

Pilot plant scale extraction of black cincau (*Mesona palustris* BL) using historical-data response surface methodology

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Abstract

This study successfully optimized the extraction time for dried ground leaf of black cincau (*Mesona palustris* BL) in extraction tank from 15 to 300 minutes, and the historical input data were used in this study. The optimum conditions for total phenolic compound (TPC), antioxidant activity and the cost of extraction process were evaluated using Historical-data Response Surface Methodology (RSM) by setting to maximum values and extract production cost to minimum values. The optimum extraction process was gained at 153.65 min or 2.5 hours with the predicted values of TPC, antioxidant activity and extraction cost were 1.636 ± 0.12 mg CAE/g, $49.57 \pm 12.26\%$, 1.733×10^6 IDR, respectively. Small deviation between experimental values and predicted values was obtained, i.e $1.574 + 0.06$ mg CAE /g, $49.51 + 12.26\%$ and $1,734 \times 10^6$ (IDR). This study showed that optimizing the extraction time in pilot-scale for black cincau extract using Historical-data RSM is accurate and feasible to be commercialized.

Keywords

Antioxidant

Black cincau

Extraction time

Historical-data-Response

surface methodology (RSM)

Pilot

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Introduction

In recent years, consumer interest in the active role of foods beyond its functional activity has increased remarkably. Foods may provide health benefits identified as food functionalities where antioxidant activity is the most used ones (Diplock *et al.*, 1999). Natural phenols, present in plant food could provide beneficial health effects mainly through their antioxidant activity (Proteggente *et al.*, 2002; Zhou and Yu, 2006). Moreover, these polyphenol compounds have been found effective in many health-related properties, such as antioxidant, anticancer, antiviral and anti-inflammatory activities (Hyardin *et al.*, 2012).

Herbs have become more popular as a polyphenol source in Indonesia in recent years. Presently, several common herbs and spices are identified to exert many beneficial physiological effects (Srinivasan, 2005). Black grass jelly (*Mesona palustris* BL) known as black cincau in Indonesia due to its translucent black color or Hsian-tsau (*Mesona procumbens* Hemsl) in China or Taiwan is a traditional food and is believed to contribute to the health benefit. Black cincau extract component consists of bioactive compounds containing phenol, flavonoids and steroid (Yen *et al.*, 2001). Polyphenol compounds have been revealed to act as immunomodulator and can effectively inhibit mitogen-stimulated proliferation of peripheral blood

muscular cells, Ig production, IL-2 and interferon gamma (IFN- γ) (Widyaningsih *et al.*, 2013).

The availability of polyphenol compounds in black cincau, as antioxidant and immunomodulator activity is ensured. However, the economic feasibility studies in industrial scale is required to attain high extraction efficiency (Chan *et al.*, 2009). Many parameters have been determined to achieve the extraction efficiency, such as extraction methods, particle size, solvent type, solvent concentration, solvent-to solid ratio, extraction temperature, extraction time and pH (Pinelo *et al.*, 2005; Banik and Pandey, 2007; Silva *et al.*, 2007; Chan *et al.*, 2009). In this study, we report the effect of extraction time on yield, total polyphenol content and total antioxidant activity of black cincau extracts using historical data-response surface method.

Response surface methodology (RSM) is an effective statistical technique for optimizing the process variables and a powerful tool which can present the optimal conditions that improve a process if it is used adequately (Fan *et al.*, 2008). RSM helps to define the effect of the independent variables, whether it is alone or in combination in the process (Bas, 2007). Thus, RSM is a useful tool for optimizing the chemical and biochemical process over the conventional one-factor-at-time approach, which is relatively time-consuming and expensive.

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had been reported (Rochmawati, 2014). However, no previous work reported the pilot plant scale study of antioxidant activity and black cincau extraction cost in Indonesia. The use of RSM with central composite design (CCD) on the optimization of extraction conditions on antioxidant activity of edible wild mushroom *Pleurotus porrigens* (Yim et al., 2012), *Schizophyllum commune* (Yim et al., 2013), at laboratory scale has been reported. Nevertheless, none experiment was performed using historical data design to optimize the antioxidant activity and the extraction cost of black cincau, particularly at pilot plant scale. Thus, this study applied the RSM approach according to historical-data design to optimize the extraction time in order to maximize total phenolic content, antioxidant activity and to minimize the extraction cost from black cincau.

Materials and Methods

Chemicals and reagents

All the chemicals and reagents used in this study were analytical grade. Reagent 2,2-diphenyl-1-picrylhydrazyl/DPPH was purchased from Sigma-Aldrich and it was obtained from Food Nutrition Laboratory, Faculty of Agricultural Technology, (Brawijaya University). Reagent Folin Ciocalteu's, ethanol, Sodium Carbonate (Na_2CO_3) were purchased from Sigma-Aldrich. Dextrin (technical grade) was purchased from Kridatama local chemical shop, in Malang, Indonesia.

Plant materials

Dried black cincau (*Mesona palustris* BL) powder and dried red ginger (*Zingiber officinale* var. rubrum) powder was purchased from PT. ASIMAS Lawang, East Java, Indonesia, with 80 mesh size and water content of 12% w.b.

Pilot plant scale extraction of black cincau using Historical data-response surface analysis: extraction conditions

Dried black grass jelly and dried red ginger were grounded with a disk mill (model FFO-15, made in China) and sieved to 80-mesh size. 24 kg of dried black grass jelly powder and 1 kg of dried red ginger powder were weighed with a digital balance (Yamata brand, Japan) and mixed. The mixture was extracted with 500 L of water in a double jacket-vacuum extractor. The extraction process was conducted at temperature of 400°C, vacuum-pressure of \pm -45 cm Hg, black grass jelly and red ginger ratio of 24:1 and solvent-to-solid ratio of 20:1. The mixture was agitated with an orbital paddle using an electric motor

Table 1. Actual and coded levels of independent variables used in the Historical-data RSM design.

Design Summary				
Study Type	Response		Runs	20
	Surface			
Initial Design	Historical Data	Blocks	No Blocks	
Factor Name	Units	Type	Low Actual High Actual	Low Coded High Coded
Extraction Time (minutes)	Numeric		15.00 300.00	- 1.00 1.00

to ensure the homogenization of the mixture during the extraction process. Actual and coded independent used in the RSM design are listed in Table 1. The extraction time was conducted from 15 minutes up to 300 minutes. The selection of 5 hours as the final extraction time is according to the routine practice extraction time operated at PT Asimas Lawang, Indonesia. Every 15 minute, the mixture was drained off from the extractor and a sample was collected and the total phenol content, antioxidant activity and the extraction cost (IDR) for 20 samples were then analyzed. This definite variable of extraction time was used as a single factor experiment and it was called historical-data method using RSM. The optimum extraction time was selected according to the lowest value of extraction cost, the optimum antioxidant activity (Hatano et al., 1989) and total phenol content (George et al., 2005).

The run of experiments were conducted in continual order to collect samples and the data were analyzed using Design Expert Software (version 7.1.5). The experimental data were then fitted to the linear-regression model using Eq. (1)

$$Y = \beta_0 + \beta_1 X + \varepsilon \quad (1)$$

Where Y_k refers to the measure predicted responses, β_0 is the intercept; β_1 is the linear interaction coefficient of the model, X is the levels of independent variables and ε is the error of experiments. The validation of predicted model was done to ensure that the predicted optimal results were not biased towards the practical value with the target responses to obtain maximum total phenol content and antioxidant activity and minimal extraction cost.

DPPH radical-scavenging activity

Antioxidant activity was determined as described

with slight modifications (Hatano *et al.*, 1989). Sample was diluted with distilled water to obtain solution with concentration of 20, 40, 60 and 80 ppm. Each sample solution (4 ml) was added to 1 ml DPPH solution (0.2 mM). The reduction of DPPH was measured at 517 nm against a blank assay for 30 minutes. The percentage of radical inhibition in medium was calculated as the difference between absorbance of the blank and the sample divided by that of DPPH control at the same time multiplied by 100 shown in Eq. (2).

$$\% \text{Inhibition} = \frac{A \text{ Blank} - A \text{ Sampel}}{A \text{ Blank}} \times 100\%$$

Total phenol content assay (TPC)

The concentration of total polyphenol was determined by Folin Ciocalteu method with some modifications (Andarwulan and Shetty, 2000). Blank solution was prepared by adding 2 ml of 96% ethanol in a 10 ml test tube. 1000 ppm of caffeic acid was used as stock solution. 50 mg of tannic acid was diluted with 50 ml of 96% ethanol. The sample was weighed approximately 1 mg and diluted with 2 ml of 96% ethanol in a 10 ml test tube. The standard solution and sample were added to 5 ml deionized water with 0.5 ml of Folin Ciocalteu reagent (50% v/v) and incubated for 5 minutes. It was then homogenized with 1 ml of sodium carbonate solution (5% v/v), and incubated at room temperature and in the dark condition for 1 hour. After incubation, the solution was homogenized once more. Then, the total phenol content was measured with a spectrophotometer at a wavelength of 725 nm. The value of total phenolic content was interpreted by milligram equivalents of caffeic acid/g extract (mg/g CAE extract).

Calculation method of extraction cost

Extraction cost was calculated based on three components: raw materials price, extraction cost service, which was mainly determined by PT. Asimas, Lawang, because PT. Asimas Lawang charged the use of all extraction facilities regarding the extraction process, and the payment for an operator who was in charged during extraction processes. Those extraction cost components were calculated in every 15 minutes of extraction time. Lists of extraction cost in detail were outlined (Yulina, 2015).

Result

Calculation of extraction cost

Details price requirement extraction:

- Crude Cincau Black = Rp 35.000 / kg

- Crude Red Ginger = Rp 80.000 / kg
- Extraction Fee = Rp 1,500,000
- UMR Malang = Rp 1,587,037 / month

Days off work per month is 26 days

Labor cost per day = Rp 1,587,037

Labor cost per month = Rp 61039.884

Older job during optimization = 10 hours = 600 minutes

Wages per Minute = USD 61039.884

Wages for 600 minutes = Rp 101.733

Details Wages:

- The waiting time boiling: 2 hours = 120 minutes
Wages = 120 minutes x Rp 101.733
= Rp 12207.960

- Preparation for 3 hours = 180 minutes
Wages = 180 minutes x Rp 101.733
= Rp 18311.940

Extraction costs per sample = Rp 1,500,000 / 20
= Rp 75,000

Total Fixed Costs = Price simplisia black grass jelly + price simplisia red ginger + wages + wages preparatory work during the waiting time boiling

A. Total Fixed Costs = Rp 840,000 + U
SD 80,000 + USD 18311.940 + Rp 12207.960
= Rp 950,519.9

B. The cost of extraction at 153.65 minutes
The cost of extraction per minute =
Rp 1,500,000 / 300 minutes
= Rp 5,000
Thus, the extraction fee in 153.65 min =
Rp 5,000 x 153.65 minutes
= USD 768 250

C. Wages operator at 153.65 minutes
Wages operator per minute = Rp 101.733 x
153.65 minutes
= Rp 15631.275
Total Cost of extraction at the time of 153.65
minutes = A + B + C
= Rp Rp 950,519.9 + 768 250 + USD 15631.275
= Rp 1,734,401.175 or 1.734 million

Table 2 presents the responses obtained from this experiments which were fitted into the first-order polynomial equations and analysis of variance (ANOVA) for responses were calculated by Design Expert (Version 7.1.5.) summarized in Table 3.

The models F value of 36.28, 37.90 and 2278.24

Table 2. Experimental design and responses of the dependent variables to extraction time.

No.	Factor	Responses		
	Extraction time (min.)	TPC (mg CAE/g)	Antioxidant activity (%)	Extraction cost (X 10 ³) (IDR ²)
1	15.00	1.435	23.13	1.027
2	30.00	1.148	33.46	1.103
3	45.00	1.427	37.35	1.180
4	60.00	1.507	50.54	1.256
5	75.00	1.507	53.18	1.333
6	90.00	1.458	43.86	1.408
7	105.00	1.623	50.90	1.485
8	120.00	1.770	53.31	1.562
9	135.00	1.732	55.09	1.639
10	150.00	1.582	56.87	1.715
11	165.00	1.612	65.19	1.792
12	180.00	1.538	61.70	1.868
13	195.00	1.659	63.04	1.945
14	210.00	1.901	61.50	2.021
15	225.00	1.880	58.35	2.098
16	240.00	1.838	61.03	2.174
17	255.00	1.916	57.54	2.251
18	270.00	1.884	62.30	2.327
19	285.00	1.762	68.00	2.404
20	300.00	1.751	64.65	2.481

1 US \$ equals to 12.977 IDR (exchange rate April, 22th, 2015)

for TPC, antioxidant activity and extraction cost, respectively (Table 3) implies that the models were significant and predicted calculation suggested that the models for responses were linear model. The results indicated that extraction time has significant linear effects on all responses ($p < 0.0001$). The coefficients of multiple determinations (R^2) value of 0.6684, 0.7142 and 0.9922 for the responses of TPC, antioxidant activity and extraction cost, respectively indicated that the linear-order polynomial models were adequately represented by the respective experimental data.

The value of R^2 for extraction cost is the best the model that fits to the predicted calculation data compared to the R^2 of TPC and antioxidant activity (Table 3). The “Predicted R-Square” of all responses is well agreed with the “Adjusted R-Square” (Table 3). Whereas, Adequate Precision, which measures the signal to noise ratio, showed that it was greater than 4 for all responses meaning that the linear model is desirable.

In the Figure 1, it was obvious that residuals demonstrate a normal distribution for all responses, where the point followed a straight line. In addition, they also showed that the models cannot be improved further through any change in the transformation of the response because data point in Figure 1 were scattered and they did not exhibit a “S-shaped” curve.

The response surface analysis (RSA) of

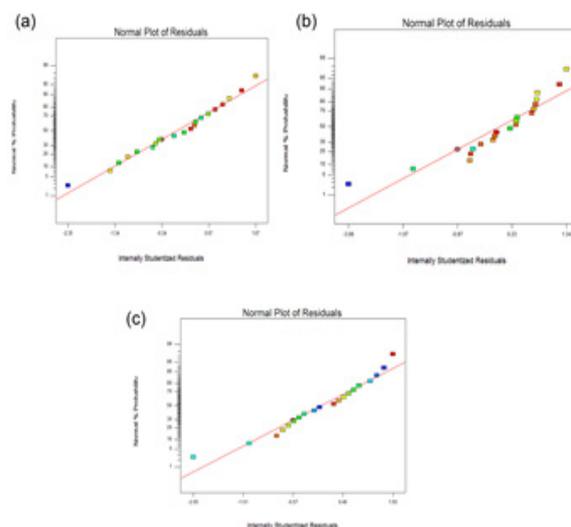


Figure 1. (a) Normal plots of the residual of (TPC); (b) antioxidant activity and (c) extraction cost.

experimental data shown in Table 3 demonstrates that extraction time has linear effect on TPC with a good regression coefficient ($R^2 = 0.6684$). The relationship between TPC and the extraction time is shown in Eq. (3)

$$Y_1 = 1.35212 + 0.00185193 X_1 \quad (3)$$

TPC of extract black cincau increased along with the extraction time in the range of 15 to 255 minutes. Beyond 255 minutes, TPC detected in the extract black Cincau showed continually decrease (Table 2).

The response surface analysis of experimental data shown in Table 3 demonstrates that extraction time has positive linear effect on antioxidant activity with a good regression coefficient ($R^2 = 0.7142$). The relationship between antioxidant activity and the extraction time is written in Eq. (4).

$$Y_2 = 26.95295 + 0.14725 X_1 \quad (4)$$

The antioxidant activity of black cincau extract increased continuously up to 165 minutes of extraction time as illustrated in Figure 2a. After that, the antioxidant activity showed decrement.

Price of raw material, extraction cost that was calculated for every 15 minutes of extraction process, and labor wages were parameters to evaluate industrial feasibility. The response surface analysis of experimental data shown in Table 3 demonstrates that extraction time has positive linear effect on extraction cost with a good regression coefficient ($R^2 = 0.9922$). The relationship between antioxidant activity and the extraction time is written in Eq. (5).

$$Y_3 = 9.4996 + 0.00510150 X_1 \quad (5)$$

Table 3. Regression coefficient of the predicted first-order polynomial models for total phenol content, antioxidant activity and extraction cost.

	Regression coefficient		
	Y_1	Y_2	Y_3
Intercept, X_0	1.35525	36.60947	0.95754
X_1 : Extraction time (min)	0.00184922	0.11073	0.00511053
Mean	1.65	54.05	1.76
Standard Deviation	0.12	6.39	0.041
R^2	0.6684	0.7142	0.9922
Adjusted R^2	0.6500	0.6983	0.9917
Pred R-Square	0.5772	0.6241	0.9913
Adeq Precision	14.034	15.627	111.210
Coefficient of variation	7.21	11.82	2.35
F-value (model)	36.28*	37.90*	2278.24*

Y_1 : Total Phenol Content (TPC); Y_2 : Antioxidant Activity; Y_3 : Extraction Cost * $p < 0.000$

Extraction time had significant positive linear effect on extraction cost as depicted in Figure 2b. As the extraction time increased in every 15 minutes, the cost of extraction positively continued to rise.

Three individual verification experiments for TPC (Y_1 , mg CAE/g), antioxidant activity (Y_2 , %) and extraction cost (Y_3 , x 106 IDR) were performed under respective extraction time for 2.5 hours. A small deviation was observed between the experimental values of $1.574 + 0.06$ mg CAE/g (Y_1), $49.51 + 12.26\%$ (Y_2) and $1,734 \times 106$ (IDR) (Y_3) and the predicted values derived from the respective regression models with degree of precision ranging from 96.21 to 99.94% or p values from 3.79 to 0.06%.

Discussion

Fittings the model

Another research reported that extraction time showed significant linear effect on FRAP and TPC of edible wild mushroom *Pleurotus porrigens* (Yim *et al.*, 2012). Widjanarko *et al.* exhibited that R^2 of viscosity of purified konjac flour (PKF) was higher than the R^2 of other responses of PKF affected by time (min.) and solvent/flour ratio (ml/g) as independent variables (Widjanarko *et al.*, 2014). Cordova *et al.* also reported that the adequate precision ratio (42.21) of RSM for lead bio-sorption on *Aspergillus terreus*, exceeded 4, indicated an appropriated signal to noise ratio (Cordova *et al.*, 2011).

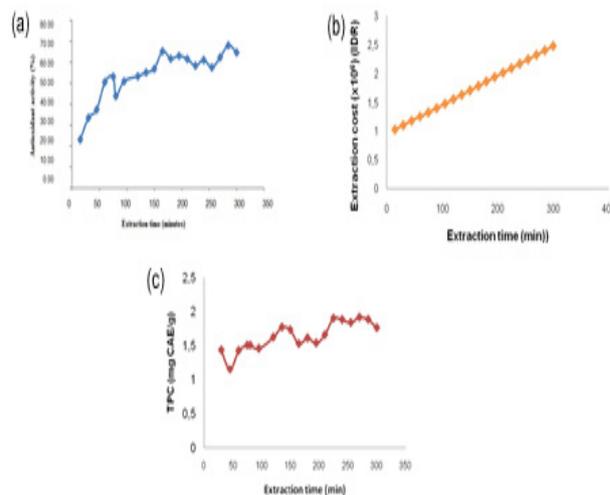


Figure 2. (a) Effect of extraction time on antioxidant activity; (b) extraction cost and (c) TPC value.

Mathematical model for TPC, antioxidant activity and extraction cost response

The fitness of the model equation for predicting the response value was verified by conducting the extraction process under the recommended optimal condition (Chan *et al.*, 2009). P-values were used as a tool for ensuring the significance of each coefficient, which in turn might indicated the interaction patterns between variables (Hou and Chen, 2008). The smaller P-value, the more significant was the corresponding coefficient. Based on Figure 1, therefore, this figure can be considered as the best models of the historical data RSM design (Jeirani *et al.*, 2013).

Response surface analysis of TPC

These phenomena in Table 2 could be well explained by Fick's second law of diffusion, predicting that a final equilibrium between solute concentrations in the solid matrix and in the solvent might be reached after a certain time (Silva *et al.*, 2007). Figure 2c showed that an increase in extraction time from 15 – 120 min was followed by an increment of TPC from 1,427 – 1,770 mg CAE/g and showed decrement after 120 min. Extraction time was main parameter in the extraction procedure. The extraction time can either be as short as few minutes or very long up to 24 hours (Lapornik *et al.*, 2005; Lee *et al.*, 2005; Chan *et al.*, 2009).

An increment of extraction time and TPC was well explained by Fick's second law of diffusion, which declares that final equilibrium will be achieved between the solute concentration in the solid matrix (plant matrix) and in the bulk solution (solvent) at a certain time. Hence, an excessive extraction time was not useful to extract more phenolic antioxidant (Silva

et al., 2007).

Ghasemzadeh and Jaafar reported that TPC value of *Pandanus amaryllifolius* Roxb extract at 5 hours extraction time showed maximum and exhibited "S-shaped" curve (Ghasemzadeh and Jaafar, 2013). Further extraction time would lead to a stable state. Prolonged extraction process might lead to phenolic oxidation due to light or oxygen exposure (Chan et al., 2009). A slight increase of TPC value at 210 min. was well explained then stating that the Folin-Ciocalteu method suffers from a number of interfering substances. The interfering substances may also react with the Folin-Ciocalteu reagent to give elevated apparent phenolic content (Prior et al., 2005).

Response surface analysis of antioxidant activity

Chew et al. claimed that the maximum antioxidant capacity of *Orthosiphon stamineus* extract was achieved at 240 minutes of extraction time and dropped till 300 minutes (Chew et al., 2011). Choi et al. found that polyphenol compound increased as increasing heating temperature and extraction time (Choi et al., 2006). Roy et al. concluded that normal cooking temperatures (75°C-100°C, 10-30 min) were detrimental to the phenolic content, antiradical and antiproliferative activities of many vegetable juices. However a mild heating (50°C, 10-30 min) could preserve 80-100% of the phenolic content (Roy et al., 2007).

Response surface analysis on extraction cost

Extraction time had significant positive linear effect on extraction cost as depicted in Figure 2c. Santos et al. concluded that it was economically viable to stop the extraction time at 9 minutes when higher amount of extracts from jabuticaba skins with the higher contents of anthocyanins was obtained (Santos et al., 2012).

The extraction cost increased as rising cost of goods manufacturing and selling cost. According to Figure 2c, a minimum extraction cost for producing black cincau extract with maximum TPC value and antioxidant activity required more than 50% of the total extraction cost calculated by RSA. Manufacturing costs are considered as substantial part of their total cost structure (Abboud and Hensley, 2003) reaching as high as 27-30% sales for manufacturers of brand-name pharmaceuticals according to previous estimations (Reinhardt, 2001).

Optimization of extraction process

Some steps were taken into account prior to predict the optimum extraction conditions. The goal of experimental region for extraction time

was set to within in range. The responses for TPC and antioxidant activity were set to maximum, and extraction cost was set to minimum during calculation process using the Design Expert version 7.0

The optimum extraction conditions were predicted at extraction time of 2.5 hours or 153.65 min. The maximum TPC value and antioxidant activity predicted by RSA were 1.636±0.12 mg CAE/g and 49.57±12.26%, respectively. The minimum extraction cost predicted by RSA was 1.733 x 10⁶ IDR.

Verification of predictive model

These verification values of prediction models were in well agreement with the predicted values with deviation less than 5%. Wu et al. stated that if the difference between predicted and experimental values is not more than 5%, it indicates that the responses model are quite accurate (Wu et al., 2006). Therefore, the historical-data RSM design was considered as an efficient statistical technique to predict the optimum extraction time.

The experimental and predictive study of the black cincau (*Mesona palustris* BL) extraction time effects in pilot plant scale on Total Phenolic Content (TPC), antioxidant activity (DPPH scavenging ability) and extraction cost (IDR) were adequately performed by using historical-data RSM. The optimum extraction conditions with regards to three responses were efficiently observed and predicted through regression coefficient of the predicted first-order polynomial models and mathematical models for three responses combined with RSM's desirability function. The experimental values generated based on optimized extraction parameters were well agreed and consistent with the predicted values. The present study provides an insight on the extraction time optimization for the maximum yield of TPC and antioxidant activity with minimum extraction cost. The results indicated that the optimal extraction time was 2.5 h with responses value for TPC, antioxidant activity, and the extraction cost were 1.636 ± 0.12 mg CAE/g, and 49.57±12.26%, 1,734 x 10⁶ (IDR), respectively. This results also proved that black cincau extraction process in pilot plant scale developed in this study is feasible to be commercialized for industrial scale.

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