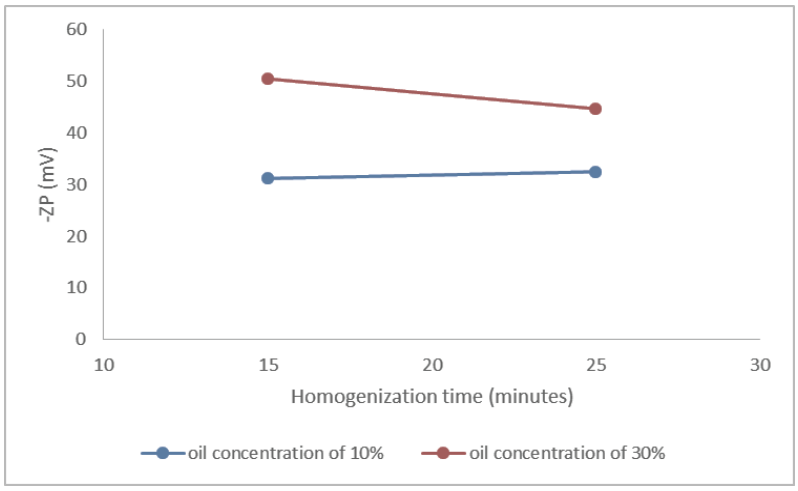

FIGURE 3. The Zeta Potential of Ginger Extract-nanoemulsion

Figure 3 shows the zeta potential value of a nano material defined as the electrokinetic value which is associated with the real magnitude of the colloidal surface charge. It also shows that the ginger nano particles obtained from the homogenization process of 15 minutes using 10% oil have a low zeta potential value, which is -31 mV (negatively charged), while ginger extract nano particles obtained from the homogenization process of 25 minutes using 10% oil have a zeta potential value of -32.5 mV. Material nano formulations that produce various zeta potential values from these nanoparticles will influence and correlate with absorption targets at the cellular level and their intracellular distribution. The target intracellular distribution can be in the form of the lysosome, mitochondria, cytoplasm and so forth. A negatively charged nanoparticle will be rapidly absorbed by fibroblast cells in the lungs, while positively charged particle nanoparticles diffuse faster in skin cells.

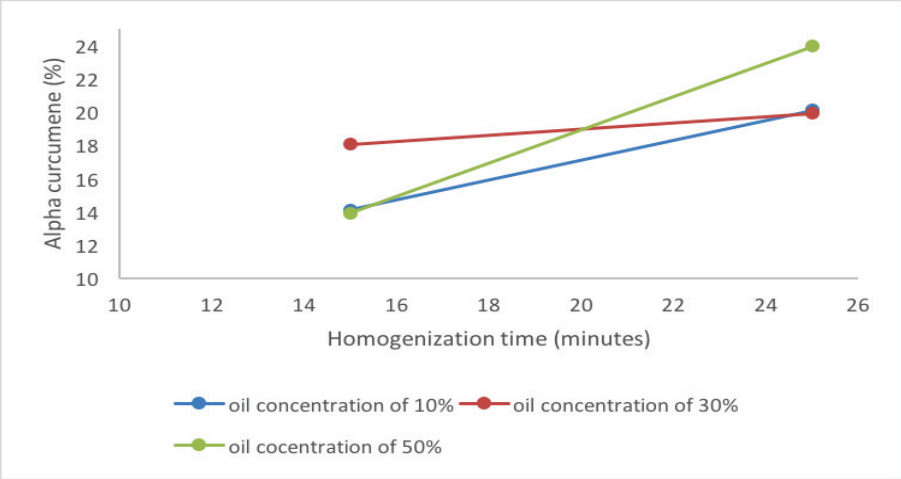

FIGURE 4. Alpha Curcumen Nanoemulsion of Ginger Extract

Figure 4 shows the alpha curcumen levels of nano emulsion ginger extract increase n line with the increase in homogenization time. It happens because the longer the homogenization time causes the longer the phase contact between the dispersion phase (oil phase) with the dispersing phase (water phase), so that it will reduce the surface tension, and dispersion phase and dispersing phase interface voltage, and the small size of dispersion phase granules facilitate spread and penetration is increasing. The decrease in surface tension and second phase interface voltage cause the mass transfer of the dispersed phase to more easily pass through both phase boundaries and diffuse, and infiltrate into the dispersing phase internally. Therefore, there is an increase in the level of nano emulsion alpha

curcumen due to the internal dispersing phase has been infiltrated by the dispersed phase. Figure 4 also shows that the greater the concentration of oil causes the acquisition of alpha curcumen nano emulsion ginger extract to increase. It happens because the increase in the concentration of oil means the increase in the number of dispersed phases.

CONCLUSION

The longer the homogenizer time, the smaller the particle size, which shows more nanoemulsions are formed. However, at certain times, the particle size will be constant. The smaller the comparison of the water-oil phase, the larger the particle size resulting from homogenization.

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