Optimization of maltodextrin concentration, drying temperature and drying time on total flavonoid content and antioxidant activity of black garlic (*Allium sativum* L.) aqueous extract powder using response surface methodology

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**Abstract.** The purpose of this research was to optimize the drying process and maltodextrin concentration of black garlic (*Allium sativum* L.) aqueous extract powder using the response surface methodology. The process conditions were based on the Box-Behnken design, with 17 kinds of variations in the drying temperature (50-70 °C), drying time (18-30 hours), and maltodextrin concentration (5-15 %). The moisture content, total flavonoid content and antioxidant activity (DPPH, ABTS, FRAP) of black garlic extract powder were significantly higher at temperature of 60 °C, drying time of 30 hours and maltodextrin concentration of 5%. In this condition, total flavonoid content (14.372 mg QE/g), DPPH radical scavenging (19.616 mg TE/g), FRAP radical scavenging activity (27.661 mg TE/g) were excellent. Overall, the drying process conditions for the optimized biological activities of black garlic extract powder were expected to be at 59.95 °C, for 30 hours with 5% maltodextrin concentration.

1. **Introduction**
Black garlic is a fermented garlic product made from raw garlic which is incubated in a range temperature of 65-80 °C and a relative humidity of 70-80 % for several days [1]. Black garlic has a stronger antioxidant activity than fresh garlic [2]. This is because fermentation process increases the bioactivity of black garlic compared to raw garlic caused by the content of polyphenol, flavonoid [3] and S-allyl-L-cysteine [4].

Black garlic has many health benefits. Black garlic contains high concentration bioactive compounds that have hepatoprotective effect [5], photoprotective effect [6], cardioprotective effect [7], anti-inflammatory effect [8], immunomodulatory [9], anti-tumour potency [4], anti-cancer potency [10], anti-allergic activity [11], anti-diabetic activity [12], and anti-hypertensive activity [13].

The compounds contained in black garlic are vulnerable or sensitive to certain environments, for example by light and heat. Therefore, it is necessary to compare the compounds. One of the methods to protect bioactive compound were encapsulation process of black garlic extract. Encapsulation is the process by which small particles consisting of droplets or liquids are coated or arranged with a polymer composition to form small particles called microcapsules or microspheres [14].

Maltodextrin is usually used for coating materials because it shows low viscosity at high solid and good water solubility. Maltodextrin have the highest flavour retention because up to 35.5 % of the...
solution could be dispersed in water without haze formation [15]. Maltodextrin was chosen due to its wide range of applications in the market. It is very simple production and control methods, as well as the probability of its widespread application in the food industries [16].

Maltodextrin applications have been found in several studies about extract powder processing, for example tamarind powder [17], Sinom beverage powder [18] and guava powder [19]. However, no proportions of maltodextrin were found that matched for coating ingredient of black garlic extract powder. The black garlic extract is dried by using hot-air dryer to extend shelf life. This dryer uses electric power which would be a source of heat. So, the stability of compounds in black garlic extract that have antioxidant activity is influenced by drying temperature and drying time. Based on those considerations, it is necessary to find a solution to study optimal drying temperature, drying time and concentration of maltodextrin to produce black garlic aqueous extract powder.

2. Material and methods

2.1. Chemicals and materials
Fresh garlic (*Allium sativum* L.) was purchased from local market in Malang, East Java, Indonesia. Maltodextrin was obtained from Xingmao with dextrose equivalent 10-12. Sodium nitrite, aluminum chloride, acetone, sodium hydroxide, methanol, hydrochloric acid, acetic acid, sodium acetate, and FeCl₃.6H₂O were obtained from Merck (Germany). The chemicals DPPH (2,2-diphenyl picrylhydrazyl), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), ABTS (2, 2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid), TPTZ (2,4,6-tripyridyl-S-triazine), quercetin, potassium persulfates were obtained from Sigma Chemicals (USA).

2.2. Instrumentation
This experiment used spectrophotometer UV-Vis (Genesys 10s), rice warmer, hot air dryer, water bath shaker (Memmert), oven (Memmert), sieve 80 mesh, blender (Phillip), analytical balance (Ohaus), magnetic stirrer (MS-H-Pro GSA), vortex (LW Scientific, Inc.) and glassware.

2.3. Experimental design
The optimum levels of the three independent variables (X₁: maltodextrin concentration; X₂: drying temperature; and X₃: drying time) were determined using the response surface methodology. Each independent variable consisted of three levels (Table 1). This experiment used a parameter design with 17 experiments and including five replicates of the central point for optimization of the drying process. The responses of this experiment were moisture content, total flavonoid content, and antioxidant activity (DPPH, ABTS and FRAP).

| Symbol | Independent variable | Unit | Code levels |
|--------|---------------------|------|-------------|
| X₁     | Maltodextrin concen. | %    | Low (-1) 10  Central (0) 15 |
| X₂     | Drying temperature  | °C   | 50          60          70 |
| X₃     | Drying time         | hours| 18          24          30 |

Table 1. Levels and code of variables chosen for Box-Behnken design.
2.4. Experimental process

2.4.1. Preparation of black garlic. The garlic was washed with tap water. The garlic was arranged in rice warmer machine (73±2 °C) for 15 days. Then, the black garlic was peeled, and the pulp was grinded into paste. For preparation of the extracts, the black garlic paste was weighed and homogenized with five times of water. The mixture was extracted by using a water bath at 90±2 °C for 5 minutes. Subsequently, the filtrate was separated from the residue by filtration using filter paper. The extracted solution was stored in a closed bottle and kept at 4 °C before experiment.

2.4.2. Black garlic powder processing. Maltodextrin (5, 10, and 15 %) was mixed with 250 ml black garlic aqueous extract per experimental design conditions and then agitated to create an aqueous solution. A hot air dryer was used to dry the resulting mixture at certain temperature (50, 60, 70 °C) for certain duration (18, 24, 30 hours). After drying, the flakes were collected and milled into powder. The black garlic powder was sieved using 80 mesh sieves. The sample was stored in a closed plastic before analyzed. The black garlic extract powders were analyzed for moisture content (%), total flavonoid content (mg QE/g), antioxidant activity (mg TE/g). Then the data is tabulated to identify the optimum conditions for the variables that produce the highest quality products.

2.4.3. Verification of model. Optimum conditions for the processing of black garlic extract powder depended on maltodextrin concentrations, drying time and drying temperature was obtained using the predictive equations of (response surface methodology) RSM with Box-Behnken design. The antioxidant activity, moisture content and total flavonoid content were measured after production of black garlic extract powders under optimal conditions. The verification phase is carried out with three replications and compared with the optimization results predicted by Design Expert version 11.1.0.1 software. The predicted and experimental values were compared to determine the validity of the model. This verification uses paired T-test with Minitab 17 software.

2.5. Analysis procedure

2.5.1. Determination of moisture content. AOAC (2007) was used to determine powder moisture in an air-circulation oven at 105 °C. The results were given in grams of water per 100 grams of powder (w/w) [20].

2.5.2. Determination of total flavonoid content (TFC). The total flavonoid content from black garlic aqueous extract powder was determined by a NaNO$_2$-AlCl$_3$-NaOH method [21]. 0.1 grams samples were dissolved in 10 ml distillation water. 1 mL of the solution was added with 0.3 mL 5% (w/v) NaNO$_2$. Five minutes later, 0.3 mL of 10% (w/v) AlCl$_3$ was added and the mixture incubated for six minutes. Then, 2.0 mL of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. Furthermore, the solution was mixed thoroughly by using vortex and stood for 30 minutes at room temperature. Finally, the absorbance of the solution was measured using a UV–Vis spectrophotometer against a blank at wave length of 325 nm. Quercetin solution (20-100 µg/ml) was used as the standard to make calibration curve. The total flavonoid contents of the dry sample were expressed as mg QE/g dry weight.
2.5.3. **DPPH radical scavenging activity measurement.** DPPH assay was analysed using a colorimetric method explained by Thaipong et al. with slightly modifications [22]. Firstly, the extracts were weighed at 0.1 gram and dissolved in volumetric flask 10 ml. Then, 2.85 mL of 0.03 mg/ml DPPH solution (in methanol) and 0.15 ml of the sample solution were mixed. Furthermore, the mixture was stood for 30 minutes in the dark at room temperature. Finally, the absorbance was analysed immediately against a blank solution (absolute methanol) at wave length of 517 nm. Trolox solution (25-200 µg/ml) was used for standard curve. Result was expressed in mg TE/g dry weight.

For each experiment, a new DPPH solution was produced.

2.5.4. **ABTS radical scavenging activity measurement.** ABTS assay of black garlic aqueous extract powder was measured with a previous method with certain modifications [22]. The reaction of 1 ml of 7.4 mM ABTS solution and 1 ml of 2.6 mM potassium persulfate aqueous solution was performed for 12 hours in the dark, to make the ABTS radical cation. The solution of ABTS radical cation was diluted by mixing 1 ml ABTS with methanol until 50 ml in volumetric flask. For each experiment, a new ABTS solution was produced. The samples were dissolved in distilled water to obtain concentrations 10 mg/ml. Then, 0.15 ml samples were mixed with 2.85 ml for 30 minutes in dark condition. After reaction, the solution absorbance was analysed at 734 nm using UV-Vis spectrophotometer. Trolox (concentration 25-150 µg/ml) was used as the standard curve.

2.5.5. **Ferric-reducing antioxidant power (FRAP) measurement.** FRAP assay was measured by a colorimetric method explained by Thaipong et al. with minor modifications [22]. FRAP reagent was prepared by mixing 25 mL of FeCl₃.6H₂O (20 mM) with 25 mL of 10 mM TPTZ solution (diluted in 40 mM HCl), and 250 mL of 300 mM acetate buffer pH 3.6 (5.1 g CH₃COONa and 20 mL CH₃COOH). Then, 3.9 mL FRAP reagent and 130 µL upper-layer solutions was mixed. Furthermore, the reaction mixture was stood for 15 minutes at 37 °C in the dark room. Finally, the absorbance of the mixture was analysed at wave length of 593 nm against a blank solution (0.15 mL of distilled water and 2.85 mL of FRAP reagent). Trolox (concentration 25-200 µg/ml) was used as the standard curve.

3. Results and discussion

3.1. Analysis of response surface method results

Response surface methodology is a mathematical and statistical technique that can be used to optimize multivariable systems by finding the true relationship between the factors and the response. The operational variables in this study were concentrations of maltodextrin (5-15%), drying temperature (50-70 °C) and drying time (18-24 hours).

Maltodextrin concentration, drying temperature and drying time are important factors in the process of making black garlic extract powder to obtain optimum conditions. These factors were optimized for minimum moisture content, maximum total flavonoid content and maximum antioxidant activity by DPPH, ABTS and FRAP methods. All data collected from experimental condition with 17 runs and predicted using response surface methodology by using Box-Behnken design (Table 2).

The model suggested by the Design Expert version 11.1.0.1 software for all responses were a quadratic model so that it produces an equation in the form of a coded variable for all response were shown in Table 3.
Table 2. The set of experiment variable based on Box Behnken design and observed responses of black garlic extract powder.

| Variables                      | Responses                          |
|-------------------------------|------------------------------------|
| No.                           | Maltodextrin concentration (%)     | Drying temperature (°C) | Drying time (hours) | Moisture content (%) | Flavonoid content (mg QE/g) | Antioxidant activity (mg TE/g) |
| 1                             | 5                                  | 50                      | 24                 | 13.8935             | 13.0848                     | 16.4857                      |
| 2                             | 15                                 | 50                      | 24                 | 9.6504              | 6.6500                      | 6.7473                       |
| 3                             | 5                                  | 70                      | 24                 | 13.5995             | 14.2345                     | 16.2770                      |
| 4                             | 15                                 | 70                      | 24                 | 8.3153              | 6.3655                      | 8.6950                       |
| 5                             | 5                                  | 60                      | 18                 | 14.3551             | 13.7020                     | 19.0594                      |
| 6                             | 15                                 | 60                      | 18                 | 7.3977              | 7.1511                      | 9.8775                       |
| 7                             | 5                                  | 60                      | 30                 | 12.2903             | 14.372                      | 26.1659                      |
| 8                             | 15                                 | 60                      | 30                 | 7.4191              | 6.9233                      | 9.0428                       |
| 9                             | 10                                 | 50                      | 18                 | 9.9785              | 8.3674                      | 9.1819                       |
| 10                            | 10                                 | 70                      | 18                 | 10.2742             | 8.6935                      | 10.9209                      |
| 11                            | 10                                 | 50                      | 30                 | 11.0717             | 8.4375                      | 9.3906                       |
| 12                            | 10                                 | 70                      | 30                 | 9.0924              | 8.7844                      | 11.4078                      |
| 13                            | 10                                 | 60                      | 24                 | 10.1352             | 7.2148                      | 9.5297                       |
| 14                            | 10                                 | 60                      | 24                 | 10.8392             | 7.1134                      | 8.0689                       |
| 15                            | 10                                 | 60                      | 24                 | 10.9642             | 6.7316                      | 8.2776                       |
| 16                            | 10                                 | 60                      | 24                 | 10.6870             | 7.0398                      | 9.1123                       |
| 17                            | 10                                 | 60                      | 24                 | 10.5278             | 6.7682                      | 9.5992                       |

Table 3. Response variables and their fitted model equations.

| Response variable                  | Fitting the coding equation of the model                                                                 |
|------------------------------------|---------------------------------------------------------------------------------------------------------|
| Moisture content (%)               | \( Y_1 = 54.32913 - 1.07599 X_1 - 1.03611 X_2 - 0.071052 X_3 - 0.0052055 X_1^2 + 0.034073 X_3 X_1 - 0.011775 X_2 X_3 + 0.0043473 X_1^2 + 0.00625332 X_2^2 - 0.024345 X_3^2 \) |
| Total Flavonoid Content (mg QE/g)  | \( Y_1 = 53.76528 - 2.12831 X_1 - 0.59742 X_2 - 1.28478 X_3 - 0.007173 X_1 X_2 - 0.000748167 X_3 X_1 + 0.0000867 X_3 X_2 + 0.10153 X_2^2 + 0.0057192 X_3^2 + 0.028478 X_3^3 \) |
| Antioxidant Activity (mg TE/g)     | \( Y_1 = 44.35217 - 4.21849 X_1 + 0.556984 X_2 - 2.38186 X_3 + 0.010782 X_1 X_3 - 0.011593 X_1^2 + 0.001159 X_2 X_3 + 0.1461646 X_3^2 - 0.005199 X_2^2 + 0.05077 X_3^2 \) |
| DPPH (mg TE/g)                     | \( Y_1 = 0.047873 X_1 - 0.02269 X_1 X_2 + 0.112665 X_2^2 - 0.032585 X_3^2 + 0.066987 X_3^3 \)          |
| ABTS (mg TE/g)                     | \( Y_1 = -118.64417 - 0.746705 X_1 + 4.71904 X_2 - 0.375673 X_3 + 0.000241 X_2 X_3 + 0.95511 X_1 X_2 + 0.00682 X_3 + 0.096268 X_3^2 - 0.037816 X_3^2 + 0.041767 X_3^3 \) |

3.1.1. Analysis of moisture content response. Moisture content is important factor that affects powder stability. It is related to powder flowability, drying efficiency, storage stability and stickiness [23]. The moisture content of black garlic extract powder varied from 7.3977 to 14.3551%. Black garlic extract powder obtained from the drying process with 15% maltodextrin concentration at 60 °C for 18 hours resulted the lowest moisture content. However, the utilization of lower maltodextrin concentrations (5%) and prolonged drying times (18 hours) at 60 °C produced the powder with the highest moisture content.
Maltodextrin concentration significantly affected the moisture content. A higher maltodextrin concentration led to the reduction of moisture content of black garlic extract powders. Maltodextrin has low molecular weight and simple molecule structure that water content in material could evaporate easily during drying process.

**Figure 1.** Effect of maltodextrin concentration, drying temperature and drying time on moisture content.

Drying temperature ($X_2$) and drying time ($X_3$) factors have significantly affect in moisture content of black garlic extract powder. Higher drying temperature and drying time promote greater losses of water that evaporated from product during drying process. The migration of water in surface of product transfer to dry air led decreasing of amount of water in product. Thus, it caused water content in product decreased [24].

Interaction between drying temperature ($X_1$) factor and drying time ($X_3$) factor also significantly affected to moisture content. The higher temperatures and longer drying time led to higher energy being transferred to the product and evaporated of a higher amount of water [25].

**3.1.2. Analysis of total flavonoid content response.** The total flavonoid content of black garlic extract powder varied from 6.3655 to 14.372 mg QE/g. The lowest total flavonoid content was obtained using 15% maltodextrin concentration at drying temperature of 70 °C for 24 hours. The combination factor of drying temperature of 60 °C, 5% maltodextrin concentration, and drying time of 30 hours, led to the highest total flavonoid content of black garlic extract powder.

Figure 2 illustrates the interactive effects of the independent variables corresponding to the total flavonoid content. Maltodextrin concentration ($X_1$) high significantly affected the total flavonoid content of black garlic extract powder. It could be seen that the total flavonoid content increased with the decrease of maltodextrin concentration.

**Figure 2.** Effect of maltodextrin concentration, drying temperature and drying time on total flavonoid content.
Drying temperature ($X_2$) significantly affected the total flavonoid content. It increased with the increase of drying temperature. Furthermore, total flavonoid content also increased with the increase of drying time ($X_3$). But drying time no significantly affected the total flavonoid content.

3.1.3. Analysis of antioxidant activity response. The antioxidant capacity of the black garlic extract powder was determined by using the DPPH, ABTS and FRAP assays. The first assay (DPPH) was conducted to measure the antioxidant capacity of the product. The ability of the black garlic extract powder to neutralize DPPH radicals was measured to be from 6.7473 to 19.6159 mg TE/g. The results obtained from this experiment indicated that drying process with 15% maltodextrin concentration, at 50 °C for 24 hours was exhibited the lowest antioxidant capacity by the DPPH assay. However, performing drying process at 60 °C for 30 hours with 5% maltodextrin concentration produced powder with the greatest ability to neutralize DPPH free radicals.

The results obtained using the ABTS assay exhibited that the black garlic extract powder contains high levels of antioxidant compound. Black garlic extract powder obtained from drying process with 15% maltodextrin concentration at 50 °C for 24 hours provided the lowest antioxidant activity toward ABTS assay (8.7351 mg TE/g). On the other hand, drying by using 5% maltodextrin concentration, temperature (70 °C) and time (24 hours) resulted product with the highest antioxidant activity according to ABTS assay (27.2953 mg TE/g).

The reducing ability of the black garlic extract powder was conducted by FRAP assay. Drying process of black garlic extract with maltodextrin concentration of 15% at 50 °C for 24 hours resulted black garlic extract powder with the lowest reducing ability based on the FRAP method (4.7868 mg TE/g). On the other hand, the use of maltodextrin with lower amounts (5%) and prolonged drying times (30 hours) at 60 °C produced the powder with the greatest activity based on the FRAP assay (27.6612 mg TE/g).

The regression equations showed the relationship between values of antioxidant activity of black garlic extract powder as responses. They were influenced by initial factors including maltodextrin concentration, drying temperature and drying times which were coded as $X_1$, $X_2$ and $X_3$, respectively.

Maltodextrin concentration ($X_1$) factor significantly affected the antioxidant activity response of DPPH, ABTS and FRAPs assays. Based on the polynomial equation, antioxidant activity decreased when the maltodextrin concentration increased. The decrease in antioxidant activity is due to the addition of maltodextrin which is higher. It was causing more total solids contained in an ingredient or product as an encapsulated or filler material so that the measured antioxidant activity will be decrease [26]. The addition of maltodextrin concentration will more influence the decrease of antioxidant activity compared with other factors because the $X_1$ coefficient is the largest.

The drying temperature factor ($X_2$) had a significant effect on the response of antioxidant activity of the DPPH and ABTS assays but did not significantly affected the FRAP assay. Based on the polynomial equation, increasing of drying temperature elevated black garlic extract powder antioxidant activity. Based on experiment conducted by Katsube et al. [28] reported that the higher of drying temperature would increasing the antioxidant activity to a certain point with the best treatment that was at a temperature of 60 °C. Antioxidant activity in black garlic was influenced by several chemical compounds. For example, polyphenols and flavonoids, are bioactive compounds whose availability is dependent on the type and duration of processing [29]. In this study, total flavonoid compounds were found to be significantly greater at the greatest drying temperature. Thus, increased flavonoid compounds resulted in higher antioxidant activity than low drying temperatures [30].

The drying time factor ($X_3$) significantly influenced the antioxidant activity response of the FRAP assay but did not significantly affect the DPPH and ABTS assays. Based on the polynomial equation, the longer the drying period was used, the higher the antioxidant activity of the product. The length of the drying process significantly affected the moisture content of the product. Longer drying time promote greater losses of water that evaporated from product during drying process, then reduce the weight and decrease moisture content of the material [31]. However, a decrease in moisture content of
the black garlic powder may be due an increase in antioxidant compounds concentration, so it also leads to increasing of antioxidant compounds in neutralizing free radicals [32].

**Figure 3.** Effect of maltodextrin concentration, drying temperature and drying time on antioxidant activity DPPH (A), antioxidant activity ABTS (B), and antioxidant activity FRAP (C).

Interaction between maltodextrin concentration factor and drying temperature (X1X2), also interaction between drying temperature and drying time (X2X3) did not significantly affect the antioxidant activity response of DPPH, ABTS and FRAP methods. The interaction of maltodextrin concentration factor and drying time significantly affected the response of the antioxidant activity of the FRAP method but did not significantly affect the DPPH and ABTS methods. Based on the polynomial equation, the interaction coefficient value of the maltodextrin concentration factor and the drying time (X1X3) was negative so it could be suggested that the higher interaction of the maltodextrin concentration and drying time would reduce antioxidant capacity. The addition higher amounts of maltodextrin resulted in a decrease of antioxidant activity, causing more total solids contained in an ingredient or product as an encapsulated or filler material so that the measured antioxidant activity will be less [2].
3.2. Optimization of the drying process

The purpose of this study was to find the optimum process parameters for minimum moisture content, maximum total flavonoid content and antioxidant activity. For optimization, the desirability function was created after limiting the preferred goal of responses during drying process of black garlic extract powder to obtain the desired quality product. Based on the desirability function, maltodextrin concentration of 5.00%, drying temperature of 69.95 °C, and drying time of 30 hours was found to be the predicted optimum condition. Table 4 showed the predicted and experimental values of the responses at optimum condition based on the quadratic model.

3.3. Verification of the model

In terms of model verification, three experiments were conducted at the recommended optimum condition with a minor adjustment in temperature drying by 59.95 °C in exchange of 60.00 °C. The experimental and predicted values are tabulated in Table 4. The experimental results were adequate with the response surface model predicted values, because the experimental values were quite near to the expected values.

Table 4. Experimental and predicted values of the responses at optimum conditions.

| Responses                          | Values  | P-value (T-test) |
|-----------------------------------|---------|-----------------|
| Moisture content (%)              | 10.810  | 10.110          | 0.068          |
| Total Flavonoid Content (mg QE/g) | 14.447  | 14.378          | 0.757          |
| Antioxidant Activity DPPH (mg TE/g) | 20.103  | 19.434          | 0.087          |
| Antioxidant Activity ABTS (mg TE/g) | 26.804  | 26.025          | 0.325          |
| Antioxidant Activity FRAP (mg TE/g) | 26.939  | 26.823          | 0.790          |

From the overall responses that have been carried out the T-test shows that the optimum condition verification value is not significantly different or not significant. This proves that the optimum conditions of black garlic extract powder processing were a maltodextrin concentration of 5%, drying temperature of 60 °C and drying time of 30 hours. These conditions with the highest desirability value have the results of response tests in accordance with predictions recommended by Design Expert 11.1.0.1 software comparison with black garlic powder using the T-test to find out the differences between the response from prediction and experimental.

4. Conclusions

The optimal conditions to obtain the minimum moisture content, the highest total flavonoid content and maximum antioxidant activity of black garlic extract powder were maltodextrin concentration of 5%, drying temperature of 60 °C and drying time of 30 hours. Moisture content and total flavonoid content at this condition was 10.81% and 14.4471 mg QE/g, respectively. The optimal antioxidant activity of black garlic extract powder obtained from this study were 20.103 mg TE/g of DPPH radical scavenging activity, 26.804 mg TE/g of ABTS radical scavenging activity, and 26.939 mg TE/g of FRAP radical scavenging activity.

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